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Original Article

Salmonella Serovars Isolated from Poultry Breeding Flocks under the Brazilian Official Control Programme Between 2016 and 2018

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ABSTRACT

The control of *Salmonella* in the poultry production chain combined with biosecurity measures is an important tool to maintain and guarantee the sanitary status of Brazilian flocks. The aim of this work was to compare official laboratory data on molecular typification of *Salmonella* isolates from poultry breeding flocks in different Brazilian states between 2016 and 2018 and identify the production category with the most positive flocks, in light of current legislation. Surveillance data of positive samples from the official Brazilian *Salmonella* Control Programme sent to Federal Agricultural Defence Laboratory of São Paulo (LFDA-SP) after molecular characterization were analysed. These data were subject to an exploratory study, undergoing a descriptive statistical analysis followed by the use of frequency and non-parametric hypothesis tests. Overall, 49 serovars were detected in poultry broiler-breeder and layer-breeder flocks. *Salmonella* ser. Heidelberg, *Salmonella* ser. Anatum, *Salmonella* ser. Newport, *Salmonella* ser. Schwarzengrund and *Salmonella* ser. Mbandaka were the five most common isolated serovars. The data shows that there is an opportunity to improve biosecurity measures in parent breeder flocks. A total of 16 serovars were identified in turkey-breeders. *Salmonella* ser. Anatum, *Salmonella* ser. Newport, *Salmonella* ser. Brandenburg, *Salmonella* ser. Litchfield, and *Salmonella* ser. Livingstone were the most common ones. The four official controlled serovars represented a small part of the isolated strains. These data demonstrate the importance of an official program in Brazil for *Salmonella* surveillance in breeder flocks combined with biosecurity measures.

INTRODUCTION

Brazil is one of the world's largest producers of animal protein, the third largest producer of broiler chicken, and the first global exporter of chicken meat and its products. Its privileged position is the result of the country's natural capacity for producing food, as well as the sanitary status of its flocks. Such position allows industries to stand out in their competitiveness up to the present days (ABPA, 2021).

The southern region of the country, comprising the states of Paraná, Santa Catarina, and Rio Grande do Sul, accounts for approximately 65% of Brazilian chicken meat production and 80% of its exports. On the other hand, the south-eastern region is responsible for the core of the country's egg production, which has been developing over the years.

Meanwhile, Brazilian turkey meat production has declined considerably after 2017, along with its exports. The southern states of Santa Catarina and Rio Grande do Sul are responsible for 99% of turkey meat exports (ABPA, 2021).

Due to the importance of poultry production, the Ministry of Agriculture, Livestock, and Food Supply (MAPA) launched the National Poultry Health Program (PNSA) in 1994 (Brasil,1994), which has allowed Brazil's poultry production to move forward. In order to maintain and guarantee the sanitary status of Brazilian flocks, biosecurity and surveillance public policies have been established (Brasil, 2003; 2007; 2016). Nevertheless, the prevention and control of pathogens in those flocks are a permanent challenge, in which the official veterinary service plays a crucial role.

Among such pathogens, *Salmonella* is a major concern in the poultry production chain worldwide, considering its importance for public and animal health (WOAH, 2021a; WOAH, 2021b). From farm to fork, there are many possible routes for *Salmonella* dissemination and contamination in poultry and their products, thus examination of each step of the process is necessary (Rajan *et al.*, 2016). Nevertheless, *Salmonella* is a sanitary barrier to the export of multiplication material (WOAH, 2021b) and poultry products, according to bilateral sanitary agreements in force. Furthermore, the World Organisation for Animal Health (WOAH) suggests that countries should identify the prevalent *Salmonella* serotypes in humans and poultry in order to establish a control programme (WOAH, 2021a). Therefore, the PNSA implemented the official *Salmonella* Control Programme (Brasil, 2003).

In this context, the control of this pathogen must start in breeding establishments, considering their potential for dissemination to the rest of the poultry production chain (Sivaramalingam *et al.*, 2013; Rajan *et al.*, 2016). The Brazilian breeding flocks are constantly monitored to be certified as being free from typhoidal and non-typhoidal *Salmonella*. To monitor *Salmonella* in the poultry production, samples are collected on a regular basis from all breeder flocks, which includes broiler-breeders, layer-breeders, turkey-breeders, and other bird breeders such as quail, duck, and geese (Brasil, 2003). Additionally, control and monitoring of *Salmonella* in commercial broilers and turkey establishments was established, as well as measures to control this pathogen in slaughterhouses (Brasill, 2016).

At present, breeding flocks are certified free from 4 (four) *Salmonella* serovars (Brasil, 2003): *Salmonella* ser. Enteritidis and *Salmonella* ser. Typhimurium, which are non typhoidal serovars and important foodborne pathogens worldwide related to poultry products (WOAH, 2021a), and *Salmonella* ser. Gallinarum e *Salmonella* ser. Pullorum, typhoidal serovars which concern poultry health and can cause severe economic losses (Markos & Abdela, 2016; Celis-Estupiñan *et al.*, 2017; De Carli *et al.*, 2017; WOAH, 2021b).

The aim of this work was to compare official laboratory data on molecular typification of *Salmonella* isolates from poultry breeding flocks from different Brazilian states dated between 2016 and 2018, received by the Federal Agricultural Defence Laboratory of São Paulo (LFDA-SP), and to identify the production category with the most positive flocks in light of the current legislation.

MATERIALS AND METHODS

The study analysed the surveillance data of Brazil's official *Salmonella* Control Programme, studying positive samples sent to Federal Agricultural Defence Laboratory of São Paulo (LFDA-SP) after molecular characterization. The samples were collected from poultry breeder flocks for certification of epidemiological units, which mostly comprises broiler-breeders, layer-breeders, and a few samples from turkey-breeders, from different states of Brazil (BRASIL, 2003). Other poultry breeder species such as quail-breeders and duck-breeders were excluded from the analyses because of the small number of samples. The data comprises 318 positive *Salmonella* samples collected in 2016, 2017, and 2018, which are used to describe the major isolated serovars and determine which breeder category had the greatest number of serovars identified.

Types of samples collected in epidemiological units

The surveillance had been performed in broiler-breeders, layer-breeders, and turkey-breeders during rearing and production period in great-grandparent, grandparent, and parent flocks.

Samples were collected between one and five days of life, in the middle of the growing period, at the beginning of production, and then every three months for periodic control of the epidemiological unit.

The type of material used for bacteriological or molecular diagnosis depends on the production stage. In the initial growing phase, dead chicks, drag swabs, and paper from the chick's transport boxes were collected. Flocks that were vaccinated against paratific *Salmonella* had their samples collected before vaccination. Cloacal swabs, fresh faeces, and boot swabs were the samples collected during the rearing period from great-grandmothers, grandmothers, and parents. In addition to these, the samples collected in breeders' flocks vaccinated with inactivated

vaccines for paratific *Salmonella* were pipped embryo, meconium, and organs. After the first certification of the epidemiological unit by the government, the control is conducted on a quarterly basis, collecting samples of cloacal swab, fresh faeces, drag and boot swabs, and pipped embryo (Brasil, 2003).

Salmonella isolation

The samples were sent to accredited laboratories in the National Accredited Laboratories Network. All isolates tested positive for *Salmonella* in accredited laboratories were sent to the official MAPA laboratory, the Federal Agricultural Defence Laboratory of São Paulo (LFDA-SP), where they are received, identified, purified, and typified by DNA microarray.

Strains purification and biochemical tests

As a confirmation of a diagnosis already made by accredited laboratories with previously isolated strains, the next step performed was purification in agar plate (Rambach). After purification, the colonies were subjected to molecular characterization by DNA microarray test.

Typing

Molecular characterization followed the methodology described in the Routine *Salmonella* Serotype Identification Check & Trace manual (CHECK-POINTS, 2020). Soon after, three to five colonies isolated on agar plate (Rambach) were collected to be typified when there was suspicion of *Salmonella* ser. Gallinarum or *Salmonella* ser. Pullorum, or a single colony for the other *Salmonellae*. The colony was homogenised in 100 µl of lysis buffer. From the 1.5 mL microtubes, the homogenate was transferred to the thermoblock previously heated to 99°C for 15 min. Afterwards, the homogenate was cooled to room temperature by vortexing. Then, three steps of DNA recognition and detection were performed, as described in the manual. The reading of *Salmonella* typification results was performed using software available in the analysis system.

Results analysis

Accredited laboratories provided the official laboratory data sheets collection and the *Salmonella* isolates on a monthly basis. To select the strains considered in this work, the following filters were applied from the database: year, state, region, type of poultry (chicken, turkey, quail and duck); category (e.g. great-grandmother, grandmother); surveillance type (certified as free or controlled epidemiological unit for *Salmonella*) and type of sample. Exploratory analysis was used on these data, followed by descriptive statistical analysis. Frequency and non-parametric tests (Kruskal-Wallis) were performed using RStudio software.

RESULTS AND DISCUSSION

Surveillance for *Salmonella* is not only important for public health, but also for the economic losses due to high morbidity and mortality in poultry flocks. Furthermore, *Salmonella* is responsible for losses and waste of animal protein that reduce global food availability in a world where hunger is a reality. Therefore, *Salmonella* control in early phases of production could help reducing these losses (FAO, 2011).

Many studies report that *Salmonella* control and prevention strategies in the poultry chain should cover the whole production chain, from farm to fork (Andino & Hanning, 2015; Rajan *et al.*, 2016; Machado *et al.*, 2020;). Sivaramalingam *et al* (2013) suggest that interventions at the breeder flock level would likely reduce transmission from breeder flocks to lower levels of the production chain and possibly also to the retail level. When a *Salmonella* Control Programme for birds is applied at all production stages, including hatcheries, it is possible to keep *Salmonella* prevalence at a low level (EFSA, 2019). Likewise, the European Commission and European Food Safety Authority (EFSA) attributed the reduction in reported human salmonellosis cases across the European Union to, at least in part, successful control of *Salmonella* in breeding hens, broiler, laying flocks, and eggs through the National Control Programmes for *Salmonella* implemented by the United Kingdom in the poultry sector (O'Brien, 2013). Moreover, the National Poultry Improvement Plan (NPIP) executed by the United States Federal Government eradicated *Salmonella* ser. Pullorum and *Salmonella* ser. Gallinarum from poultry flocks (Andino & Hanning, 2015). As other countries, in order to reduce *Salmonella* in the poultry production chain, the Brazilian government implemented a National Surveillance Program for *Salmonella* in breeder flocks (Brasil, 2003).

The present study analysed data from the National Surveillance Program for *Salmonella* and the result (Table 1) has shown a great variety of serovars isolated from poultry broiler-breeder and layer-breeder flocks in the main producing states. Some isolates could not be characterised and were described as *Salmonella* spp., while others were characterised as two possible

Table 1 – *Salmonella* serovars isolated per state from poultry broiler-breeder and layer-breeder flocks, through the Brazilian National Surveillance Programme between 2016 and 2018.

Region	State°	Serovar/year		
		2016	2017	2018
	DF	Glostrup	NI*	NI*
Midwest	GO	Enteritidis Havana Heidelberg Infantis Muenster or Reading Newport Schwarzengrund	Mbandaka Ohio Schwarzengrund	Javiana Mbandaka Montevideo Saintpaul
	MS	Coeln Minnesota Schwarzengrund	Braenderup Livingstone Newport Saintpaul Tennessee	*Salmonella* subspecie non-*enterica*
	MT	Alachua Corvallis Idikan Infantis Livingstone Meleagridis Miami Morehead Saintpaul *Salmonella* subspecie non-enterica Schwarzengrund	Corvallis *Salmonella* subspecie non-*enterica* Saintpaul Tennessee	Saintpaul
North	RO	NI*	Javiana	NI*
	RR	Anatum	NI*	NI*
	TO	Miami	NI*	NI*
Northeast	BA	Miami	Anatum	NI*
	MA	NI*	NI*	Saintpaul
	PB	Muenster or Reading Senftenberg	Sandiego Senftenberg	NI*
	PE	NI*	*Salmonella* spp.	NI*
South	PR	Anatum Enteritidis Give Heidelberg Mbandaka Rissen Salmonella 4,[5],12:d:- Schwarzengrund Senftenberg	Anatum Coeln Corvallis Give Heidelberg Infantis Javiana Newport Ohio Panama Saintpaul Salmonella 1, 4, [5],12:i:- Schwarzengrund Senftenberg Typhimurium	Agona Anatum Braenderup Coeln Corvallis Gallinarum Give Heidelberg Infantis Javiana Mbandaka Molade Muenchen Newport Panama Rissen Saintpaul Schwarzengrund Senftenberg Thompson Typhimurium
	RS	Javiana	Give	Montevideo

Table 1 – *Salmonella* serovars isolated per state from poultry broiler-breeder and layer-breeder flocks, through the Brazilian National Surveillance Programme between 2016 and 2018.

		Minnesota Montevideo Senftenberg Tennessee Worthington	Mbandaka Saintpaul *Salmonella* spp. Schwarzengrund Senftenberg Stanley	Newport Panama Stanley
	SC	Anatum Genovar 14975 Genovar 15101 Gloucester Javiana Livingstone Mbandaka Minnesota	Anatum Braenderup Brandenburg Give Heidelberg Idikan Livingstone Panama *Salmonella* spp.	Agona Brandenburg Gallinarum Heidelberg Litchfield- Pullorum *Salmonella* spp. Senftenberg
Southeast	MG	Corvallis Panama Sandiego Schwarzengrund	Give	NI*
	RJ	Senftenberg	NI*	NI*
	SP	Anatum Gallinarum Heidelberg Javiana Minnesota Schwarzengrund	Gloucester Livingstone Minnesota Senftenberg Typhimurium	Anatum Gallinarum Gloucester Havana Heidelberg Isangi Livingstone Mbandaka Minnesota Rissen Sandiego Senftenberg

*NI: not isolated.

DF: Distrito Federal; GO; Goiás; MS: Mato Grosso do Sul; MT: Mato Grosso; RO: Rondônia; RR; Roraima; TO: Tocantins, BA: Bahia; MA: Maranhão; PB: Paraíba; PE: Pernambuco; PR: Paraná; RS: Rio Grande do Sul; SC: Santa Catarina; MG: Minas Gerais; RJ: Rio de Janeiro; SP: São Paulo.

serovars due to limitations of the microarray assay (CHECK-POINTS, 2020).

Overall, 49 different serovars were detected in poultry broiler-breeder and layer-breeder flocks throughout the three analysed years. *Salmonella* ser. Heidelberg (10,7%), *Salmonella* ser. Anatum (6,3%), *Salmonella* ser. Newport (6,3%), *Salmonella* ser. Schwarzengrund (5,9%) and *Salmonella* ser. Mbandaka (5,1%) were the five most common isolated in poultry breeder flocks, accounting for 34,4% of all characterised strains (Figure 1). According to our findings, a Canadian study based on their official data on those types of breeder flocks, between 1998 and 2008, identified the majority of the serovars isolated in Brazil (Sivaramalingam *et al.*, 2013). *Salmonella* ser. Heidelberg was the most common serovar in both studies.

In terms of Brazilian regions, 39 distinct serovars were identified in the Southern region, where

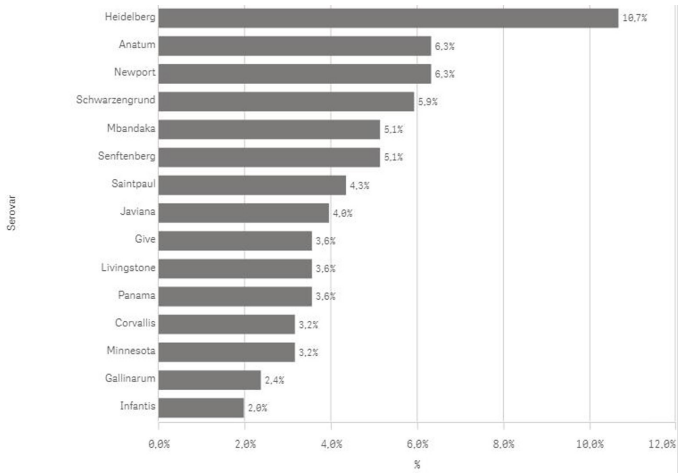


Figure 1 – Fifthteen most common serovars isolated in poultry broiler-breeder and layer-breeder flocks between 2016 and 2018.

most breeders' establishments are located. In the Midwestern region, 25 distinct serovars were identified, while in the southeast region there were 18 distinct serovars. On the other hand, only four and six distinct serovars were isolated through the analysed years in the Northern and Northeastern regions, respectively (Table 1). The most common isolated serovars in the Southern region were *Salmonella* ser. Heidelberg, *Salmonella* ser. Newport, and *Salmonella* ser. Anatum. *Salmonella* breeder data in Brazil are scarce and a great number of studies are based on broiler data. Some of those studies have demonstrated the presence of *Salmonella* ser. Heidelberg in broiler establishments in the Southern region (Pandini *et al.*, 2015; Voss-Rech *et al.*, 2015) which emphasises that this serovar is more resistant than others in the environment (Voss-Rech *et al.*, 2019). Indeed, our study shows that *Salmonella* ser. Heidelberg has been constantly isolated in this region, especially in the two main producer states, Paraná and Santa Catarina (Figure 2).

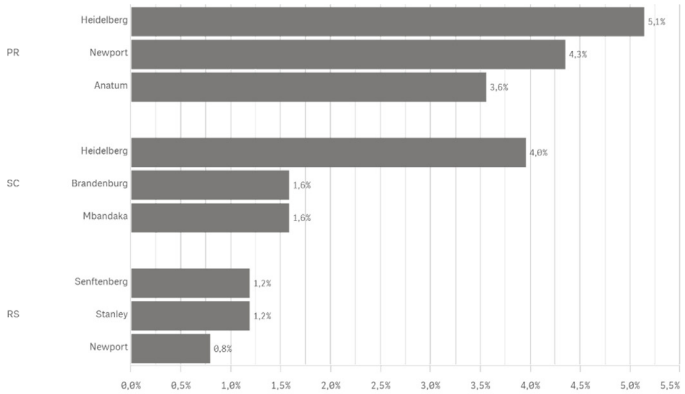
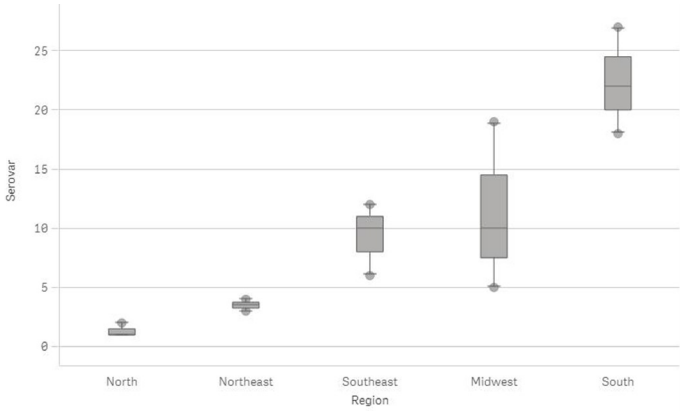


Figure 2 – Most common serovars isolated in the Southern region of Brazil in poultry broiler-breeder and layer-breeder flocks between 2016 and 2018.

Our results demonstrated that the number of serovars identified in Paraná and São Paulo has increased through the analysed years (Table 1). A review was performed on the number of poultry establishments through those years and the increase in the variety of serovars could not be related to the increase in the number of poultry producing establishments. On the other hand, layer breeder population and egg production have clearly risen in those years, which might have contributed for the increase of *Salmonella* isolations in breeder flocks; particularly in São Paulo, where the production of laying hens is predominant (ABPA, 2021). Another possibility to explain the increase in the number of serovars detected is a failure in the biosecurity programs, which may facilitate the spread of local *Salmonella* strains (De Carli *et al.*, 2017).

Some serovars are common in broiler-breeders regardless of the production region. Data analysis has shown that the frequency of appearance of different serovars in poultry production in the regions of Brazil is statistically significant. The Southern and Midwestern regions presented the greatest variety of serovars throughout the analysed period.



Kruskal-Wallis chi-squared = 12.049, df = 4, p-value = 0.01699.

Figure 3 – Frequency of distinct serovar in Brazil per region in poultry broiler-breeder and layer-breeder flocks between 2016 and 2018.

The aim of Brazilian breeding flock's *Salmonella* certification is to eradicate the four official controlled serovars from great-grandparent and grandparent flocks; to eradicate *Salmonella* ser. Pullorum and *Salmonella* ser. Gallinarum from parent flocks; and to control *Salmonella* ser. Enteritidis and *Salmonella* ser. Typhimurium in parent flocks (Brasil, 2003). In addition to the *Salmonella* surveillance programme, poultry breeder establishments have also implemented compulsory biosecurity measures in order to prevent and control pathogens (Brasil, 2007).

The four serovars controlled by the official surveillance programme, *Salmonella* ser. Enteritidis, *Salmonella* ser. Typhimurium, *Salmonella* ser. Gallinarum and *Salmonella* ser. Pullorum, represent a small part of the strains isolated (5,5%). Among those serovars, *Salmonella* ser. Gallinarum was the most common one isolated, followed by *Salmonella* ser. Typhimurium, *Salmonella* ser. Enteritidis, and *Salmonella* ser. Pullorum (Figure 4). The results show that the *Salmonella* control programme combined with biosecurity measures (Brasil, 2007) is fulfilling its purpose, even though the aim of the official control program has not been achieved.

Regarding the four serovars, a Canadian survey showed that less than 1% of *Salmonella*-positive isolates in breeders between 1998 and 2008 were

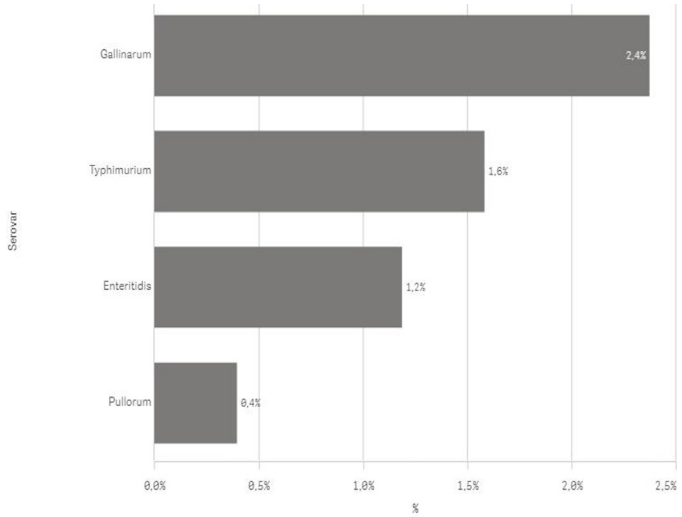


Figure 4 – Percentage of controlled serovars isolated in Brazil in poultry broiler-breeder and layer-breeder flocks between 2016 and 2018.

Salmonella ser. Enteritidis, whereas *Salmonella* ser. Gallinarum and *Salmonella* ser. Pullorum were not isolated. Although in the same study *Salmonella* ser. Typhimurium was the fourth most common serovar, it represented only 2.8% of the isolates (Sivaramalingam *et al.*, 2013). Costa *et al.* (2013) suggested that the decline in the prevalence of *Salmonella* ser. Enteritidis and the lack of identification of *Salmonella* ser. Pullorum and *Salmonella* ser. Gallinarum in commercial poultry carcass and poultry products in Brazil might reflect the actions of the government programs geared to its control. Nevertheless, those serovars can still be isolated, which may indicate biosecurity gaps.

Salmonella phylogenetic analysis shows common populations and similar strains of *Salmonella* ser. Enteritidis between humans and chickens, considering that poultry products are the primary vehicle of this serovar transmission to humans, this may be attributable to infected breeding flocks (Li *et al.*, 2021). Moreover, the control of *Salmonella* ser. Gallinarum and *Salmonella* ser. Pullorum can be complicated due to vertical transmission, as hens can become sub clinically infected carriers and pass the infection on to their embryos (Berhanu & Fulasa, 2020). Consequently, a lot of effort still needs to be made to eradicate and control those serovars in the poultry chain.

Poultry breeder categories differed in the number of positive epidemiological units for *Salmonella*. The percentage of positive *Salmonella* epidemiological units is much higher in parents than in other categories such as great-grandparent and grandparent poultry in all three years. Even in the rearing period, parent-breeder was the category with the most *Salmonella* positive epidemiological units identified, despite the fact that the same legal biosecurity measures are required for all breeder categories (Table 2).

Table 2 – Percentage of *Salmonella* positive epidemiological unit per broiler-breeder and layer-breeder category and year in Brazil.

Breeder category	(%) Positive epidemiological units /year		
	2016	2017	2018
Great-grandparent - rearing period	0	0	0
Great-grandparent	1	0	0
Grandparent - rearing period	0	0	1
Grandparent	9	0	1
Parent - rearing period	0	11	15
Parent	90	89	83

Current knowledge about risk/protective factor categories in relation to the farming system of breeder flocks to improve *Salmonellae* control highlight outdoor access, group size, stocking density, genetic, farm hatching, existence of enrichment (such as perches, windows, nest boxes), type of litter, factors relating to biosecurity measures (e.g. disinfection, pest control), and occurrence of other diseases. Also, animal welfare indicators listed were stress (other than heat stress), heat stress, activity/behaviour, body status (e.g. foot pad dermatitis - FPD) and other diseases (EFSA *et al.*, 2019).

Although biosecurity legal requirements are the same for these different breeder categories (Brasil, 2007), great-grandparents flocks probably have a higher sanitary status, which implies more strict biosecurity measures. Based on these study data, it is possible to assume that the correct application of biosecurity measures may differ between categories. Therefore, data shows that there is an opportunity to improve biosecurity measures in parent breeder flocks.

When the data about turkey breeder flocks was analysed, a total of 16 serovars were identified. The most common isolated serovars were *Salmonella* ser. Anatum, *Salmonella* ser. Newport, *Salmonella* ser. Brandenburg, *Salmonella* ser. Litchfield, and *Salmonella* ser. Livingstone, representing 72% of identified strains. It is important to highlight that none of the official controlled serovars were detected (Table 3).

The data presented on Table 3 differs from other studies which isolated *Salmonella* ser. Typhimurium and *Salmonella* ser. Enteritidis in turkey facilities, even though with very low prevalence (O´Brian, 2013; Sivaramalingam *et al.*, 2013). Another research

Table 3 – *Salmonella* serovars isolated per state from poultry turkey-breeder flocks, through the Brazilian National Surveillance Programme between 2016 and 2018.

Year	Breeder category	State/Serovar				
		GO	MG	SP	RS	SC
2016	Great-grandparent	NI*	NI*	NI*	NI*	Newport
	Parent	Anatum Brandenburg Genovar 14973 (poss. Javiana) Genovar 15101 Heidelberg Schwarzengrund	Anatum Genovar 15083 Newport	NI*	Anatum Brandenburg Genovar 6255 Livingstone Senftenberg	NI*
2017	Parent	NI*	NI*	NI*	Brandenburg Give Senftenberg	Anatum
2018	Parent - rearing period	NI*	NI*	NI*	Corvallis	
	Parents	NI*	NI*	NI*	Corvallis Senftenberg Tennessee	Agona Anatum Litchfield Newport

*NI: not isolated.

regarding official data with *Salmonella* isolates from turkey carcasses was realised in the Southern region of Brazil, between 2004 and 2006, and identified the occurrence of *Salmonella* ser. Enteritidis and *Salmonella* ser. Typhimurium in turkey products (Palmeira *et al.*, 2016). Brazil's turkey production is currently represented by a few establishments. The lack of isolation of official serovars in this study may be caused by the ongoing official certification focused on the eradication and control of those serovars and the decrease in Brazilian turkey production (ABPA, 2021).

We analysed different types of samples used to certify the breeder flocks. It was possible to asses that most *Salmonella* positive isolates were from drag swabs, boot swabs, and pipped embryo samples (Table 4).

Regarding different sample types used to isolate *Salmonella* in breeders, Berghaus *et al.* (2012) conducted a serial survey of 49 broiler breeder farms across the United States of America. Different sample types such as drag swabs, boot swabs, and litter samples yielded comparable prevalence of *Salmonella* positive results, while the prevalence was significantly lower for the slat and egg belt sponge samples. The study by Kahya *et al.* (2013) compared different types of samples for *Salmonella* detection in chicken layer breeder flock, and positive *Salmonella* samples were 8% out of pooled cloacal swab, 90,9% out of pooled embryonated chicken egg, and 21,4% of pooled wet faeces. PCR and ISO 6579 culture methods results were 100% in agreement (100% sensitivity and specificity) with culture results for all sample types. These results showed that pipped embryo was the most meaningful sample for *Salmonella* detection from breeder flocks, followed by wet faeces.

As expected, *Salmonella* ser. *Gallinarum* and *Salmonella* ser Pullorum were isolated from organs. According to the WOAH (2021b), *post mortem* tissues are preferable to isolate those serovars. It is important to highlight *Salmonella* ser. *Gallinarum* isolationfrom dead embryo. This matrix is easy to collect and include in poultry industries' biosecurity programs. Finally, *Salmonella* ser. Enteritidis, was detected from boot and drag swabs and *Salmonella* ser. Typhimurium was isolated from a large number of sample sources, like day old chicks, meconium, boot, and drag swabs. These data reinforce the importance of collecting tissues to detect typhoid *Salmonella* and suggest that the embryo has a great value in monitoring the presence of this pathogen.

CONCLUSION

Salmonellosis is a disease of economic and public health importance throughout the world, especially for those countries that are major exporters of poultry products, such as Brazil, requiring constant monitoring of the prevalent strains and their characteristics. Especially in the case of Brazil, the adoption of the verticalized poultry production model requires special attention in relation to the control of poultry salmonellosis in breeder flocks.

Table 4 – Type of samples collected and corresponded *Salmonella* serovars isolated by year in broiler-breeder and layer-breeder in Brazil.

Type of sample	Sampling year/serovar		
	2016	2017	2018
Boot swabs	Anatum Enteritidis Genovar 14975 Gloucester Heidelberg Idikan Infantis Javiana Livingstone Minnesota Muenster or Reading Newport Saintpaul Schwarzengrund Senftenberg	Anatum Give Gloucester Heidelberg Idikan Infantis Minnesota Newport Panama Saintpaul Schwarzengrund Senftenberg Stanley Typhimurium	Agona Anatum Corvallis Give Gloucester Heidelberg Isangi Javiana Litchfield Livingstone Mbandaka Minnesota Montevideo Muenchen Newport Saintpaul Senftenberg Stanley
Chick box liners	Minnesota Senftenberg	NI*	Livingstone Newport Rissen Sandiego *Salmonella* subspecie non-*enterica*
Cloacal swabs	Miami Panama Schwarzengrund Senftenberg	Sandiego Senftenberg	NI*
Pipped embryo	Alachua Corvallis Gallinarum Infantis Livingstone Mbandaka Meleagridis Miami Minnesota Schwarzengrund	Braenderup Corvallis Newport Ohio Panama *Salmonella* spp. Senftenberg	Agona Anatum Gallinarum Heidelberg Mbandaka Newport Panama Schwarzengrund Senftenberg Thompson
Dead day-old chicks	Glostrup Genovar 15101 Senftenberg	Heidelberg Livingstone Typhimurium	Montevideo Newport
Drag swabs	Anatum Enteritidis Give Havana Heildelberg Miami Montevideo Morehead *Salmonella* 4,[5],12:d:- *Salmonella* subspecie non-enterica Sandiego Schwarzengrund Tennessee	Anatum Braenderup Brandenburg Coeln Corvallis Give Heidelberg Javiana Livingstone Mbandaka Newport Ohio Panama Saintpaul *Salmonella* subspecie non-enterica *Salmonella* spp. Schwarzengrund Tennessee	Agona Anatum Braenderup Coeln Give Havana Heidelberg Infantis Javiana Mbandaka Molade Newport Panama Saintpaul Sandiego Schwarzengrund Senftenberg Typhimurium
Faecal	Coeln Miami	Anatum Heidelberg	NI*

Table 4 – Type of samples collected and corresponded *Salmonella* serovars isolated by year in broiler-breeder and layer-breeder in Brazil.

Meconium	Mbandaka Meleagridis Muenster or Reading Rissen Senftenberg	Anatum Corvallis Give Mbandaka Tennessee Typhimurium	Livingstone Senftenberg
Organs	Corvallis *Salmonella* subspecie non-*enterica*	Saintpaul *Salmonella* 1, 4, [5], 12:i:-	Brandenburg Gallinarum Heidelberg Pullorum *Salmonella* spp.
Litter swab	Javiana Worthington	Senftenberg	NI*

*NI: not isolated.

Our results showed that the number of serovars has increased between 2016 and 2018 in the main broiler and layer poultry producer states; the frequency of distinct serovars in the different regions of Brazil were statistically significant; parent-breeders was the category with the most *Salmonella* positive epidemiological units identified; and *Salmonella* ser. Enteritidis, *Salmonella* ser. Typhimurium, *Salmonella* ser. Gallinarum, and *Salmonella* ser. Pullorum represent a small part of the isolated strains.

Therefore, the present study demonstrates the importance of an official program in Brazil for the surveillance for salmonella in breeder flocks, especially when combined with biosecurity measures designed to prevent and control the disease.

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