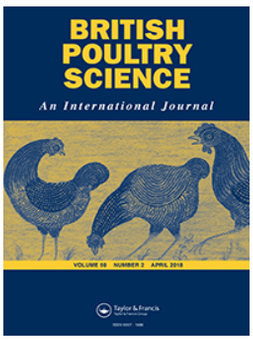

British Poultry Science

ISSN: 0007-1668 (Print) 1466-1799 (Online) Journal homepage: https://www.tandfonline.com/loi/cbps20

Salmonella Gallinarum field isolates and its relationship to vaccine strain SG9R

P. K. V. Koerich, B. B. Fonseca, E. Balestrin, V. Tagliari, P. G. Hoepers, C. Ueira-Vieira, I. Oldoni, R. H. Rauber, L. Ruschel & V. P. Nascimento

To cite this article: P. K. V. Koerich, B. B. Fonseca, E. Balestrin, V. Tagliari, P. G. Hoepers, C. Ueira-Vieira, I. Oldoni, R. H. Rauber, L. Ruschel & V. P. Nascimento (2018) *Salmonella* Gallinarum field isolates and its relationship to vaccine strain SG9R, British Poultry Science, 59:2, 154-159, DOI: 10.1080/00071668.2017.1406062

To link to this article: https://doi.org/10.1080/00071668.2017.1406062

Accepted author version posted online: 15 Nov 2017.
Published online: 08 Dec 2017.

BRITISH POULTRY SCIENCE, 2018
VOL. 59, NO. 2, 154–159
https://doi.org/10.1080/00071668.2017.1406062

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Salmonella Gallinarum field isolates and its relationship to vaccine strain SG9R

P. K. V. Koerich[a,b], B. B. Fonseca[c], E. Balestrin[d], V. Tagliari[b], P. G. Hoepers[c], C. Ueira-Vieira[b], I. Oldoni[b], R. H. Rauber[b], L. Ruschel[e] and V. P. Nascimento[a]

[a]Departamento de Medicina Animal da Faculdade de Veterinária da Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; [b]Instituto de Genética e Bioquímica da Universidade Federal de Uberlândia, Uberlândia, Brazil; [c]Faculdade de Medicina Veterinária da Universidade Federal de Uberlândia, Uberlândia, Brazil; [d]Programa de Pós Graduação em Biologia Celular e Molecular Aplicada à Saúde d Universidade Luterana do Brasil, Canoas, Brazil; [e]Faculdade de Agronomia e Medicina Veterinária da Universidade de Passo Fundo, Passo Fundo, Brazil

ABSTRACT

1. The aim of the present study was to determine if the 9R-strain of the *Salmonella* Gallinarum live vaccine was responsible for having fowl typhoid outbreaks in chicken flocks from both chicken and turkey breeders as well as to verify the antimicrobial resistance of the isolates from the outbreaks.
2. The triplex polymerase chain reaction, standard antimicrobial test, beta-lactamase genes identification and Ion Torrent PMG whole-genome sequence were used in the field isolates and in the vaccine strain of *S.* Gallinarum.
3. The 60 tested isolates were not from vaccine origin and manifested high resistance to drugs from macrolide and quinolone groups. Whole-genome sequencing (WGS) and single nucleotide polymorphism analysis on selected isolates for core genes from *Salmonella enterica* confirmed the wild origin of these isolates and showed two possible sources of *S.* Gallinarum in the studied outbreaks.
4. *S.* Gallinarum isolated from fowl typhoid outbreaks in the studied period were not caused by the use of the SG9R live vaccine. The source of strains sequenced was diverse.

ARTICLE HISTORY
Received 23 January 2017
Accepted 9 October 2017

KEYWORDS
Antimicrobial resistance; fowl typhoid; poultry; epidemiology; whole-genome sequence

Introduction

Salmonella enterica subsp. *enterica* from the serotype Gallinarum biovar causes fowl typhoid in chickens, turkeys and several other avian species (Shivaprasad 2000). Fowl typhoid is an acute or chronic septicaemia disease that usually affects adults, all ages being susceptible. The disease can be transmitted in several ways, but infected birds are the most important means of perpetuation and spread of the bacteria. Birds may infect not only their own generation by horizontal transmission but also succeeding ones through egg transmission (Beach and Davis 1927; Nobrega and Bueno 1942; Hall et al. 1949; Kwon et al. 2010). Although official data concerning fowl typhoid in many European countries, USA, Australia and Japan indicate that they are disease free in commercial flocks, the occurrence may be underestimated because cases are more likely to occur in backyard flocks (Barrow and Freitas Neto 2011). Fowl typhoid is still of considerable economic importance to the poultry industry in many countries from Africa, Asia, Central and South America (Jones et al. 2001).

The *S.* Gallinarum live vaccine strain (SG9R) has been used to control fowl typhoid in many regions where the disease is endemic. In Brazil, this vaccine has been used to control fowl typhoid in laying hens (Feberwee et al. 2001; Lee et al. 2005). Vaccine use is not allowed in chicken and turkey breeders and broiler flocks. The mutation of LPS biosynthesis genes and the molecular basis of SG9R attenuation (including virulence gene expression) are not understood. It has been suggested that either SG9R or SG9R variants might be the cause of some outbreaks of disease in poultry. This hypothesis is supported by the isolation of SG9R-like rough strains from cases of fowl typhoid in SG9R-vaccinated chickens in Korea. Results of pulsed-field gel electrophoresis (PFGE) and multiple-locus variable number tandem repeat analysis on isolates from an outbreak of fowl typhoid in Belgium indicated that these were almost identical to the strain used in the vaccine (Kwon and Cho 2011; Immerseel et al. 2013).

Brazil makes an important contribution to international poultry meat production and the majority of its production is concentrated in the southern states (UBABEF (União Brasileira de Proteína Animal) 2015). Despite advances of poultry rearing in Brazil, outbreaks of fowl typhoid still occur sporadically and may cause more than 50% mortality. Establishing whether outbreaks are related to the earlier use of SG9R or cross-contamination is problematic.

In the present study, multiplex polymerase chain reaction (PCR) and bacterial whole genome sequencing (WGS) were used as epidemiological tools to determine if SG9R was the source of the outbreaks. WGS has proved to be a powerful tool for the investigation of outbreaks caused by *S. enterica*, demonstrating epidemiological agreement and higher resolution than PFGE, the traditional method for *Salmonella* strain subtyping (Deng et al. 2015; Scaltriti et al. 2015; Taylor et al. 2015). In addition, an important antimicrobial resistance profile for the characterisation of isolates was established.

The aim of this study was to differentiate isolates from chicken and turkey outbreaks in breeders and broiler flocks from SG9R vaccine strains, to determine the antimicrobial resistance of the isolates, and to use WGS and single nucleotide polymorphism (SNP) analysis on core genes to identify the origin of selected isolates.

CONTACT B. B. Fonseca biafonseca@ufu.br, Rua Ceará sem número, bloco 2D, segundo andar, sala 48, CEP: 38402-018, Campus Umuarama, Uberlândia, Brazil

Material and methods

Sixty *S.* Gallinarum field isolates from outbreaks of fowl typhoid in southern Brazil between 2011 and 2014, two vaccine strains and *S.* Gallinarum NCTC10532 were tested. These outbreaks isolates were selected due to the high number of laying hen farms in this region of Brazil. Field isolates were cultured from turkey breeders (1), chicken breeders (22), broilers (2) and turkey finisher flocks (35). Vaccine strains were isolated from two different brands: SG9R, Nobilis®SG9R (MSD Animal Health, Boxmeer, Holland) and Cevac® *S.* Gallinarum (Ceva Santé Animale, Libourne, France).

S. Gallinarum identification was based on the lack of motility, carbohydrate fermentation, amino acid decarboxylation and agglutination tests using *Salmonella* antisera (Biorad Laboratories Inc., Marnes-La-Coquette, France) as described by Ewing (1986) and Grimont and Weill (2007). The *S.* Gallinarum was confirmed with a commercial low-density DNA microarray (Check-Points, Wageningen, The Netherlands) following the manufacturer's instructions. Single colonies were stored in cryotubes with 50% glycerol at −20°C.

The disc-diffusion method was used to test antibiotic susceptibility according to the criteria established by the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute (CLSI) 2012). The antimicrobials tests (Oxoid®) are listed as follows, disc content indicated in parentheses: gentamicin (10 µg), enrofloxacin (5 µg), trimethoprim–sulfamethoxazole (1.25/23.7 µg), ceftiofur (30 µg), erythromycin (15 µg), tetracycline (30 µg), norfloxacin (10 µg), amoxicillin (10 µg), florfenicol (30 µg), oxitretacycline (30 µg), lincomycin–spectinomycin (109 µg), streptomycin (300 µg), colistin (10 µg), spyramycin (100 µg), apramycin (15 µg), neomycin (200 µg), and fosfomicyn (200 µg). Antimicrobial agents were selected based on their use in poultry production for other diseases such as colibacillosis, currently or in the past. Isolates were classified as multi-drug resistant when they showed resistance to greater than or equal to three classes of antimicrobial agents (Cohen et al. 2008).

A DNA microarray for identification of extended spectrum β-lactamases (ESBL) and plasmid-mediated cephalosporinase (AmpC) genes was used. All isolates from fowl typhoid outbreaks were tested with Check-MDR CT_{101} (Check-Points, Wageningen, The Netherlands) for the presence of CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9,CTX-M-25, TEM and SHV, and CMY II, DHA, FOX, ACC, ACT/MIR, CMY I/MOX. The DNA extraction was performed according to the manufacturer's instructions using the mini-kit QIAamp DNA (Qiagen, Valencia, CA).

Gene detection was performed following the manufacturer's instructions. Tubes were inserted in the single-channel ATR03 array tube reader upon completion of the detection reaction, and images acquired and interpreted with the software supplied by the manufacturer (Check-Points, Wageningen, The Netherlands) (Cuzon et al. 2012).

For genomic DNA isolation, 1 µl loops of bacterial culture grown on blood agar plates were collected and suspended in 200 µl of PrepMan™Ultra (Applied Biosystems Inc., Norwalk, CT). The suspension was vortexed for 10–30 s to dissolve the bacterial culture and heated at 100° C for 10 min for lysis. Subsequently, samples were centrifuged at 16 000*g* for 3 min. The supernatant containing bacterial DNA was used immediately or transferred to a new tube and stored in the freezer at −20°C until use.

Triplex PCR was used to differentiate the field and vaccine isolates. Salmonella isolates were analysed by triplex PCR to different *S.* Gallinarum and SG9R strains used in live vaccines. Kang et al. (2011, 2012) described the methodology and primer set. Triplex PCR assays were performed in a 25-µl reaction mixture composed of 1× reaction buffer, 1.5 mM $MgCl_2$, 200 µmol of each deoxynucleotide triphosphate (dNTP), 400–1000 nmol of each primer and 1 unit of Taq DNA polymerase, 100-bp DNA marker (Invitrogen, Life Technologies Carlsbad, CA, USA). PCR conditions were initial denaturation for 5 min at 94°C followed by 30 cycles of 30 s at 94°C, 30 s at 64°C, and 30 s at 72°C and final extension step of 7 min at 72°C. PCR products were analysed by electrophoresis on a 2% agarose gel (Invitrogen, Carlsbad, CA, USA).

Selected isolates from chicken breeders from the state of Rio Grande do Sul, vaccine strains and a control *strain* SGNCTC10532 were sequenced by the Ion Torrent Platform, 200-bp fragment library kit using one 314 chip run per sample. Field isolates named SG01, SG02 and SG03 (Property A Brazil-RS) were cultivated using liver or spleen (Property A), SG04 Property B Brazil-RS from liver (Farm B), SG05 Property C Brazil-RS from spleen (Farm C), SG06 and SG07 Property D Brazil-RS from liver and ovarian follicles (Farm D). Samples collected in farm A were from two different outbreaks, SG03 (Property A Brazil-RS) from the first outbreak and SG01 and SG02 from the second. Sequenced isolates from 5 outbreaks represented 4 different chicken breeder farms (A, B, C and D) located in the same State, Rio Grande do Sul, distinct cities and next to properties that used the live vaccine SG9R in laying hens.

All samples were mapped against a reference genome from strain 287/91 (NC_011274) using tmap 4.4, the Torrent Variant Caller version 4.4 was used to find the single nucleotide variants (SNV) between the samples and the reference genome; for each sample, we generated one VCF file. Both pieces of software were part of the sequencer software suite.

Only highly confident SNV with quality score above 100 were selected. The GATK software was used to combine all the filtered VCF into a single multi-sample VCF with 336 variants. A custom script was used to convert the multi-sample VCF into a matrix in phylogeny interference package (PHYLIP) format (Felsenstein 1981). A phylogenetic analysis was performed with DNAML from PHYLIP version 3.696 using the matrix. The program MEGA 7 (Kumar et al. 2016) generated the SNP tree. *De novo* assembly was performed using SPAdes version 3. The contigs generated were aligned with the software Mauve version 2.3.1 to generate the multi-alignment graph. Prokka analysis was performed with software Prokka version 1.12-beta. The graph was created using gnuplot.

Results

The primer sets in the triplex PCR assay produced two amplicons of 174 and 252 bp specific to wild-type *S.* Gallinarum for all isolates from outbreaks of fowl typhoid and three amplicons of 119, 174 and 252 bp specific to SG9R from vaccines (Figure 1). These results indicated that the field isolates from outbreaks of fowl typhoid were not of vaccine origin.

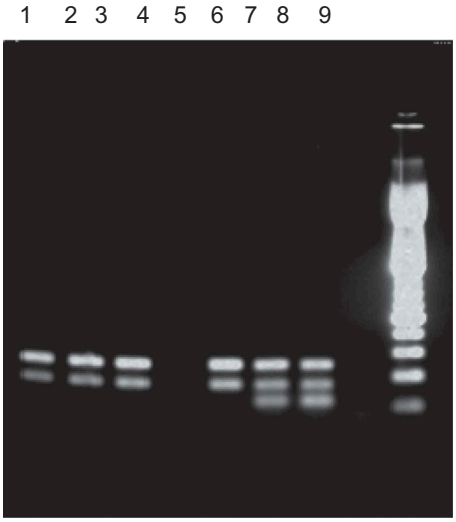

Figure 1. Triplex PCR for differentiation of field isolates *Salmonella* Gallinarum and strain SG9R. Lane 1, 2, 3 and 5: *S.* Gallinarum from outbreaks; lane 4: negative control: *Escherichia coli* ATCC 25922; lane 5: *S.* Gallinarum control strain NCTC 10532; lane 6: *S.* Gallinarum vaccine strain Nobilis SG 9R; lane 7: *S.* Gallinarum vaccine strain CEVAC®; lane 8: MIX, Master Mix Control; lane 9: 100-pb DNA marker (Invitrogen, Life Technologies Carlsbad, CA, USA).

WGS (Table 2) demonstrated that samples SG04 and SG05 had gene variants twice the size of the other samples. The genome covered was 100% except for SG04 (Table 1). The statistical and Prokka analysis of the isolates are shown in Table 2. *De novo* analysis and alignment of the contigs with the reference genome (NC_011274) showed that the region between 400 kbp and 1.2 Mbp is structurally variable. Larger variations in this region are observed in samples SG01, SG02 and SG03, SG04 and SG05. Phylogenetic analysis was performed based on SNP on core genes of *S.* Gallinarum as proposed by Leekitcharoenphon et al. (2012). Samples SG04 and SG05 presented greater genome variation than other samples and the reference (Figure 2). The vaccine CEVAC exhibited some DNA regions with possible duplications. The core gene number and percentage for each genome sequenced are in Table 3.

The analysis of the whole genome from samples compared to the reference showed that sample SG01 had a large inversion (>1.5 Mb) in its genome. The sample SG02, SG04, SG05 and the vaccine SG Cevac®-CEVA exhibited a small inversion (<210 kb) in genome segments (Figure 2).

Variant analysis found 2379 SNPs in core genes. The majority of SNPs were observed in samples SG04 and SG05; 2216 core genes showed SNPs only in samples SG04 and SG05.

In the antimicrobial susceptibility test, the vaccine strains were resistant to streptomycin, erythromycin and spiramycin. All isolates were resistant to at least one antimicrobial agent. Field isolates showed 33 resistance patterns and from the 60 isolates tested, only 6 were not classified as multi-drug resistant. Tested isolates showed high resistance against macrolide and quinolone groups, i.e. spiramycin (100%), erythromycin (96%), norfloxacin (90%) and enrofloxacin (83%), intermediate or low rate of resistance for streptomycin (62%), amoxicillin (35%), gentamicin (45%), tetracycline (33%), oxytetracycline (30%), neomycin (30%), colistin (27%), florfenicol (25%), apramycin (23%), ceftiofur (8%), trimethoprim–sulfamethoxazole (7%) and lincomycin–spectinomycin (1.6%). All isolates were sensitive to fosfomycin. Considering the sites of antibiotic action, 70% of the isolates were resistant to at least one antimicrobial that acts on the cell wall (ceftiofur, amoxicillin, fosfomycin and colistin) but the percentage was 35% if amoxicillin was excluded; 90% of the isolates were resistant to at least one of the antimicrobials that inhibit nucleic acid synthesis (enrofloxacin and norfloxacin); all isolates were resistant to at least one antimicrobial that blocks protein synthesis.

Table 1. Covered genome assembled using *S. enterica* serovar Gallinarum strain 287/91 (NC_011274) as reference genome.

Sample	Missed bases	Covered reference (%)	Mean coverage (%)
SG Nobilis®9R-MSD	46	100.00	33
SG Cevac®-CEVA	51	100.00	24
SG01 Property A Brazil-RS	6744	100.00	64
SG NCTC10532	6644	100.00	58
SG02 Property A Brazil-RS	6981	100.00	29
SG03 Property A Brazil-RS	6677	100.00	62
SG04 Property B Brazil-RS	372 092	99.96	64
SG05 Property C Brazil-RS	6749	100.00	69
SG06 Property D Brazil-RS	6749	100.00	69
SG07 Property D Brazil-RS	6652	100.00	30

Discussion

According to Brazil's National Plan of Poultry Health, the use of live vaccines or antimicrobial therapy to control *S.* Gallinarum and *Salmonella* Pullorum is forbidden in poultry breeder and meat flocks and all flocks testing positive must be killed. However, vaccine strain SG9R is allowed and used extensively in laying hens in southern Brazil. There is evidence that fowl typhoid outbreaks can be caused by the vaccine strain SG9R (Kwon and Cho 2011; Immerseel et al. 2013). Triplex PCR and WGS showed that the *S.* Gallinarum isolates from fowl typhoid outbreaks studied here were not caused by vaccine strains. Therefore, the causes of these outbreaks need to be investigated, though probably these result from the lack of biosecurity.

Table 2. Summary statistics of draft genome sequence and Prokka analyses.

Isolates	Contigs	Length (bp)	C + G content (%)	N_{50}	SNP	SNP core genes	Core genes	CDS	tRNA	rRNA
SG01 Property A	453	4991 390	52.66	280 501	292	159	2870	4970	67	8
SG02 Property A	47	4693 797	52.16	195 541	268	146	2873	5113	65	9
SG03 Property A	62	4697 270	52.17	244 002	300	164	2869	4889	69	8
SG04 Property B	62	4594 785	52.17	170 830	43 521	27 717	2874	4564	83	10
SG05 Property C	61	4589 223	52.21	170 942	39 172	24 816	2877	4775	78	11
SG06 Property D	51	4701 129	52.17	224 458	299	161	2871	5095	71	9
SG07 Property D	41	4695 181	52.17	229 107	281	150	2872	5186	68	9
SG NCTC10532	53	4699 060	52.18	224 411	311	162	2870	4970	67	8
SG Nobilis®9R-MSD	45	4700 254	52.18	224 372	9	5	2869	5230	65	7
SG Cevac®-CEVA	92	4704 328	52.18	115 867	17	6	2869	5111	71	15
Reference 287/91 NC_011274		4658 679	52.18				2438	3970	75	22

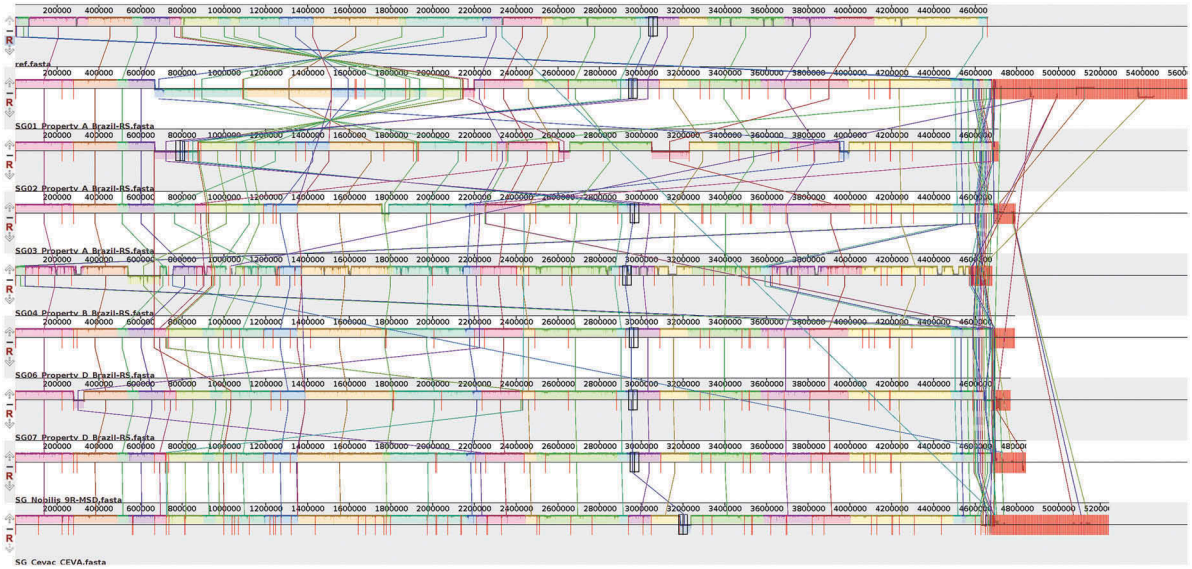

Figure 2. A whole-genome alignment of *Salmonella* Gallinarum strains from Brazilian Property and controls. Mauve algorithm was used for the alignment of 9 genomes. Regions with the same colour indicate high similarity and connect by the same colour bars. The genome was drawn to scale based on the reference *S.* Gallinarum strain SGNCTC10532. Each sample was mapped against the reference NC_011274 using Ion Torrent's tmap. The graphic shows the result of bed tool's genome Cov of each sample. The figure was created using gnuplot.

Table 3. Core gene number and percentage for each genome.

Sample	Core genes	%
core genes	2882	100.0
10C_vacina	2869	99.5
11C_vacina	2869	99.5
1A_liver	2870	99.6
1C_controle	2870	99.6
2A_spleen	2873	99.7
3A_liver	2869	99.5
4B_liver	2874	99.7
5C_spleen	2877	99.8
6C_deleted	2874	99.7
6D_liver	2871	99.6
7D_ovary	2872	99.7

The set of 2882-core gene cluster for *S. enterica* (Leekitcharoenphon et al. 2012) in the reference genome was used to compare the samples and obtain the SNP tree. The SNP tree evaluation on core genes has proven to provide a high-quality approach to epidemiologic studies (Leekitcharoenphon et al. 2014).

Bacterial WGS was used in this study for epidemiological investigation which has been shown to be a powerful tool for investigating outbreaks caused by *S. enterica*, providing great epidemiological concordance and higher resolution than PFGE, the traditional method for *Salmonella* subtyping (Deng et al. 2015; Scaltriti et al. 2015; Taylor et al. 2015). WGS offers the ultimate sensitivity for strain typing and can provide insight into phylogenetic relationships between strains (Immerseel et al. 2013). The WGS and *de novo* analysis showed that samples could be classified in three main groups. One was formed by samples SG04 and SG05, the second by vaccine strains and the third by other field isolates (Figure 3). Samples from property B Brazil-RS and SG05 (Property C Brazil-RS) are from different properties, but located in the same region, and the outbreaks occurred with 1 month of each other. Although we do not know the origin of these salmonella isolates, it is possible to speculate that it is due to the presence of free animal carriers that transit between the two farms. Practitioners suspected that Farm C was contaminated from Farm B by a truck of feedstuff (data not shown). Outbreaks at Farms A and D may be associated with the same origin as the isolates were similar. However, there was no epidemiological relationship between isolates from Farms A and D (data not shown). In

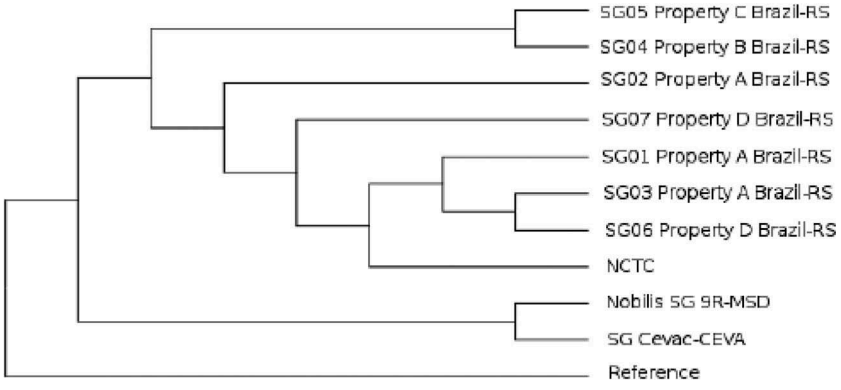

Figure 3. Phylogenetic analysis among samples selected isolates, vaccine strains (SG Nobilis 9R MSD and SG Cevac Ceva) and a control strain *S.* Gallinarum NCTC10532 based on the SNP in the genome sequenced.

Farm A, sample SG03 (Property A Brazil-RS) came from an outbreak that occurred 1 year before the outbreaks that provided samples SG01 (Property A Brazil-RS) and SG02 (Property A Brazil-RS). This fact may explain why sample SG03 (Property A Brazil-RS) did not show the same inversions as samples SG01 (Property A Brazil-RS) and SG02 (Property A Brazil-RS). This suggests that these had different origins indicating lack of biosecurity at these farms. Samples SG06 (Property D Brazil-RS) and SG07 (Property D Brazil-RS) from farm D are from the same outbreak and occurred 15 months after outbreaks that provided samples SG01 (Property A Brazil-RS) and SG02 (Property A Brazil-RS) but no epidemiological relationship between them could be found, despite similarities in these isolates.

The most frequent resistance profile: norfloxacin, enrofloxacin, streptomycin, erythromycin, spiramycin (8/60), was found in 2012 and 2013, from outbreaks of fowl typhoid in turkey finishers, broiler breeders and broilers from different southern Brazilian States. The occurrence of *S.* Gallinarum resistant to the macrolides, erythromycin and spiramycin is in agreement with other studies (Chu and Chiu 2006; Kang et al. 2010). Despite the intermediate rate of resistance to fluoroquinolones, i.e. enrofloxacin reported earlier, high prevalence of resistance to norfloxacin was not reported. Resistance against aminoglycosides reported in this study is consistent with Kang et al. (2010) that compared isolates of *S.* Gallinarum from South Korea in 2002–2007 and found increased resistance to quinolones and aminoglycosides. Resistance to β-lactam antibiotics, amoxicillin (35%) and ceftiofur (8%) and tetracycline has not been reported before for *S.* Gallinarum. However, reduced susceptibility to β-lactams and tetracycline has been reported in Brazil for diverse strains of *Salmonella* spp., mainly isolated from the poultry environment (Mattiello et al. 2015). Despite presence of resistance to the third-generation cephalosporin, ceftiofur and β-lactam amoxicillin, genes for ESBL and plasmid-mediated cephalosporinase were not found indicating that the resistance observed in these isolates is not related to the genes surveyed. In research on *Salmonella* spp. isolated from farm animals in the USA from 1999 to 2003, the majority of resistance to ceftiofur was associated with $bla_{\text{CMY-2}}$-encoding plasmids (Frye and Fedorka-Cray 2007). In this report, the plasmid-mediated CMY-2 was absent from the isolates tested.

Although use of antibiotics for treatment of fowl typhoid is not allowed in Brazil, the high rate of resistance reported here demonstrates that, as in other countries, antibiotic use is frequent. Many antibiotics have been found to be effective at reducing mortality in fowl typhoid outbreaks but are not able to eliminate infection from the flock since birds remain infected after the treatment and can be reinfected from the local environment (Gordon and Tucker 1957; Barrow and Freitas Neto 2011). The profile of this antibiotic resistance in isolates from the present study from commercial poultry may be a consequence of the continued use of antimicrobials for treating poultry disease problems such as *Escherichia coli* or arthritis.

The present study suggests that *S.* Gallinarum isolated from fowl typhoid outbreaks in the studied period were not caused by the use of the live vaccine SG9R. Strains sequenced had similarities in some outbreaks, even when the epidemiological relationship could not be found. The study highlights the presence of antimicrobial resistance to the main classes of antibiotics used in the poultry industry.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Barrow, P. A., and O. C. Freitas Neto. 2011. "Pullorum Disease and Fowl Typhoid-New Thoughts an Old Diseases: A Review." *Avian Pathology* 40: 1–13. doi:10.1080/03079457.2010.542575.

Beach, J. R., and D. E. Davis. 1927. "Acute Infection in Chicks and Chronic Infection of the Ovaries of Hens Caused by the Fowl Typhoid Organisms." *Hilgardia* 2: 411–424. doi:10.3733/hilg.v02n12p411.

Chu, C., and C. H. Chiu. 2006. "Evolution of the Virulence Plasmid of Non-Typhoid Salmonella and Its Association with Antimicrobial Resistance." *Microbes and Infection* 8: 1931–1936. doi:10.1016/j.micinf.2005.12.026.

Clinical and Laboratory Standards Institute (CLSI). 2012. "Performance Standards for Antimicrobial Susceptibility Testing." In *22nd Informational Supplement. CLSI document M100-S22*, 19087-1898. Wayne, PA: CLSI.

Cohen, A. L., S. K. Fridkin, S. S. Huang, J. A. Jernigan, E. Lautenbach, S. Oriola, K. M. Ramsey, C. D. Salgado, and R. A. Weinstein. 2008. "Recommendations for Metrics for Multidrug-Resistant Organisms in Healthcare Settings: SHEA/HICPAC Position Paper." *Infection Control & Hospital Epidemiology* 29: 901–913. doi:10.1086/591741.

Cuzon, G., T. Naas, P. Boagaerts, Y. Glupczynski, and P. Nordmann. 2012. "Evaluation of a DNA Microarray for the Rapid Detection of Extended-Spectrum β-lactamases (TEM, SHV and CTX-M), Plasmid-Mediated Cephalosporinases (Cmy-2-Like, DHA, FOX, ACC-1, ACT/MIR and CMY-1- like MOX) and Carbapenemases (KPC, OXA-48, VIM, IMP and NDM)." *Journal of Antimicrobial Chemotherapy* 67: 1865–1869. doi:10.1093/jac/dks156.

Deng, X., N. Shariat, E. M. Driebe, C. C. Roe, B. Tolar, E. Trees, P. Keim, et al. 2015. "Comparative Analysis of Subtyping Methods against a Whole-Genome-Sequencing Standard for Salmonella Enterica Serotype Enteritidis." *Joural Clinical Microbiology* 53: 212–218. doi:10.1128/JCM.02332-14.

Ewing, W. H. 1986. *Edwards and Ewing's Identification of Enterobacteriaceae*, 536. 4th ed. New York, NY: Elsevier Science Publishing.

Feberwee, A., T. S. De Vries, E. G. Hartman, J. J. De Wit, A. R. W. Elbers, and W. A. De Jong. 2001. "A Vaccination against *Salmonella Enteritidis* in Dutch Commercial Layer Flocks with Vaccine Based on a Live *Salmonella Gallinarum* 9R Strain: Evaluation of Efficacy, Safety, and Performance of Serologic Salmonella Tests." *Avian Disease* 45: 83–91. doi:10.2307/1593015.

Felsenstein, J. 1981. "Evolutionary Trees from DNA Sequences: A Maximum Likelihood Approach." *Journal of Molecular Evolution* 17 (6): 368–376. doi:10.1007/BF01734359.

Frye, J. C., and P. J. Fedorka-Cray. 2007. "Prevalence, Distribution and Characterization of Ceftiofur Resistance in Salmonella Enterica Isolated from Animals in the USA from 1999 to 2003." *International Journal of Antimicrobial Agents* 30: 134–142. doi:10.1016/j.ijantimicag.2007.03.013.

Gordon, R. F., and J. F. Tucker. 1957. "The Treatment of Chronic Carriers of *Salmonella Pullorum* with Furazolidone." *British Veterinary Journal* 113: 99–111. doi:10.1016/S0007-1935(17)46151-3.

Grimont, P. A. D., and F. Weill. 2007. *Antigenic Formulae of the Salmonella Serovars*, 166. 9th ed. Paris. France: WHO Collaborating Centre for Reference and Research on Salmonella. Institut Pasteur.

Hall, W. J., D. H. Legenhausen, and A. D. Macdonald. 1949. "Studies on Fowl typhoid.1.Nature and Dissemination." *Poultry Science* 28: 344–362. doi:10.3382/ps.0280344.

Immerseel, F. V., D. J. Studholme, V. Eeckhaut, M. Heyndrickx, J. Dewulf, I. Dewaele, V. S. Hoorebeke, et al. 2013. "*Salmonella* Gallinarum Field Isolates from Laying Hens are Related to the

Vaccine Strains SR9R." *Vaccine* 31: 4940–4945. doi:10.1016/j.vaccine.2013.08.033.

Jones, M. A., P. Wigley, K. L. Page, S. D. Hulme, and P. A. Barrow. 2001. "*Salmonella Enterica* Serovar Gallinarum Requires the *Salmonella* Pathogenicity Island 2 Type III Secretion System but Not the Salmonellapathogenicity Island 1 Type III Secretion System for Virulence in Chickens." *Infection and Immunity* 69: 5471–5476. doi:10.1128/IAI.69.9.5471-5476.2001.

Kang, M. S., A. Kim, B. Y. Jung, M. Her, W. Jeong, Y. M. Cho, J. Y. Oh, Y. J. Lee, J. H. Kwon, and Y. K. Kwon. 2010. "Characterization of Antimicrobial Resistance of Recent Salmonella Enterica Serovar Gallinarum Isolates from Chickens in South Korea." *Avian Pathology* 39: 201–205. doi:10.1080/03079451003767261.

Kang, M. S., Y. K. Kwon, A. Kim, K. M. Lee, B. K. An, E. A. Song, J. H. Kwon, and G. S. Chung. 2011. "Differential Identification of *Salmonella Enterica* Subspecie Enterica Serovar Gallinarum and Pullorum Based on Polymorphic Regions of *glgC* and *speC* Genes." *Veterinary Micobiology* 147: 181–185. doi:10.1016/j.vetmic.2010.05.039.

Kang, M. S., Y. K. Kwon, A. Kim, J. Y. L. E. E. Oh, M. J. Kim, B. K. An, E. G. Shin, J. H. Kwon, and C. K. Park. 2012. "Differential Identification of *Salmonella Enterica* Serovar Gallinarum Biovars Gallinarum and Pullorum and the Biovar Gallinarum Live Vaccine Strain 9R." *Veterinary Micobiology* 160: 491–495. doi:10.1016/j.vetmic.2012.05.041.

Kumar, S., G. Stecher, and K. Tamura. 2016. "MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets." *Molecular Biology and Evolution* 33 (7): 1870–1874. doi:10.1093/molbev/msw054.

Kwon, H. J., and S. H. Cho. 2011. "Patogenicity of SG 9R, a Rough Vaccine Strain against Fowl Typhoid." *Vaccine* 29: 1311–1318. doi:10.1016/j.vaccine.2010.11.067.

Kwon, Y. K., A. Kim, M. S. Kang, M. Her, B. Y. Jung, K. M. Lee, W. Jeong, B. K. An, and J. H. Kwon. 2010. "Prevalence and Characterization of Salmonella Gallinarum in the Chicken in Korea during 2000 to 2008." *Poultry Science* 89 (2): 236–242. doi:10.3382/ps.2009-00420.

Lee, Y. J., I. P. Mo, and M. S. Kang. 2005. "Safety and Efficacy of *Salmonella Gallinarum* 9R Vaccine in Young Laying Chickens." *Avian Pathology* 36: 362–366. doi:10.1080/03079450500180895.

Leekitcharoenphon, P., C. Friis, F. M. Aarestrup, and D. W. Ussery. 2012. "Genomic Variation on Salmonella Enterica Core Genes for Epidemiological Typing." *BMC Genomics* 13: 1–11. doi:10.1186/1471-2164-13-88.

Leekitcharoenphon, P., E. M. Nielsen, R. S. Kaas, O. Lund, and F. M. Aarestrup. 2014. "Evaluation of Whole Genome Sequencing for Outbreak Detection of *Salmonella Enterica*." *Plos One* 9: 1–11. doi:10.1371/journal.pone.0087991.

Mattiello, S. P., G. Drescher, V. C. Barth, C. A. Ferreira, and S. D. Oliveira. 2015. "Characterization of Antimicrobial Resistance in Salmonella Enterica Strains ISOLATED FROM Brazillian Poultry Production." *Antonie Van Leeuwenhoek* 108: 1227–1238. doi:10.1007/s10482-015-0577-1.

Nobrega, P., and R. C. Bueno. 1942. "On the Presence of Salmonella Gallinarum in Fresh Eggs of Hens Recognized as Chronic Carriers of Fowl Typhoid." *Arquivos Do Instituto Biológico* 13: 17–20.

Scaltriti, E., D. Sassera, F. Comandatore, M. Morganti, C. Mandalari, S. Gaiarsa, C. Bandi, et al. 2015. "Differential Single Nucleotide Polymorphism-Based Analysis of an Outbreak Caused by Salmonella Enterica Serovar Manhattan Reveals Epidemiological Details Missed by Standard Pulsed-Field Gel Electrophoresis." *Journal Clinical Microbiology* 53 (4): 1227–1238. doi:10.1128/JCM.02930-14.

Shivaprasad, H. L. 2000. "Fowl Typhoid and Pullorum Disease." *Revue Scientifique Et Technique* 19: 405–424. doi:10.20506/rst.19.2.1222.

Taylor, A. J., V. Lappi, W. J. Wolfgang, P. Lapierre, M. J. Palumbo, C. Medus, and D. Boxrud. 2015. "Characterization of Foodborne Outbreaks of Salmonella Enterica Serovar Enteritidis with Whole-Genome Sequencing Single Nucleotide Polymorphism-Based Analysis for Surveillance and Outbreak Detection." *Journal Clinical Microbiology* 53: 3334–3340. doi:10.1128/JCM.01280-15.

UBABEF (União Brasileira de Proteína Animal). *Relatório Anual* 2015. Accessed: 09th February, 2016. In: http://abpa-br.com.br/files/RelatorioAnual_UBABEF_2015_DIGITAL.pdf