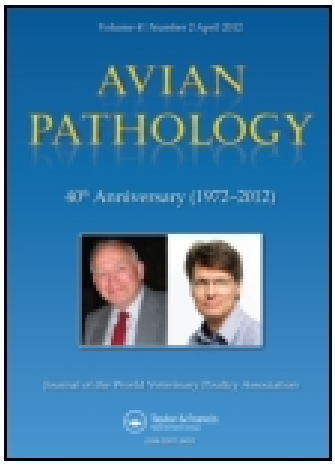

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Distribution of *Salmonella* serovars and antimicrobial susceptibility of *Salmonella* Enteritidis from poultry in Zimbabwe

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A study was carried out to determine the distribution and antimicrobial susceptibility profiles of *Salmonella* serovars from chickens from large-scale commercial (LSC), small-scale commercial (SSC), and rural free-range (RFR) farms of Zimbabwe. Pooled cloacal swabs were collected for culture and isolation of *Salmonella* spp. A chi-square test was used to assess distribution differences of salmonellas among the farming sectors. Approximately 10% (283/2833) of the swabs were positive for *Salmonella enterica*, with only subspecies *enterica* (98.6%) and *arizonae* (1.4%) being detected. The prevalence of *S. enterica* varied significantly ($P < 0.05$) among areas, with Harare (27.8%) and Buhera (1.3%) recording the highest and the least prevalence, respectively. *S. enterica* was only isolated from LSC and SSC farms, with the former having a significantly ($P < 0.001$; $\chi^2 = 155.3$) higher prevalence than the latter. *S. arizonae* was only isolated from the SSC farms while none were obtained from the RFR farms. The serovars isolated were *Salmonella* Enteritidis (72.8%), Group C (20.1%), Group B (4.2%), *Salmonella* Typhimurium (1.1%) and *Salmonella* Gallinarum (0.4%). *S.* Enteritidis predominated in the urban/periurban areas. Approximately 26% (53/206) of *S.* Enteritidis isolates were resistant to one or more antimicrobial agents. Resistance to tetracycline was the most common, while no resistance was detected for furazolidone, neomycin and trimethoprim-sulfamethoxazole. There were 12.1% multi-drug-resistant *S.* Enteritidis isolates, and the resistance to ampicillin/kanamycin was predominant. The identification of multi-drug-resistant *S.* Enteritidis is of public health concern. Thus, stringent control of *S.* Enteritidis will reduce the public health risk of human salmonellosis.

Introduction

The genus *Salmonella* is divided into two species, *Salmonella enterica* and *Salmonella bongori*, with the former being further split into six subspecies: *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV) and *indica* (VI) (Brenner *et al*., 2000). Except for *S. enterica* subsp. *enterica* and *S. enterica* subsp. *arizonae*, the majority of these *Salmonella* subspp. are associated with reptiles and the environment (Brenner *et al.*, 2000). *S. enterica* subsp. *enterica*, which is further split into more than 2500 serovars, is mainly associated with infections in birds, warm-blooded animals and humans, and is a recognized zoonotic pathogen. *S. enterica* subsp. *arizonae* is associated with acute septicaemia (arizonosis) in turkeys (Crespo *et al*., 2004) and occasionally in chickens (Silva *et al*., 1980) but it is widely distributed in reptilian and mammalian species (Oros *et al.*, 1998; Ramsay *et al*., 2002).

Salmonella infections occur frequently in poultry and are difficult to control, especially in intensive farming systems (Filho *et al*., 2009). Poultry salmonellosis may result in acute or chronic infections, but most probably will result in sub-clinical infection, leading to contamination of poultry meat and their products, with a subsequent increase in the risk of food poisoning in humans (Foley *et al*., 2008). In recent decades, the global prevalence of zoonotic gastrointestinal diseases has risen dramatically (Rodriguez *et al*., 1990; Willocks *et al*., 1996; Papadopoulou *et al*., 2009). This increase in the prevalence and incidence of human non-typhoidal salmonellosis has been mainly attributed to *Salmonella* Typhimurium, *Salmonella* Enteritidis and other serovars (Kariuki *et al*., 2006). These *Salmonella* serovars have largely been associated with consumption of poultry meat, food products made from eggs, and occasionally other food products especially of animal origin (Foley *et al*., 2008).

In Zimbabwe, increased frequency of isolation of *S.* Enteritidis in both large-scale commercial (LSC) poultry and small-scale commercial (SSC) poultry has been reported (Karenga, 1997; Matope *et al*., 1998). This rise in isolation frequency is probably a true reflection of an increase in flock prevalence in Zimbabwe. Since

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chickens and chicken products are widely consumed in both rural and urban populations, this further increases the zoonotic risk of *S.* Enteritidis. Furthermore, the global upsurge of multi-drug-resistant *Salmonella* strains has led to failures of treatment of human salmonellosis (Van Duijkeren & Houwers, 2000), necessitating the need to introduce strategies to monitor and control the spreading of such strains between animals and humans. The objective of this paper was therefore to determine the distribution of *Salmonella* serovars and the antimicrobial susceptibility patterns of *S.* Enteritidis from chickens from LSC, SSC and rural free-range (RFR) farms of Zimbabwe.

Materials and Methods

Study sites. The study was conducted in three randomly selected rural districts (Buhera, Murewa and Wedza) and six urban/periurban areas (Harare, Bulawayo, Chinhoyi, Chitungwiza, Masvingo and Mutare). These study areas in Zimbabwe are located between the lines of latitude 27°0′ to 32°38′E and 17°20′ to 20°07′S. They were selected for the study because they represented the varied agro-ecological areas of the country and involved the three different chicken farming types.

Study design and sampling. A cross-sectional study was conducted between 2003 and 2005 using a multistage sampling technique to identify primary, secondary and tertiary sampling units. Rural districts and urban/periurban areas were identified as primary sampling units, while poultry farms (large-scale commercial [LSC], small-scale commercial [SSC] and rural free range [RFR]) and the chickens were secondary and tertiary sampling units, respectively. The LSC farms were defined as those having more than 10,000 birds while SSC farms were defined as those having 1000 to 10,000 birds. Birds were chosen randomly, and approximately 5% of the chickens were sampled for the LSC and SSC farms while for the RFR farms all were sampled if the number of birds per household was 10 or less. Cloacal swabs were collected from all selected birds and pooled (in groups of five) in 10 ml Rappaport-Vassiliadis broth (Oxoid, Basingstoke, Hampshire, UK), a *Salmonella*-enrichment broth, and the bottles were transported to the laboratory at approximately 4°C for further investigation. The details of the cloacal swabs collected from each of the study areas and farming types are presented in Table 1.

Bacterial culture and identification. The swabs in Rappaport-Vassiliadis broth were incubated aerobically at 37°C for 18 to 24 h. A loopful of each broth was then streaked onto xylose lysine desoxycholate agar (Oxoid) and plates were incubated aerobically at 37°C for a further 18 to 24 h. The xylose lysine desoxycholate plates were examined for typical *Salmonella* pink colonies with a black centre. The colonies were then investigated using triple sugar iron agar (Oxoid), urea agar (Oxoid) and lysine decarboxylase broth (DIFCO Becton, Dickinson and Company, Franklin Lakes, NJ, USA) tests. A positive reaction on triple sugar iron agar (red slant/yellow but with H_2S production) and lysine decarboxylase broth (purple and yellow in test and control, respectively) and a negative reaction (yellow) in the urea agar were used for presumptive identification of *Salmonella* spp. The isolates were further identified using biochemical tests (catalase, motility, growth in potassium cyanide medium), sugar fermentation (dulcitol, lactose, mannitol, salicin and trehalose) with gas production from glucose, citrate utilization, indole production, O-nitrophenyl-b -D-galacto-pyranoside ONPG and malonate utilization, essentially as outlined by Barrow & Feltham (1993). The identified *Salmonella* isolates were then serotyped using polyvalent (O) somatic antisera and a range of specific flagellar (H) antisera (both phases 1 and 2) (Murex Diagnostics Ltd, Dartford, Kent, UK) to identify the different serovars according to the Kauffman and White scheme. However, since flagellar (H) antisera (for both phase 1 and 2) were only available for a limited number of serovars, it was not possible to identify all *Salmonella* isolates to serovar level, but only to serogroup level.

Table 1. *Distribution of pooled cloacal swabs collected according to area and poultry farming sector.*

Area	Samples processed from each of the farming sectors			
	LSC	SSC	RFR	Total
Buhera	0	106	279	385
Bulawayo	164	109	0	273
Chinhoyi	145	105	0	250
Chitungwiza	172	110	0	282
Harare	167	103	0	270
Masvingo	163	105	0	268
Murewa	37	104	284	425
Mutare	154	106	0	260
Wedza	26	107	287	420
Total	1028	955	850	2833

Antimicrobial susceptibility testing. The antimicrobial susceptibility testing was determined by the disc diffusion method on diagnostic sensitivity test agar (CM261; Oxoid). Overnight cultures were inoculated into 5 ml nutrient broth (Oxoid) and the turbidity of the inoculum adjusted to approximately 1.5×10^8 colony-forming units/ml corresponding to 0.5 McFarland standard. Cultures (approximately 1 ml) were then inoculated onto diagnostic sensitivity test agar, spread evenly and allowed to stand at 37°C for 5 min, and then antimicrobial discs were applied onto the surface of the medium using a disc dispenser (Oxoid) and plates were incubated at 37°C for 18 to 24 h. Antimicrobial susceptibility testing was carried out on seven antimicrobials; ampicillin (10 μg), enrofloxacin (Baytril) (100 μg), furazolidone (80 μg), kanamycin (10 μg), neomycin (120 μg), septran (trimethoprim/sulfamethoxazole) (100 μg) and tetracycline (80 μg). The quality control for susceptibility was performed using the reference strain *Escherichia coli* (ATCC 25922). The zones of inhibition were interpreted as susceptible, intermediate and resistant according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2009).

Data analysis. Data analysis was carried out using SPSS 16.0 for Windows to generate prevalence of isolates according to area and farming sector. The chi-square (χ^2) test was used to assess differences between areas and farming sectors, and values of $P < 0.05$ were considered significant. For the purpose of data analysis, the isolates of *S.* Enteritidis showing intermediate reactions were considered sensitive. The number of *S.* Enteritidis isolates resistant to various antibiotics was calculated and the result was expressed as a percentage.

Results

Salmonella serovar distribution. A total of 2833 pooled samples (14,165 individual chicken cloacal swabs) were collected and approximately 10% (283/2833) were positive for *Salmonella* spp. (Table 2). Only two subspecies were isolated with *S. enterica*, accounting for 98.6% (279/283) of the isolates, with the remainder 1.4% (4/283) being *S. arizonae*. *S. enterica* subsp. *enterica* was isolated from all areas studied and its prevalence varied significantly ($P < 0.05$) among these areas, with the highest (27.8%) and the lowest (1.3%) being recorded from Harare and Buhera, respectively (Table 2). In contrast, *S. enterica* subsp. *arizonae* was isolated from only three areas (Table 2) and its prevalence varied from 0.2 to 0.5%.

Salmonella enterica subsp. *enterica* was isolated from the LSC and the SSC farms, with the former recording a significantly ($P < 0.001$; $\chi^2 = 155.3$) higher prevalence compared with the latter (Table 2). *S. arizonae* was only

Table 2. *Classification of* Salmonella *subspecies according to area and farming sector.*

Area	Total samples	*S. enterica* (%) (subspecies I)	*S. arizonae* (%) (subspecies IIIa)	Number (%) of total isolates
Harare	270	75 (27.8)^{A}	0	75 (27.8)
Bulawayo	273	47 (17.2)^{B}	0	47 (17.2)
Mutare	260	41 (15.8)^{B}	0	41 (15.8)
Masvingo	268	42 (15.7)^{B}	0	42 (15.7)
Chinhoyi	250	37 (14.8)^{B}	0	37 (14.8)
Chitungwiza	282	15 (5.3)^{C}	0	15 (5.3)
Murewa	425	10 (2.4)^{D}	2 (0.4)	12 (2.8)
Wedza	420	7 (1.7)^{D}	1 (0.2)	8 (1.9)
Buhera	385	5 (1.3)^{D}	1 (0.3)	6 (1.6)
Total	2833	279 (9.8)	4 (0.1)	283 (9.99)
LSC	1028	241 (23.4)	0	241 (23.4)
SSC	955	38 (3.98)	4 (0.42)	42 (4.4)
RFR	850	0 (0.0)	0 (0.0)	0 (0.0)

Prevalence within the same column with different uppercase superscript letters are significantly ($P < 0.05$) different.

isolated from the SSC farms while no isolates were obtained from the RFR farms (Table 2).

The isolated serovars were *S.* Enteritidis, *S.* Gallinarum, *S.* Typhimurium and Group B and Group C species. The overall prevalence of *S.* Enteritidis was 7.3%, followed by Group C (2%), and the rest had an overall prevalence less than 0.5% (Table 3). *S.* Enteritidis contributed 72.8% (206/283) of the total isolates, followed by Group C (20.1%; 57/283) and Group B (4.2%; 12/283), while *S.* Typhimurium contributed 1.1% (3/283) and *S.* Gallinarum 0.4% (1/283). The area to area prevalence of *S.* Enteritidis varied significantly ($P < 0.05$), with Harare recording the highest (20.7%; 56/270) while Wedza ($\sim$1%; 4/420) reported the lowest prevalence (Table 3). Group C prevalence varied from 0.3 to 4.8% while that of Group B varied from 0 to 2.2% and the rest had a prevalence of less than 1%.

S. Enteritidis was isolated from the LSC and the SSC farms with the former recording a significantly ($P < 0.001$; $\chi^2 = 119.5$) higher prevalence compared with the latter (Table 3). *S.* Typhimurium, Group B and Group C were also isolated from both groups of farms while *S.* Gallinarum was only isolated from SSC farms (Table 3).

A total of 25.7% (53/206) of *S.* Enteritidis isolates were found to be resistant to one or more antimicrobial agents, with 88.7% (47/53) of these originating from LSC farms. Resistance to tetracycline was the most common, while no resistance was detected for furazolidone, neomycin and trimethoprim-sulfamethoxazole (Table 4). Multiple antimicrobial resistance was detected in 12.1% (25/206) of the *S.* Enteritidis isolates, where the resistance to ampicillin/kanamycin was predominant (Table 5).

Discussion

As observed elsewhere (Esteban *et al*., 2008; Foley *et al*., 2011), this study confirmed the predominance of *S. enterica* subsp. *enterica* from LSC compared with SSC poultry farms, while the absence of *Salmonella* from RFR chickens has been a consistent finding in other studies (Mdegela, 1998). The intensification of chicken rearing in LSC farms is likely to be associated with increased risk of spread of *Salmonella* among chickens in an infected farm, and this probably explains the higher isolation frequency in these farms. The less intensified chicken production system in the SSC and RFR farms greatly reduces the risk of spread of *Salmonella* spp. It is therefore most probable that the prevalence of *Salmonella* spp. in indigenous RFR chickens is low or negligible. Although poor growth rates and low yields of meat and eggs are some of the disadvantages associated with indigenous RFR chickens, possible disease tolerance and high-quality meat and eggs are great advantages. Thus, in addition to management practices, the differences in chicken lines amongst the LSC, SSC and RFR farms could have contributed to the observed differences in the prevalence of these *Salmonella* spp. Similarly, the *Salmonella* prevalence tended to be high in urban/periurban areas practicing LSC and SCC poultry farming using mainly exotic chickens, compared with rural districts practicing a free-range production system using indigenous chickens.

In previous studies, *S. arizonae* has been isolated mainly in turkeys where it is associated with acute septicaemia of poults (Crespo *et al*., 2004), but may cause infections in chickens (Silva *et al*., 1980) that are possibly reared closely together with infected turkeys. Although turkey production is common especially in areas where RFR and SSC chicken farming is practiced,

Table 3. *Distribution of* Salmonella *serovars isolated according to study area and farming sector.*

Area	Total samples	S.E. (%)	S. P/G.	S.T.	Group B	Group C	Others
Harare	270	56 (20.7)	0 (0.0)	0 (0.0)	6 (2.2)	13 (4.8)	0 (0.0)
Bulawayo	273	34 (12.5)	0 (0.0)	2 (0.7)	1 (0.4)	10 (3.7)	0 (0.0)
Chinhoyi	250	31 (12.4)	0 (0.0)	0 (0.0)	0 (0.0)	6 (2.4)	0 (0.0)
Mutare	260	32 (12.3)	0 (0.0)	0 (0.0)	2 (0.8)	7 (2.7)	0 (0.0)
Masvingo	268	32 (11.9)	0 (0.0)	1 (0.4)	1 (0.4)	8 (3.0)	0 (0.0)
Murewa	425	7 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.7)	2 (0.5)
Chitungwiza	282	6 (2.1)	0 (0.0)	0 (0.0)	2 (0.7)	7 (2.5)	0 (0.0)
Wedza	420	4 (1.0)	1 (0.2)	0 (0.0)	0 (0.0)	2 (0.5)	1 (0.2)
Buhera	385	4 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	1 (0.3)
Total	2833	206 (7.3)	1 (0.04)	3 (0.11)	12 (0.4)	57 (2.0)	4 (0.14)
LSC	1028	181 (17.6)	0 (0.0)	1 (0.1)	9 (0.9)	50 (4.9)	0 (0.0)
SSC	955	25 (2.6)	1 (0.1)	2 (0.2)	3 (0.3)	7 (0.7)	4 (0.4)

Data presented as *n* (%). S.E., *Salmonella* Enteritidis; S.P/G., *Salmonella Pullorum-Gallinarum*; S.T., *Salmonella* Typhimurium; Group B, *Salmonella* group B; Group C, *Salmonella* group C; Others, Other *Salmonella* species.

Table 4. *Antimicrobial resistance profiles of* S. *Enteritidis isolates from LSC and SSC farms in Zimbabwe.*

	LSC isolates ($n = 181$)		SSC isolates ($n = 25$)		Total ($n = 206$)	
Antibiotic	Number resistant	% resistant	Number resistant	% resistant	Number resistant	% resistant
Amoxicillin	11	6.1	3	12.0	14	6.8
Ampicillin	0	–	0	–	0	0
Enrofloxacin	4	2.2	3	12.0	7	3.4
Erythromycin	5	2.8	0	–	5	2.4
Furazolidone	0	–	0	–	0	–
Kanamycin	8	4.4	0	–	8	3.9
Neomycin	0	–	0	–	0	–
Tetracycline	19	10.5	0	–	19	9.2
Trimethoprim/sulfamethoxazole	0	–	0	–	0	–
Total	47	26.0	6	24.0	53	25.7

this type of management could not be established in those farms that tested positive for *S. arizonae*.

The observed predominant distribution of *S.* Enteritidis generally agrees with previous reports in Zimbabwe (Karenga, 1997; Matope *et al*., 1998) and in other parts of the world (Rodriguez *et al*., 1990; Sakai & Chalermchaikit, 1996; Capita *et al*., 2007). The reasons for the preponderance of *S.* Enteritidis are not clear, but may be linked to the intensification of poultry production which could have changed the population characteristics of *Salmonella* present in poultry flocks (Velge *et al*., 2005; Foley *et al*., 2008). It has been suggested that the eradication of *S.* Gallinarum opened up an ecological niche that allowed the introduction of *S.* Enteritidis in poultry flocks (Baumler *et al*., 2000). For instance, *S.* Enteritidis phage type 4 is especially adapted to egg transmission (Hirsh, 1999), which tends to propagate it in poultry flocks, ultimately outcompeting other serovars (Velge *et al*., 2005). The increased prevalence of *S.* Enteritidis in poultry has been associated with a concomitant global pandemic of human food poisoning due to increased demand for poultry, eggs and egg-based products (Rodriguez *et al*., 1990). However, the prevalence of *S.* Enteritidis in some developed countries, in both poultry and humans, has been recently declining, probably due to vaccination of poultry flocks and implementation of other control measures (Van Immerseel *et al*., 2009).

Notwithstanding the predominance of *S.* Enteritidis, the isolation of *S.* Typhimurium highlighted its significance not only in the chicken industry but also as an important zoonotic pathogen. *S.* Typhimurium infects a wide range of hosts and is frequently associated with septicaemic infections in poultry, farm animals and humans (Kingsley & Baumler, 2000).

Table 5. *Proportions of the total* S. *Enteritidis isolates showing multiple antimicrobial resistance.*

	Multi-drug-resistant isolates	
Antibiotics	Number resistant	% resistant
Ampicillin/tetracycline/kanamycin	6	2.9
Tetracycline/ampicillin	6	2.9
Erythromycin/tetracycline	3	1.5
Ampicillin/kanamycin	9	4.4
Kanamycin/tetracycline	1	0.5
Total	25	12.1

Although an increase in the isolation frequency of group C serovars from other animal species has been reported previously (CVL Report, 2001), further studies are required to ascertain the serovars that are associated with poultry in Zimbabwe. Amongst *Salmonella* group C serovars Hadar and Montevideo have been reported to be common in poultry in other regions (Berrang *et al*., 2006; Foley *et al*., 2008; Dogru *et al*., 2010).

We reported a low prevalence *S.* Gallinarum in SSC farms but not in LSC and RFR chicken farms. Our results support the findings of other studies where *S.* Gallinarum was not isolated in free-range chickens (Mdegela, 1998; Jafari *et al*., 2007). However, in other studies, a low prevalence of *S.* Gallinarum has been reported in free-range chickens as well as in intensively managed chickens (Aragaw *et al*., 2010). The reasons for the apparently low prevalence of *S.* Gallinarum are not known but could be attributed to the implementation of control programmes in many countries of the world (Velge *et al*., 2005; Foley *et al*., 2011). In Zimbabwe, such control measures were implemented in the 1970s and 1980s (DVS Report, 1984) and this could have significantly reduced infection in poultry. However, the fact that *S.* Gallinarum mainly produces systemic infection and is poorly excreted in faeces (Rabsch *et al*., 2000; OIE, 2008) may reduce its isolation frequency when cloacal swabs are used for culture. Furthermore, the fact that some strains of *S.* Gallinarum (biotypes Gallinarum and Pullorum) may produce atypical biochemical results, such as production of gas from glucose fermentation and lysine decarboxylase (OIE, 2008), could have precluded their identification.

In support of earlier observations (Ward *et al*., 1990; Ling *et al*., 1998; Natasi *et al*., 2000), it has been noted in this study that most of the isolates of *S.* Enteritidis were susceptible to all the antimicrobial agents investigated, indicating that antimicrobial resistance may be attributable to a relatively low number of resistant strains circulating in the poultry farms. However, considering that 25.7% of the isolates exhibited resistance to one or more antimicrobial agents is of public health concern, caused by a potential spread of resistant clones. Unprecedented global increases of human salmonellosis due to *S.* Enteritidis have been attributed to consumption of poultry meat, eggs and poultry products. Although the percentage (12.1%) of multi-drug-resistant *S.* Enteritidis reported in this study was lower than that in previous studies (Capita *et al*., 2007; Dogru *et al*., 2010), we provided further support for previous studies (Berrang *et al*., 2006; Papadopoulou *et al*., 2009) that resistance to

ampicillin, kanamycin and tetracycline appeared to be fairly common. The increased resistance particularly to tetracycline may be due to continuous selection of resistant clones as a result of frequent and indiscriminate use of antibiotics in these poultry farms (WHO, 1997). Plasmids encoding for tetracycline, ampicillin, and kanamycin resistance may be gained by *S.* Enteritidis (Frost *et al.*, 1989). Further studies are required to determine the nature of these resistance plasmids and the conditions that favour selection of antimicrobial resistance in chickens in Zimbabwe.

In conclusion, *Salmonella* spp. are prevalent in LSC and SSC farms, but not in RFR farms. *S.* Enteritidis is the predominant isolate, and the prevalence of *S.* Typhimurium and other unclassified *Salmonella* spp. is low, while *S.* Gallinarum is sporadic. Most *S.* Enteritidis isolates were susceptible to ampicillin, enrofloxacin, erythromycin, furazolidone, kanamycin, neomycin, tetracycline and trimethoprim-sulfamethoxazole antimicrobial agents. However, antimicrobial resistance was highest to tetracycline. Multi-drug-resistant strains of *S.* Enteritidis have been identified, with resistance to ampicillin/kanamycin being predominant. Stringent control of *S.* Enteritidis and proper monitoring of antimicrobial usage especially in LSC chicken farms will reduce the public health risk of human salmonellosis.

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