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Full Length Research Paper

Occurrence and antimicrobial resistance of Salmonella serotypes isolates recovered from poultry of Western Paraná, Brazil

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This study was conducted to determine the occurrence and antimicrobial resistance of Salmonella serotypes isolates from feces, feed and broiler litter in poultry broilers in western Paraná between 2006 and 2010. The antigenic characterization and identification of serotypes confirmed 118 samples of Salmonella spp., belonging to 38 different serotypes. The most common serotypes identified were S. enteritidis (16.1%), S. heidelberg (5.9%), S. typhimurium (5.9%), S. hadar (5.0%), S. albany (4.2%), S. enterica (4.2) and S. saintpaul (4.2%). The serotypes of Salmonella spp. isolates were examined for resistance against 13 antibiotics using the disc diffusion method. Thirty-six samples (30.5%) were susceptible to all antimicrobials, and among the 82 resistant strains, there were 25 different resistance patterns. Serotypes' multi-resistant to streptomycin, nalidixic acid and tetracycline (20.7%), nalidixic acid (13.4%) and nalidixic acid and tetracycline (11.0%) were found at higher frequency. The results revealed high prevalence of Salmonella spp. mainly S. enteritidis (16.1%) in aviary environment and percentage of strains' resistant to conventional antibiotics.

Key words: Salmonella enteritidis, broilers, antimicrobial susceptibility, poultry.

INTRODUCTION

The poultry industry is currently one of the major sectors of the Brazilian agro-industrial complex that is expanding constantly and highlighted in the international market. Paraná is the state that mostly produces chickens in Brazil; quality, sanitation and price are the main factors contributing to increased productivity (UBA, 2012). However, the chicken meat is the main vehicle for transmission of *Salmonella*, responsible for huge losses

in the poultry industry (Téo and Oliveira, 2005). Due to the ability to colonize the digestive tract of animals and to present various reservoirs involved in fecal excretion and environmental pollution, researchers around the world agree that it is virtually impossible to eradicate *Salmonella* spp. from poultry and, therefore, there must be a constant control and monitoring of all serotypes, including those involved in the health of poultry and

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consumers (Andreatti Filho et al., 2009).

The use of synthetic antimicrobial helps to improve poultry health and reduces the rearing time. But, the indiscriminate use of subtherapeutic concentrations for various purposes, such as growth promoters, therapeutics and prophylactics provides the selection and prevalence of different serotypes of *Salmonella* and other microorganisms resistant to antibiotics. This reduces their effectiveness in clinical cases, besides contributing to the presence of residues in meat and meat products (Bila and Dezotti, 2003; Sakaridis et al., 2011).

It is well known that an increase is occurring in the isolation of Salmonella serotypes prevalent and resistant to synthetic antimicrobials all over the world. For example, a few studies were found reporting the isolation of salmonella serotypes with high-level resistance in Brazil (Silva and Duarte, 2002; Cardoso et al., 2006; Ribeiro et al., 2007; Souza et al., 2011), Spain (Carramiñana et al., 2004), Lithuania (Ruzauskas et al., 2005), Nigeria (Okolli et al., 2006), United States (Alali et al., 2010) and Greece (Sakaridis et al., 2011). These studies reported that Salmonella from poultry source are resistant to a wide range of antimicrobials and confirmed that poultry are important reservoirs of multidrug-resistant Salmonella. Between 1999 and 2008, there were 254 outbreaks of salmonellosis in Paraná, and eggs, meat and their derivative products are the main food connected to these outbreaks (Kottwitz et al., 2010). Although salmonellosis is one of the foodborne diseases more common in Paraná, there is little information of the occurrence and frequency of different Salmonella serotypes involved with outbreaks of this disease, emphasizing the importance of this work.

The aim of this study was to evaluate the occurrence and antimicrobial resistance profile of serotypes of *Salmonella* spp. in samples isolated from batches of broilers in Western Paraná.

MATERIALS AND METHODS

Sampling and serotype identification

Between 2006 and 2010 about 396 samples isolated from feces (cloacal swabs), broiler litter (drag swab) and feed (flour/feed inputs) were analyzed; 118 strains of the genus *Salmonella* were confirmed from two different aviaries with capacity of 5000 chickens, both located in the western region of Paraná. The samples were provided by the Veterinary Laboratory MercoLab (Cascavel / PR, Brazil) and the complete antigenic characterization and identification of serotypes were performed by the Adolfo Lutz Institute (São Paulo, Brazil). The occurrence was determined by observing the most frequent serotypes among the numbers of samples, arranging them in descending order.

Antimicrobial susceptibility test

The samples were recovered on Brain-Heart Infusion broth (BHI) (HIMEDIA ®) at 37°C for 18 h before testing. The antimicrobial susceptibility was evaluated according to the recommendations of

the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2007). The antimicrobial agents tested included (LABORCLIN®): nalidixic acid (30 μg), ampicillin (10 μg), chloramphenicol (30 μg), cephalothin (30 μg), ciprofloxacin (5 μg), enrofloxacin (10 μg), streptomycin (10 μg), imipenem (10 μg), gentamicin (10 μg), norfloxacin (10 μg) sulfazotrin (25 μg), tetracycline (30 μg), and tobramycin (10 μg). All samples that showed intermediate resistance were considered sensitive to avoid overestimation of resistance. Reference strains of Salmonella Typhimurium ATCC 14028 (American Type Culture Collection) and Escherichia coli ATCC 25922 were used. The results obtained were compared with the data in Table 2A of the document M100-S17 (CLSI, 2007). The strains with resistance to three or more antibiotics were considered as multi-resistant.

RESULTS

38 different serotypes were found of a total of 118 isolates distributed. The main serotypes isolated from broiler litter, diet and feces in poultry were *S. enteritidis* (16.1%), followed by *S. heidelberg* (5.9%), *S. typhimurium* (5.9%), *S. hadar* (5.0%), *S. albany* (4.2%), *S. enterica* (4.2) and *S. saintpaul* (4.2%) (Table 1).

Regarding the source of *Salmonella* spp. samples, 73 strains were from broiler litter (61.9%), 23 from poultry feed (19.5%) and 22 from poultry feces (18.6%) (Table1).

In strains isolated from broiler litter, 15 different serotypes were identified, with *S. enteritidis* being the most frequent serotype, followed by *S. typhimurium*, *S. hadar*, *S. heidelberg*, *S. enterica*, *S. derby*, *S. mbandaka*, *S. infants*, *S. livingstone* and *S. kentucky*. About the diet, among the 13 different serotypes isolated, the most frequent were *S. enteritidis*, *S. heidelberg*, *S. panama*, *S. corvallis*, *S. senftenberg* and *S. gafsa*. In relation to the feces, among the 22 serotypes, *S. enteritidis* was the most common serotype, followed by *S. heidelberg*, *S. albani*, *S. enterica* and *S. saintpaul* (Table 1).

About the antimicrobial susceptibility and resistance of samples, it was observed that 36 samples (30.5%) were susceptible to all antimicrobials tested, while 82 (69.6%) were resistant to at least one antimicrobial agent, namely: 15 (12.7%) were resistant to one antimicrobial agent, 22 (18.6%), resistant to two, 30 (25.4%), to three, 14 (11.9%), to four and 1 (0.8%) was resistant to five antibiotics (Table 2). 45 of samples showed multidrug resistance (that is, resistance to three or more antimicrobials) (Table 2).

As for the 82 samples that showed resistance to at least one antimicrobial, 55 were reported to nalidixic acid, 54 to streptomycin, 53 to tetracycline, 14 to ampicillin, 8 to chloramphenicol, 7 to gentamicin, 5 to enrofloxacin, 4 to cephalothin, 3 to norfloxacin, 3 to sulfazotrin, 2 for ciprofloxacin and 1 to imipenem. No serotype was resistant to tobramycin and none of the samples tested showed 100% resistance to all antimicrobials used (Table 3). 25 different antimicrobial resistance patterns were found: 7 was associated with 1 or 2 antimicrobials and 18 was associated with three or more antimicrobials

Table 1. Distribution of 118 samples of *Salmonella* spp. isolated from broiler farms, according to the occurrence and source of isolation in the period 2006-2010 of Brazil.

Isolation sources	Serotypes	N° (%)	Feces	Litter	Feed
	Enteritidis	19 (16.1)	6	9	4
	Heidelberg	7 (5.9)	2	3	2
All sources	Typhimurium	7 (5.9)	1	5	1
7111 3001003	Hadar	6 (5.0)	1	4	1
	Albany	5 (4.2)	2	2	1
	Saintpaul	5 (4.2)	2	2	1
	Enterica	5 (4.2)	2	3	0
	Mbandaka	4 (3.4)	1	3	0
	Newport	3 (2.5)	1	2	0
Feces and litter	Cerro	2 (1.7)	1	1	0
	Tennesse	3 (2.5)	1	2	0
	Cubana	2 (1.7)	1	1	0
	Lexigton	2 (1.7)	1	1	0
	Give	2 (1.7)	0	1	1
Litter and feed	Derby	4 (3.4)	0	3	1
	Braenderup	2 (1.7)	0	1	1
	Gallinarium	2 (1.7)	0	1	1
	Infantis	3 (2.5)	0	3	0
	Livingstone	3 (2.5)	0	3	0
	Kentucky	3 (2.5)	0	3	0
	Schwarzengrund	2 (1.7)	0	2	0
	Brandenburg	2 (1.7)	0	2	0
	Anatum	2 (1.7)	0	2	0
	Montevideo	2 (1.7)	0	2	0
Litter	Ohio	2 (1.7)	0	2	0
Littei	Agona	2 (1.7)	0	2	0
	Minnesota	2 (1.7)	0	2	0
	Pullorum	1 (0.8)	0	1	0
	Muenchen	1 (0.8)	0	1	0
	Morehead	1 (0.8)	0	1	0
	Worthington	1 (0.8)	0	1	0
	Grumpensis	1 (0.8)	0	1	0
	Orion	1 (0.8)	0	1	0
	Gafsa	2 (1.7)	0	0	2
	Corvallis	2 (1.7)	0	0	2
Feed	Panamá	2 (1.7)	0	0	2
	Senftenberg	2 (1.7)	0	0	2
	Rissen	1 (0.8)	0	0	1
Total		118 (100)	22 (18.6)	73 (61.9)	23 (19.5)

(multidrug resistance patterns) (Table 3). More frequent were those resistant to tetracycline (17 patterns), streptomycin (16 patterns) and nalidixic acid (13 patterns). Also, more frequent were those resistant to nalidixic acid-streptomycin-tetracycline (17 samples), those resistant

only to nalidixic acid (11 samples) and those resistant to nalidixic acid-streptomycin (9 samples) (Table 3). Among the 16 samples of *S. enteritidis* isolated, 100% were resistant to nalidixic acid, 8 (50%) to tetracycline, 7 (44%) to streptomycin and 2 (12.5%) to enrofloxacin

Number of	N	lumber of isola	ates (or strair	ns)	
antimicrobials	Feces	Litter	Feed	Total (%)	
Susceptible	3	20	13	36 (30.5)	
1	3	8	4	15 (12.7)	
2	3	14	5	22 (18.6)	
3	6	23	1	30 (25.4)	
4	5	9	0	14 (11.9)	
5	1	0	0	1 (0.8)	

Table 2. Number of antibiotic resistance of 118 *Salmonella* isolates in different sources in broiler farms in Brazil in the period 2006-2010.

(Table 3). The 6 samples of *S.* Hadar showed resistance to three different patterns of resistance: tetracycline-streptomycin, nalidixic acid-tetracycline, and nalidixic acid-tetracycline-streptomycin. As *S. hadar*, all isolates of *S. typhimurium* showed three different patterns of resistance: Gentamicin-nalidixic acid, streptomycintetracycline-nalidixic acid and nalidixic acid-streptomycin-tetracycline-gentamicin (Table 3).

About the multidrug resistance patterns, the serotypes Mbandaka and Saintpaul showed the highest amount of patterns Norfloxacin-tetracycline-streptomycin, streptomycin-tetracycline-chloramphenicol, ciprofloxacinampicillin-streptomycin-norfloxacin-chloramphenicol to Mbandaka, and streptomycin-nalidixic acidchloramphenicol, tetracycline-streptomycin-nalidixic acidsulfazotrin-tetracyclinechloramphenicol and streptomycin-nalidixic acid to Saintpaul. After these, the serotypes, S. typhimurium, S. enteritidis, S. enterica, S. albany, S. newport and S. anatum showed 2 multidrug resistance patterns and the serotypes, S. livingstone, S. give, S. hadar, S. kentucky, S. infantis, S. tenesee, S. heidelberg, S. schwarzengrund and S. montevideo showed only 1 multidrug resistance pattern (Table 3).

DISCUSSION

The occurrence of antimicrobial resistance in zoonotic bacteria such as *Salmonella* has major public health implications, and drug resistance is almost an inevitable consequence of the use of antimicrobial drugs in food-producing animals. The monitoring of occurrence of multiresistant *Salmonella* serotypes in poultry is critical to the protection of human and veterinary health (Therelfall, 2002).

The presence of the main serotype, *S. enteritidis* in poultry was also confirmed by other researchers in various regions of Brazil, such as Paraná (Souza et al., 2011), São Paulo (Hofer et al., 1998; Lima et al., 2009), Bahia, Ceará, Goiás, Paraná, Mato Grosso, Mato Grosso do Sul e Santa Catarina (Kanashiro et al., 2005), Rio Grande do Sul (Ribeiro et al., 2007) and the other states in the Northeast (Duarte et al., 2009). Similarly, in other

countries, the occurrence of *S. enteritidis* isolated from broilers in Portugal (Antunes et al., 2003), Spain (Fernandez -Rubio et al., 2009) Lithuania (Ruzauskas et al., 2005) and United States (Burkholder et al., 2008) was observed.

Regarding the source of *Salmonella* spp. samples in broiler litter, the high incidence of *Salmonella* spp. may be due to the repeated use of the same litter for several flocks of broiler, enhancing the dissemination of *Salmonella* spp. (Chernaki-Leffer et al., 2002). In addition, the humidity content of the broiler litter and consequently temperature, are factors that contribute to the proliferation of microorganisms (Mcward and Taylor, 2000).

The serotypes isolated from broiler litter were found by Andreatti Filho et al. (2009) who reported *S. enteritidis*, *S. infants* and *S. kentuky* present in broiler litter in the state of São Paulo, Brazil, and also Souza et al. (2011) who researched the resistance profile of quinolones against strains of *Salmonella* isolated from poultry farms in Paraná and found out that out of the 123 isolates, 90 belonged to *S. enteritidis*.

Contamination of broiler litter becomes a problem for poultry chain, that if infected by *Salmonella*, there is the propensity to transmit it to the poultry and then to humans through consumption of meat and meat products. So the control of dissemination of *Salmonella* is dependent on the control of transmission sources (Frederick and Huda, 2011). One reason that may explain the presence of *S. enteritidis* in poultry refers to the high incidence of mealworm (*Alphitobius diaperinus*) considered a pest and transmission vector of *Salmonella* (Crippen et al., 2012). Therefore, the mealworm elimination in broilers is considered an important measure for controlling the salmonellosis in poultry (Leffer et al., 2010).

About the diet, Hofer et al. (1998) found variation in serotypes isolated from raw materials and feed in seven different regions of Brazil; the most common being *S. montevideo*, *S. senftenberg*, *S. havana*, *S. mbandaka*, *S. tennessee*, *S. infants* and *S. agona*. The authors mention that probably the large number of serotypes recognized results from the mixture of a large number of inputs of same rating but from different sources; this probably

Table 3. Antibiotic resistance of 82 Salmonella isolates from broiler farm.

No.	<u> </u>			R	Resistance to *							Samples	0	
resistance	Amp	Cip	Clo	Cef	Enr	Gen	lmi	Nal	Sul	Str	Tet	Nor	(%)	Serovars
4								+					11 (13.4)	Enteritidis (7), Heidelberg (1), Kentucky (1), Minnesota (2)
1											+		4 (4.9)	Enterica (1), Mbandaka (1), Cubana (1), Derby (1)
	+			+									1 (1.2)	Lexington (1)
						+		+					4 (4.9)	Typhimurium (3), Infantis (1)
2	+										+		1 (1.2)	Agona (1)
2										+	+		7 (8.5)	Hadar (1), Derby (3), Corvalis (1), Enterica (1), Panama (1)
								+		+			9 (11.0)	Enteritidis (1), Hadar (3), Muenchen (1), Gallinarium (1), Pullorum (1), Infantis (1), Cubana (1)
	+			+							+		2 (2.4)	Livingstone (2)
				+			+				+		1 (1.2)	Give (1)
								+		+	+		17 (20.7)	Typhimurium (3), Enteritidis (6), Hadar (2), Kentucky (1), Enterica (2), Infantis (1), Tennnesee (2)
3					+			+			+		2 (2.4)	Enteritidis (2)
			+					+		+			1 (1.2)	Saintpaul (1)
	+									+	+		5 (6.1)	Heidelberg (5)
	+							+			+		2 (2.4)	Albany (2)
	+									+	+	+	1 (1.2)	Mbandaka (1)
	+		+							+	+		1 (1.2)	Mbandaka (1)
					+			+		+		+	1 (1.2)	Newport (1)
						+		+		+	+		1 (1.2)	Typhimurium (1)
4			+					+		+	+		3 (3.6)	Saintpaul (1), Schwarzengrund (1), Enterica (1)
4					+			+		+	+		1 (1.2)	Anatum (1)
		+			+			+		+			1 (1.2)	Newport (1)
								+	+	+	+		2 (2.4)	Saintpaul (1), Anatum (1)
			+			+				+	+		2 (2.4)	Albany (2)
			+						+	+	+		1 (1.2)	Montevideo (1)
5	+	+	+							+		+	1 (1.2)	Mbandaka (1)
Total samples	14	2	8	4	5	7	1	55	3	54	53	3	82	

^{* 36} isolates were susceptible to antimicrobials: Amp: Ampicillin; Cef: Cefalotin; Cip: Ciprofloxacin; Clo: Chloramphenicol; Enr: Enrofloxacin; Imi: Imipenem; Gen: Gentamicin; Nal: Nalidixic acid; Nor: Norfloxacin; Str: Streptomycin; Sul: Sulfazotrin; Tet: Tetracycline; Tob: Tobramycin.

being the same reason for the difference between the serotypes isolated from this study. In relation to feces, the data of the present study disagree with Oliveira et al. (2006), who analyzing samples of feces and carcasses of broilers in the state of Ceará, Brazil observed contamination by *Salmonella* spp. only in the carcasses. This suggests that the cages might be considered free of *Salmonella* contamination. This study also disagrees with Kottwitz et al. (2008), searching the incidence of *Salmonella* spp. in poultry production chain in isolates from feces, cloacal swabs and eggs in Paraná, found prevalence of *S. enterica* (67.0% of isolates) and absence of *S. enteritidis*.

The differences between studies of the occurrence, prevalence and serotypes resistance profiles of *Salmonella* in poultry can be explained by the fluctuation of dominant serotypes that occurs between geographical regions; and additionally, the amplitude of serotypes inside the same region may be related to propagation of *Salmonella* by feed, derived from supplies from different locations, collaborating to increase the diversity of serotypes (Hofer et al., 1997). Also, the differences between the data of prevalence of *Salmonella* serotypes can be associated to the age of the samples, differences in origin, variation in sampleolation, sampling procedures, poultry contamination, differences in sample size, among others (Parveen et al., 2007).

About the susceptibility and resistance of samples against antimicrobials, Castagna et al. (2001), researching the resistance of *Salmonella* isolated from swine, found that the highest rates of resistance found were to tetracycline and ampicillin; agreeing in the sense that these were among the main antimicrobials that *Salmonella* were resistant to. It is suggested that the highest levels of antimicrobial resistance occur with the antimicrobials available and used on the market for longer period, since it increases the possibility of the selection of resistant microorganisms.

About the resistance of the serotypes to enrofloxacin and ciprofloxacin, these data agree with Sakaridis et al. (2011), who analyzed the susceptibility of *Salmonella* samples isolated from chicken carcasses in Northern Greece against synthetic antimicrobials, and found low resistance to these antimicrobials. Considering the multiresistance to the antimicrobials, Sakaridis et al. (2011) reported high rates of resistance to tetracycline, streptomycin and nalidixic acid, corroborating with this study. The authors attributed the increased resistance of some antimicrobial agents compared to others to the constant and intensive use of these agents as therapeutic and preventive in flocks.

In the study of Lima et al. (2009) for antimicrobial susceptibility of isolates from poultry products, *S. gallinarum* and *S. pullorum* were resistant to nalidixic acid, but sensitive to tetracycline, partially corroborating with this study. It is known that serotypes that can produce large losses to poultry industry include *S.*

gallinarum, which causes clinical disease known as typhoid fever, and S. pullorum that causes pulorosis; both show resistance to tetracycline and nalidixic acid, constantly used in poultry.

About the resistance of S. enteritidis to antimicrobials. the results agree with Vaz et al. (2010) who isolated S. enteritidis from outbreaks of salmonellosis and products related to poultry and reported lower rates of resistance to nalidixic acid, streptomycin and tetracycline. Also, the result of Cardoso et al. (2006) mentions, besides ciprofloxacin, the susceptibility to gentamicin, norfloxacin and sulfazotrin of strains isolated from chicken carcasses in the state of Rio Grande do Sul is similar to our results. Medeiros et al. (2011), researching the resistance of 18 Salmonella serotypes isolated from frozen broiler carcasses in 15 Brazilian cities against antimicrobials, found that strains of S. enteritidis were resistant to all at antibiotics tested, various levels, including antimicrobials used in this study. Also, the highest levels of bacterial resistance to antimicrobial agents were assigned to Streptomycin, florfenicol, sulfonamide, nalidixic acid, ampicillin, ceftiofur, aztreonam, cefoxitine, gentamicin and tetracycline, cephalotin, partially corroborating with our results. This is because these are among the antibiotics that are more (Salmonella), but none were resistant to all antimicrobial agents tested.

It is noteworthy that *S. enteritidis* has emerged as a major problem in poultry and public health in Brazil from the 1990s, probably introduced by the importation of hatching eggs and a-day-old chicks infected. Moreover, the expansion of the Brazilian poultry industry concomitant with the indiscriminate use of antimicrobials in poultry has created favorable conditions for the maintenance and proliferation of *S. enteritidis* as well as induced the maintenance of positive batches (Silva and Duarte, 2002).

The resistance shown by *S. hadar* to three different patterns of multidrug resistance corroborates with the values previously reported by Antunes et al. (2003) and Ribeiro et al. (2007), who although having performed works on carcasses and other poultry products, found that all isolates of *S. hadar* were resistant to antimicrobials, with high rates of resistance to tetracycline, nalidixic acid, enrofloxacin and streptomycin.

About the resistance patterns shown by *S. typhimurium*, Bauer-Garland et al. (2006) reported the transmission of *S. typhimurium* multidrug-resistant and sensitive, noting that multi-resistant *Salmonella* are more virulent under selection pressure, which allows them to diffuse more quickly from animal to animal and also not responding well to antimicrobial therapy. About *S. mbandaka*, in a study on the characterization of class 1 integrons and antibiotic resistance genes in multidrug resistant *S. enterica* from foodstuff was reported genes resistant to ampicillin, chloramphenicol, nalidixic acid, sulfamethoxazole/trimethoprim, streptomycin and tetra-

cycline (Ribeiro et al., 2011). This corroborates the results of this study. No reports of susceptibility and resistance of this serotype to antimicrobials were found. This is the first study on resistance and susceptibility of S. mbandaka; and, since this serotype 3 showed patterns of multidrug resistance, it suggests a greater attention needs to be given to it to control it. About the serotype S. saintpaul, there are no reports of its occurrence in poultry environment or their antimicrobial resistance profile, which is similar to the first report.

The results from this study highlight the need for continuous monitoring in the poultry industry regarding the presence of *Salmonella* spp., mainly *S.* enteritidis, which is more prevalent and already recognized as a major problem in the poultry sector. It is also important to make good use of antimicrobials (since *Salmonella* serotypes exhibit different patterns of resistance and variation in sensitivity to antimicrobials tested) to replac the commonly used antibiotics such as streptomycin, nalidixic acid, tetracycline and chloramphenicol, once a high number of strains showed resistance to these antimicrobials.

We also suggest a plan of actions to prevent outbreaks of *Salmonella* and the adoption of strict hygienic and sanitary measures throughout the production chain. They include exchange of poultry litter broiler frequently and tighter control of quality of feed inputs, besides the control of the mealworm which represents a critical factor for the reduction of *Salmonella* spp. in flocks.

It is concluded from this study that S. enteritidis was the most prevalent serotype of Salmonella in poultry of Western Paraná, Brazil. For resistance, most isolates were resistant to two or more antibiotics, especially when considering the ones mostly used in the poultry sector.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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