

ORIGINAL ARTICLE

Distribution of *Salmonella* Serovars and Antimicrobial Susceptibility from Poultry and Swine Farms in Central Vietnam

A. A. Lettini¹, T. Vo Than², E. Marafin¹, A. Longo¹, K. Antonello¹, P. Zavagnin¹, L. Barco¹, M. Mancin¹, V. Cibi¹, M. Morini³, M. Dang Thi Sao², T. Nguyen Thi², H. Pham Trung², L. Le², T. Nguyen Duc² and A. Ricci¹

¹ Istituto Zooprofilattico Sperimentale delle Venezie, National Reference Laboratory for Salmonellosis, Legnaro (PD), Italy

² Central of Vietnam Veterinary Institute, Nha Trang, Vietnam

³ International Cooperation Office, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy

Impacts

- This study has estimated the prevalence of *Salmonella* spp. and their antimicrobial susceptibilities on poultry and swine farms, sampled in Central Vietnam.
- Our results showed that *Salmonella* prevalence was significantly high both in poultry and swine farms sampled; MDR *Salmonella* incidence was high, corresponding to 59.6% of *Salmonella* totally isolated.
- This updated report on the occurrence of *Salmonella* from Central Vietnam, underlines a need for integrating these data on *Salmonella* epidemiology in Central Vietnam with in-depth molecular analysis on the prevalence of resistance determinants as well as with data on *Salmonella* occurrence in corresponding meat products.

Keywords:

Salmonella prevalence on livestock; Central Vietnam; antimicrobial resistance

Correspondence:

Antonia Ricci. Istituto Zooprofilattico Sperimentale delle Venezie, National Reference Laboratory for Salmonellosis, Legnaro (PD), Italy. Tel.: +390498084296; Fax: +390498830268; E-mail: aricci@izsvenezie.it

Received for publication July 28, 2015

doi: 10.1111/zph.12265

Summary

This study was conducted to estimate the prevalence of *Salmonella* spp. and their antimicrobial susceptibilities on poultry and swine farms, sampled in 2 regions in Central Vietnam. A total of 67 poultry farms and 46 swine farms were sampled in a period of 5 months (from September 2012 to January 2013). *Salmonella* spp. was prevalent in 46.3% and 71.7% of poultry and swine farms, respectively. Altogether, 99 non-typhoidal *Salmonella* were isolated and the most common serovars were *Salmonella* Weltevreden (19%), followed by *Salmonella* Typhimurium (12%) and *Salmonella* 4,[5],12:i:- (11%). Overall, 71 of 99 (72%) *Salmonella* isolates were resistant to at least one of the 14 antimicrobial agents tested. Both in poultry and swine farms, high levels of resistance were observed for ampicillin, chloramphenicol, ciprofloxacin, sulphamethoxazole and tetracycline. The presence of *Salmonella* isolates from poultry and swine farms which were resistant to different classes of antimicrobials suggests that alternative control measures to antimicrobials should be implemented. Moreover, an effective policy should be promoted to encourage a prudent use of these agents in animal farming in Vietnam.

Introduction

Salmonella spp. cause one of the most important enteric diseases worldwide. The global human health impact of non-typhoidal *Salmonella* bacteria is estimated at 93.8 million illnesses annually. Of these illnesses, an estimated 80.3

million are foodborne and associated with 155 000 deaths (Majowicz et al., 2010).

Food-producing animals such as poultry, pigs and cattle play a crucial role as primary reservoirs of *Salmonella* and vehicles for salmonellosis worldwide. This is especially so in developing countries which are often characterized by poor

food safety measures (Lai et al., 2014). Because of the ability of *Salmonella* to contaminate meat during slaughter and to survive in fresh meats and meat products that are not thoroughly heated, nowadays, animal products still constitute a main vehicle of transmission in developing countries (Adeyanju and Ishola, 2014; Tadesse and Gebremedhin, 2015). In Central Vietnam, few data regarding the prevalence and distribution of *Salmonella* spp. in food-producing animals are available (Ta et al., 2014). However, a number of studies investigated the presence of *Salmonella* in food samples in different areas of Vietnam, such as the Mekong Delta or North of Vietnam (Thai et al., 2012; Tu et al., 2015).

Within Vietnam, access to antimicrobials is relatively difficult to control despite regulations that are supposed to limit access (Report antibiotics use, 2009). In addition, there appears to be a general lack of knowledge about the purpose and proper usage of antibiotics by farmers (Pham et al., 2015). These factors, coupled with the high burden of human infectious disease, have made this country, as other developing countries, a potential hot spot for the emergence of drug resistance (Nguyen et al., 2013).

Animal production is an increasingly important industry in Vietnam and one in which antibiotics are extensively used for several purposes including therapeutics, prophylaxis and growth promotion (GARP, 2009). Of all pharmaceutical products used in the animal sector, 70% are antibiotics (GARP - Global Antibiotic Resistance Partnership, 2009). Since Vietnam joined the World Trade Organization (WTO), regulation of antibiotic use in animals has increased and certain antibiotics, such as chloramphenicol and fluoroquinolones, have been banned (MARD, 2010). Surveillance for antibiotic residues in meat as well as in fish reveals frequent misuse of antibiotics in Vietnam (Pham et al., 2013, 2015). Thus, the use of antibiotics in livestock might contribute to the increase of antimicrobial-resistant bacteria in humans via the food chain.

In Asian countries, these problems remain poorly investigated, especially in the food and animal fields (Tran et al., 2004, 2006; Tabo et al., 2013; Tu et al., 2015). Hence, the aim of this study was to determine the prevalence of *Salmonella* spp. in poultry and pig farms located in the Binh Dinh and Khanh Hoa provinces of Central Vietnam. In addition, the antimicrobial resistance patterns of *Salmonella* isolates were investigated.

Materials and Methods

Study location and sampling

The survey was conducted between September 2012 and January 2013 in rural areas of two provinces located in Central Vietnam: Binh Dinh (between latitude 14°41'59.82" and 13°30'56.22"N and longitude

108°55'33.88" and 108°58'32.60", with a population of about 1.51 million people, whilst 6.3 million poultry, 665 900 pigs and 267 700 cattle and buffalo are farmed in an area of approximately 6000 km²) and Khanh Hoa (between latitude 12°51'56.10" and 11°48'16.70"N and longitude 109°22'12.28" and 109° 7'41.44", of 5200 km², 1.192 million people, 2.3 million poultry, 113 300 pigs and 76 400 cattle and buffalo).

The samplings were distributed in poultry and swine farms according to their size and production categories, and on each farm, the number of samples collected was proportional to the farm size, with a maximum of seven samples per farm (five faeces and two dusts). Poultry samples were collected from broiler, laying hen and breeder farms, whereas swine samples were collected from fattening (17 from rural, nine from industrial), farrow-to-weaner (11 from rural, five from industrial) and sow (three from rural, one from industrial) (Table 1).

Two types of farms, common in the area under study, were sampled: industrial farms, which involve large-scale or commercial production of high-performance types of live-stock, and rural ones which are based on the rearing of a small number of animals of local breeds in rural villages.

All samples were transported in a cooler box to the laboratory at the Central Vietnam Veterinary Institute (CVVI, Nha Trang, Vietnam), within 24 h of sampling and stored at 4°C.

Salmonella isolation

Salmonella isolation was carried out according to ISO 6579:2002 (Annex D) (Anon., 2007) within 24 h after arrival at the laboratory. In particular, from each sample up to five presumptive *Salmonella* colonies were chosen for biochemical confirmation.

Table 1. Prevalence of *Salmonella* spp. in farms sampled in Central Vietnam

Animal	Category	No. of samples (No. of farms)	No. of <i>Salmonella</i> positive samples (No. of positive farms)
Poultry	Breeder	42 (6)	4 (2)
	Broiler	248 (41)	30 (18)
	Laying hen	140 (20)	32 (11)
	Subtotal	430 (67)	66 (31)
Swine	Fattening	138 (16)	27 (9)
	and sow		
	Fattening	85 (26)	65 (23)
	Sow	18 (4)	2 (1)
	Subtotal	241 (46)	94 (33)
	Total	671 (113)	160 (64)

Salmonella serotyping and phage typing

All *Salmonella* were serotyped according to the White–Kauffmann–Le Minor scheme by slide agglutination method (Grimont and Weill, 2007) at the National and OIE Reference Laboratory for *Salmonella* (NRL-IZSVe, Legnaro, Italy).

Salmonella isolates for which it was not possible to identify a definitive serovar by traditional agglutination were examined by a molecular serotyping assay based on a bead suspension array (xMAP® *Salmonella* Serotyping Assay, SSA, Luminex Corp., Austin, TX, USA) to infer their antigenic formula. SSA is a molecular serotyping assay addressing a set of target genes involved in the expression of the most common *Salmonella* serotype-specific antigens. Multiplex PCR is used to amplify *Salmonella* antigen gene target sequences and incorporate a biotin label in the amplicons. The amplified target sequences are then captured by direct hybridization onto bead sets coupled with complementary oligonucleotide capture probes and finally labelled with a fluorescent reporter for detection on the Bio-Plex analyzer (Bio-Rad, Hercules, CA, USA) (Fitzgerald et al., 2007; Dunbar et al., 2015).

All *S. Enteritidis* and *S. Typhimurium* (including *S. 4, [5], 12:i:-*) isolates were phage-typed using the protocol previously reported (Anderson et al., 1977; Rabsch, 2007) and according to the recommendations of Public Health England (Colindale, London, United Kingdom). The definitive phage typing of *S. Enteritidis* and *S. Typhimurium* (including *S. 4, [5], 12:i:-*) was performed using the standard set of 17 and 38 typing phages, respectively, received from Public Health England.

The isolates showing a phage pattern that did not conform to any recognized phage type were designated as ‘react but did not conform’, and isolates that did not react with any of the typing phages were designated as ‘non-typeable’.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of *Salmonella* isolates was tested against 14 antimicrobials by determining the minimum inhibitory concentration (MIC) using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (CLSI, 2009) in combination with the semi-automatic Sensititre system (TREK Diagnostic Systems, Cleveland, OH, USA). The antimicrobials tested were as follows: ampicillin (AMP), ceftazidime (CAZ), cefotaxime (FOT), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), florfenicol (FFN), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfamethoxazole (SMX), tetracycline (TET) and trimethoprim (TMP). The EUCAST epidemiological cut-offs were used (EUCAST, 2015) except for SMX and KAN where the

CLSI break points were considered (CLSI - Clinical and Laboratory Standards Institute, 2009). *Escherichia coli* ATCC 25922 was used as a control strain. Antimicrobial resistance was defined as resistance to one or more classes of antimicrobials (there were 8 classes among the 14 antimicrobials; WHO, 2011), whereas multidrug resistance (MDR) was defined as a resistance to four or more classes of antimicrobials.

Statistical analysis

Exact confidence intervals (CI) were calculated for the estimated prevalences. The Z-test was performed to verify the significant differences between prevalences. The *P*-value <0.05 was considered significant. SAS 9.3 software (SAS Institute Inc., Cary, NC, USA) was used to analyse data.

Results

Sampling

A total of 67 poultry farms were sampled in the two Vietnamese provinces studied, grouped according to animal species and production categories as reported in Table 1. Among the poultry farms studied, broiler farms accounted for 61.2% of those sampled (41/67), laying hen farms for 30% (20/67) and the breeders for 9% (6/67). Overall, 430 (310 faeces and 120 dust) samples were collected from poultry farms.

With regard to pigs, 46 swine farms were sampled as described in Table 1. The great majority of swine samples were collected from fattening farms (56.5%, 26/46), followed by swine farms with both farrow-to-weaner (34.8%, 16/46) and sow farms being the least represented (8.7%, 4/46). In total, the 241 swine farm samples were composed of 167 faecal and 74 dust samples, respectively.

Salmonella isolation and serovar distribution

According to the total samples collected both in poultry and swine farms, the *Salmonella* prevalence observed in faecal samples was significantly higher than the prevalence observed in dust samples ($P < 0.001$) (Table 2). *Salmonella* spp. was isolated from 31 of the 67 [46.3%, CI (33.99; 58.88)] poultry farms sampled in both Vietnamese provinces. Among these farms, 18 of 44 [40.9%, CI (26.33; 56.75)] rural farms and 13 of 23 [56.5%, CI (34.49; 79.80)] industrial farms were positive for *Salmonella*. Among the 16 serovars identified from poultry farms, the five most frequent were as follows: *S. Weltevreden* (25%), *S. Limete* (10.4%), *S. Typhimurium* (14.6%), *S. Javiana* (8.3%) and *S. Enteritidis* (8.3%) (Table 3).

Concerning swine farms, the *Salmonella* prevalence was 71.7% (CI [56.54; 80.01]) (33 of 46 farms sampled).

Table 2. Prevalence of *Salmonella* spp. in farms grouped by sample material, and significant differences reported between types of sample

Type of sample	Poultry farms (67)			Swine farms (46)		
	No. positive farms	Percentage [CI]	P-value	No. positive farms	Percentage [CI]	P-value
Faeces	28	41.79% CI [29.85; 54.48]	$P < 0.001$	31	67.39% CI [51.98; 80.47]	$P < 0.001$
Dust	11	16.42% CI [8.49; 27.48]		12	26.09% CI [14.27; 41.13]	

Table 3. Serovars of *Salmonella* spp. isolated by animal species

Serotype	No. of isolates (%)		
	Poultry	Swine	Total
<i>S. Weltevreden</i>	12 (25)	7 (13.7)	19
<i>S. Typhimurium</i>	7 (14.6)	5 (10)	12
<i>S. 4,[5],12:i:-</i>	3 (6.2)	8 (15.7)	11
<i>S. Limete</i>	5 (10.4)	2 (4)	7
<i>S. Derby</i>	1 (2.1)	6 (12)	7
<i>S. Panama</i>	0	7 (13.7)	7
<i>S. Javiana</i>	4 (8.3)	2 (4)	6
<i>S. Albany</i>	1 (2.1)	3 (6)	4
<i>S. Enteritidis</i>	4 (8.3)	0	4
<i>S. Newport</i>	0	3 (6)	3
<i>S. Apeyeme</i>	2 (4.2)	0	2
<i>S. Bareilly</i>	0	2 (4)	2
<i>S. Brunei</i>	1 (2.1)	1 (2)	2
<i>S. Give</i>	2 (4.2)	0	2
<i>S. Kedougou</i>	1 (2.1)	1 (2)	2
<i>S. Lexington</i>		2 (4)	2
<i>S. Rissen</i>	1 (2.1)	1 (2)	2
<i>S. Stanley</i>	2 (4.2)	0	2
<i>S. Cerro</i>	1 (2.1)	0	1
<i>S. Kentucky</i>	1 (2.1)	0	1
<i>S. India/S. Panama</i> (*)			1
Total	48 (48.5)	51 (51.5)	99

**Salmonella* isolate with an incomplete and unrecognized antigenic formula phenotypically. This isolate was indistinguishable from *S. India* and *S. Panama* when examined by the molecular SAA approach used.

Table 4. Phage types observed for *Salmonella* isolated in this study

Source	Serovar	Phage type
Poultry	<i>S. Typhimurium</i> (7)	DT120 (3), DT 193 (2), NT (2)
	<i>S. 4,[5],12:i:-</i> (3)	DT 120 (2), DT 193 (1)
	<i>S. Enteritidis</i> (4)	NT (3), RDNC (1)
Swine	<i>S. Typhimurium</i> (5)	DT120 (4), NT (1)
	<i>S. 4,[5],12:i:-</i> (8)	DT120 (1), DT 193 (5), NT (2)

NT: non-typeable.

RDNC: react but does not conform.

Phage type distribution

Altogether, three *Salmonella* Enteritidis were of phage type non-typeable (NT) and the fourth was phage-typed as RDNC (react but does not conform), isolated from poultry farms.

For *S. Typhimurium* isolated from poultry, two isolates were NT, three were phage-typed as DT120 and the remaining two as DT193. Similarly, four of five *S. Typhimurium* isolated from swine were phage type DT120, whilst the remaining isolate was NT (Table 4).

With regard to *S. 4,[5],12:i:-* isolated from poultry farms, two were phage type DT120 and one isolate was DT193. Concerning swine farms, the predominant phage type was DT193 displayed by five isolates, one was DT120 and two were NT (Table 4).

Determination of antimicrobial resistance profiles

The phenotypic antimicrobial resistance profiles of the *Salmonella* serovars isolated from poultry and swine farms in this study are shown in Table 5.

With regard to poultry, 15 of 48 *Salmonella* isolates were fully susceptible to the antimicrobials tested. The antimicrobial to which the greatest percentage of isolates were resistant was sulfamethoxazole (60.4%), followed by tetracycline (58.3%), ciprofloxacin (54.2%), ampicillin (45.8%), chloramphenicol (41.7%) and trimethoprim (37.5%) (Fig. 1). Generally, fewer poultry isolates were resistant to streptomycin (35.4%), florfenicol (31.3%), nalidixic acid (25%), gentamicin (18.8%), kanamycin (10.4%) and colistin (6.3%) (Fig. 1). All poultry *Salmonella* isolates were susceptible to cefotaxime and ceftazidime.

Salmonella spp. were isolated from 22 of 31 rural farms [71%, CI (51.96; 85.78)] and 11 of 15 industrial farms [73.3%, CI (44.90; 92.21)]. Fourteen *Salmonella* serovars were isolated from the swine farms. The five most prevalent serovars were as follows: *S. 4,[5],12:i:-*, *S. Weltevreden*, *S. Panama*, *S. Derby* and *S. Typhimurium* (15.7%, 13.7%, 13.7%, 11.8% and 9.8%, respectively).

The molecular SSA approach could not determine the serovar for all *Salmonella* isolates tested. For one swine isolate, the O antigen assay detected the somatic group D, but it was unable to discriminate between group D₁ and D₂. The H antigen assay detected the flagellar antigens I_v and 1,5, and thus, the molecular antigenic formula of this isolate was indistinguishable from *S. India* and *S. Panama* (Table 3).

Table 5. Characterization of antimicrobial resistance profiles observed among *Salmonella* serovars isolated

Serovars (poultry/swine)	No. of isolates	Resistance profile	No. of antimicrobial classes
<i>S. Apeyeme</i> (1/0), <i>S. Weltevreden</i> (2/0)	3	Su	1
<i>S. Derby</i> (0/1), <i>S. Weltevreden</i> (0/1)	2	T	1
<i>S. Cerro</i> (1/0)	1	CipNal	1
<i>S. Weltevreden</i> (2/2)	4	Tcip	2
<i>S. Rissen</i> (0/1)	1	AsuT	3
<i>S. Weltevreden</i> (1/0)	1	SuTCip	3
<i>S. Derby</i> (1/0)	1	CTCipNal	3
<i>S. 4,[5],12:i:-</i> (1/1)	2	ASSuT	4
<i>S. Enteritidis</i> (3/0)	3	SSuTCol	4
<i>S. Derby</i> (0/1)	1	SuTCFCip	4
<i>S. Albany</i> (0/1)	1	CFSuTmpCipNal	4
<i>S. Kentucky</i> (1/0)	1	ASSuTCip	5
<i>S. Typhimurium</i> (1/1), <i>S. Derby</i> (0/2), <i>S. Bareilly</i> (0/2)	6	ASuTCFCip	5
<i>S. Enteritidis</i> (1/0)	1	ASSuTCipNal	5
<i>S. Typhimurium</i> (1/0)	1	ASuTmpCFCip	5
<i>S. Apeyeme</i> (1/0), <i>S. Kedougou</i> (1/0)	2	ASuTTmpCFCip	6
<i>S. 4,[5],12:i:-</i> (0/1)	1	ASSuTTmpCF	6
<i>S. Derby</i> (0/1)	1	SSuTTmpCFCip	6
<i>S. Albany</i> (1/2), <i>S. Give</i> (1/0),	4	ASuTTmpCFCipNal	6
<i>S. Panama</i> (0/1)	1	SSuTTmpCFGCip	6
<i>S. 4,[5],12:i:-</i> (0/1)	1	ASuTCFGKCipNal	6
<i>S. Typhimurium</i> (0/2)	2	ASSuTCFGKCipNal	6
<i>S. Kedougou</i> (0/1)	1	ASSuTCFGCipNal	6
<i>S. Typhimurium</i> (3/0)	3	ASSuTTmpCGCip	7
<i>S. Javiana</i> (3/0)	3	ASSuTTmpCFCip	7
<i>S. Limete</i> (1/0)	1	ASSuTTmpCGCipNal	7
<i>S. Give</i> (1/0)	1	ASSuTTmpCFCipNal	7
<i>S. Panama</i> (0/6), <i>S. 4,[5],12:i:-</i> (0/1), <i>S. Derby</i> (0/1), <i>S. India/Panama</i> (0/1)	9	ASSuTTmpCFGCip	7
<i>S. Typhimurium</i> (1/0), <i>S. 4,[5],12:i:-</i> (1/0)	2	ASuTTmpCFGKCipNal	7
<i>S. Typhimurium</i> (1/2), <i>S. Javiana</i> (0/1), <i>S. 4,[5],12:i:-</i> (1/4), <i>S. Weltevreden</i> (1/0)	10	ASSuTTmpCFGKCipNal	7
Total <i>n</i> ^o of isolates	71		

Abbreviations: A, ampicillin; C, chloramphenicol; Cip, ciprofloxacin; F, florfenicol; G, gentamicin; K, kanamycin; Nal, nalidixic acid; S, streptomycin; Su, sulphamethoxazole; T, tetracycline; Tmp, trimethoprim.

Among the 51 swine isolates, 13 (25.5%) were fully susceptible to the antimicrobials tested. The antimicrobial to which the greatest percentage of swine isolates were resistant was tetracycline (70.6%), followed by sulfamethoxazole (64.7%), ciprofloxacin (62.7%), chloramphenicol (60.8%), florfenicol (60.8%), ampicillin (58.8%), streptomycin (43.1%), trimethoprim (41.2%) and gentamicin (39.2%). Overall, fewer swine isolates were resistant to nalidixic acid and kanamycin [(25.5% and 17.6%, respectively); Fig. 1]. As observed for poultry isolates, all *Salmonella* isolated from swine were fully susceptible to cefotaxime and ceftazidime. No resistance to colistin was observed.

As far as MDR was concerned, no differences were observed between isolates from rural or industrial farms,

both for poultry or swine species. However, a significantly higher proportion of industrial swine farms (78.6%) harboured *Salmonella* isolates which exhibited MDR than did industrial poultry farms (44%) ($P < 0.05$).

In addition, nine of 19 *S. Weltevreden* isolated in this study were resistant to at least one of the antimicrobials tested. Among these, only one isolate possessed a MDR profile.

Discussion

This study provided data on *Salmonella* prevalence and antibiotic resistance in livestock farms located in the Central area of Vietnam. *Salmonella* spp. were isolated from 46.3% and 72% of poultry and swine farms, respectively.

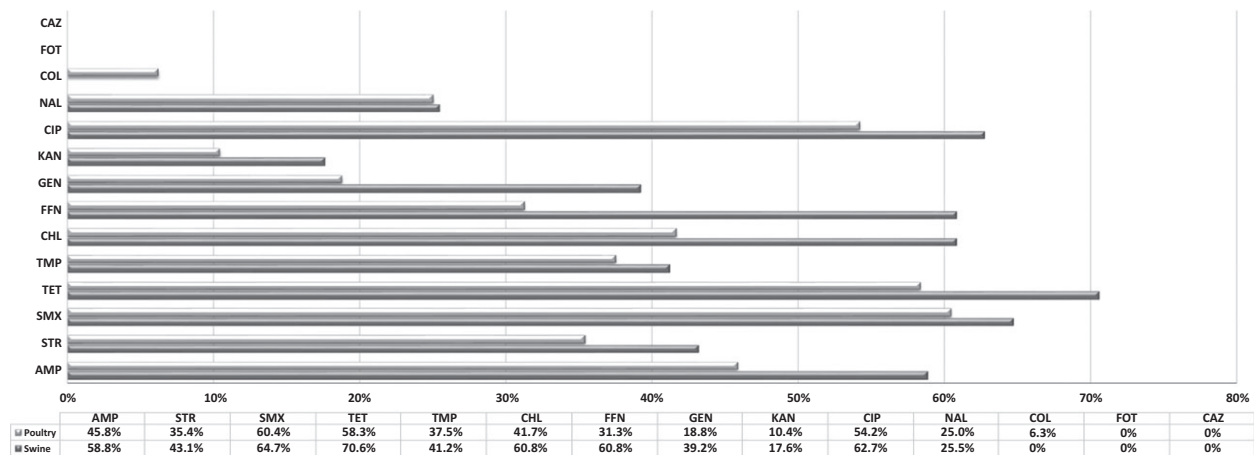


Fig. 1. Antimicrobial resistance rates (%) observed among *Salmonella* isolates from poultry and swine farms.

These prevalences are comparable with those reported in previous studies conducted in Vietnam (Ellerbroek et al., 2010; Ta et al., 2014).

Lack of information about the *Salmonella* serovars circulating in animal populations hampers the implementation of effective control measures (Dorneles et al., 2010).

The patterns of the most common *Salmonella* serovars identified in the present study are quite consistent with previous reports on this source in Vietnam (Tran et al., 2004; Ogasawara et al., 2008). In particular, the prevalence of *S. Weltevreden* (19.1%) is consistent with other reports conducted in South-East Asian countries (Aarestrup et al., 2003; Tran et al., 2004). However, the percentage of antimicrobial resistant *S. Weltevreden* observed in this study (47%) was higher than that of reported in previous studies (less than 10%) (Aarestrup et al., 2003; Huong et al., 2014).

Overall, our data showed a high prevalence of *S. Typhimurium* and *S. 4,[5],12:i:-* both in poultry and swine farms, consistent with trends described in Western countries, as well as in other developing countries in South-East Asia (Barco et al., 2011; Hendriksen et al., 2011; EFSA and ECDC, 2015).

The high percentages of *Salmonella* isolates with antimicrobial resistance to sulfamethoxazole, tetracycline, ampicillin or chloramphenicol overall observed in our study are also comparable to those described in previous Vietnamese reports (Vo et al., 2010; Thai et al., 2012; Huong et al., 2014) and in other Asian countries (Yang et al., 2010).

Conversely, with regard to ciprofloxacin, we stress that a higher percentage of resistant *Salmonella* was detected in our study, both from poultry (54.2%) and swine samples (62.7%), than in other Vietnamese studies (Van et al., 2007; Vo et al., 2010).

Consistent with this evidence, a worldwide reduced susceptibility to fluoroquinolones is occurring (Veldman et al., 2011; Zhang et al., 2014).

Our findings suggest that integrating these data on *Salmonella* epidemiology and dissemination in Central Vietnam will help guide management practices and regulations concerning restrictions on the use of antibiotics critical to human health within poultry and swine farms.

Acknowledgements

This work was funded by the World Organization for Animal Health (OIE) Twinning Project - IZSVe and Central Vietnam Veterinary Institute (CVVI). The authors wish to thank Alberto Chiaretto for his precious support and help in preparing the data, Marta Vettore and Nadia Micoli for their support in the management of the twinning OIE project and Alberto Masiero for his technical support.

References

- Aarestrup, F. M., M. Lertworapreecha, M. C. Evans, A. Bangtrakulnonth, T. Chalermchaikit, R. S. Hendriksen, and H. C. Wegener, 2003: Antimicrobial susceptibility and occurrence of resistance genes among *Salmonella enterica* serovar Weltevreden from different countries. *J. Antimicrob. Chemother.* 52, 715–718.
- Adeyanju, G. T., and O. Ishola, 2014: *Salmonella* and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *Springerplus* 12, 139. doi:10.1186/2193-1801-3-139.
- Anderson, E. S., L. R. Ward, M. J. De Sa, and J. D. H. De Sa, 1977: Bacteriophage-typing designations of *Salmonella* Typhimurium. *J. Hyg. (Lond.)* 78, 297–300.
- Anon., 2007. Detection of *Salmonella* spp. in Animal Feces and in Environmental Samples from the Primary Production Stage (ISO 6579:2002 Annex D). International Organization for Standardization, Geneva, Switzerland.
- Barco, L., A. A. Lettini, E. Ramon, A. Longo, C. Saccardin, M. C. Dalla Pozza, and A. Ricci, 2011: A rapid and

- sensitive method to identify and differentiate *Salmonella enterica* serotype Typhimurium and *Salmonella enterica* serotype 4,[5],12:i:- by combining traditional serotyping and multiplex polymerase chain reaction. *Foodborne Pathog. Dis.* 8, 741–743.
- CLSI - Clinical and Laboratory Standards Institute, 2009: Performances standards for antimicrobial susceptibility testing. 19th Informational Supplement, M100 S19, CLSI, Wayne, PA, USA.
- Dorneles, C., J. M. Correa, S. Mendes, S. Haas, M. L. Tiba, and R. Campanher, 2010: Microbiological analysis of the foods involved in foodborne disease outbreaks occurring in the Rio Grande do Sul State, Brazil. *R. Bras. Biosci.* 8, 44–48.
- Dunbar, S. A., V. B. Ritchie, M. R. Hoffmeyer, G. S. Rana, and H. Zhang, 2015: Luminex[®] multiplex bead suspension arrays for the detection and serotyping of *Salmonella* spp. *Methods Mol. Biol.* 1225, 1–27.
- EFSA and ECDC - European Food Safety Authority and European Centre for Disease Prevention and Control, 2015: The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2013. *EFSA J.* 13, 3991. [162 pp.], doi:10.2903/j.efsa.2015.3991
- Ellerbroek, L., D. Narapati, T. N. Phu, N. Poosaran, R. Pinthong, A. Sirimalaisuwan, P. Tshering, R. Fries, K. H. Zessin, M. Baumann, and A. Schroeter, 2010: Antibiotic resistance in *Salmonella* isolates from imported chicken carcasses in Bhutan and from pig carcasses in Vietnam. *J. Food Prot.* 73, 376–379.
- EUCAST - The European Committee on Antimicrobial Susceptibility Testing, 2015: Breakpoint tables for interpretation of MICs and zone diameters. Version 5.0, 2015. <http://www.eucast.org>
- Fitzgerald, C., M. Collins, S. van Duyn, M. Mikoleit, T. Brown, and P. Fields, 2007: Multiplex, bead-based suspension array for molecular determination of common *Salmonella* serogroups. *J. Clin. Microbiol.* 45, 3323–3334.
- GARP - Global Antibiotic Resistance Partnership, 2009: Global Antibiotic Resistance Partnership-Vietnam Inaugural Meeting September 4–5, 2009, Hanoi, Viet Nam.
- Grimont, P. A., and F. X. Weill, 2007: Antigenic formulae of the *Salmonella* serovars, WHO Collaborating Centre for Reference and Research on *Salmonella*, 9th ed. Institut Pasteur, Paris, France.
- Hendriksen, R. S., A. R. Vieira, S. Karlsmose, D. M. Lo Fo Wong, A. B. Jensen, H. C. Wegener, and F. M. Aarestrup, 2011: Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. *Foodborne Pathog. Dis.* 8, 887–900.
- Huong, L. Q., A. Forslund, H. Madsen, and A. Dalsgaard, 2014: Survival of *Salmonella* spp. and fecal indicator bacteria in Vietnamese biogas digesters receiving pig slurry. *Int. J. Hyg. Environ. Health* 217, 785–795.
- Lai, J., C. Wu, J. Qi, Y. Wang, H. Wang, Y. Liu, and J. Shen, 2014: Serotype distribution and antibiotic resistance of *Salmonella* in food-producing animals in Shandong province of China, 2009 and 2012. *Int. J. Food Microbiol.* 180, 30–38.
- Majowicz, S. E., J. Musto, E. Scallan, F. J. Angulo, M. Kirk, S. J. O'Brien, T. F. Jones, A. Fazil, and R. M. Hoekstra, 2010: International Collaboration on Enteric Disease 'Burden of Illness' Studies. The global burden of non-typhoidal *Salmonella* gastroenteritis. *Clin. Infect. Dis.* 50, 882–889.
- MARD - Ministry of Agriculture and Rural Development 2010: Ministerial Circular No. 20/2010/TT-BNNPTNT of 02 April 2010 on supplement and amend the Circular No. 15/2009/TT-BNN dated 17/3/2009 of the Minister of MARD promulgating the list of drugs, chemicals and antibiotics banned or restricted use.
- Nguyen, K. V., D. N. T. Thi, A. Chandna, T. V. Nguyen, C. V. Pham, P. M. Doan, A. Q. Nguyen, C. K. Thi Nguyen, M. Larson, S. Escalante, B. Olowokure, R. Laxminarayan, H. Gelband, P. Horby, H. B. Thi Ngo, M. T. Hoang, J. Farrar, T. T. Hien, and H. F. Wertheim, 2013: Antibiotic use and resistance in emerging economies: a situation analysis for Viet Nam. *BMC Public Health* 10, 1158. doi:10.1186/1471-2458-13-1158.
- Ogasawara, N., T. P. Tran, T. L. Ly, T. T. Nguyen, T. Iwata, A. T. Okatani, M. Watanabe, T. Taniguchi, Y. Hirota, and H. Hayashidani, 2008: Antimicrobial susceptibilities of *Salmonella* from domestic animals, food and human in the Mekong Delta, Vietnam. *J. Vet. Med. Sci.* 70, 1159–1164.
- Pham, D. K., G. Degand, C. Douny, G. Pierret, P. Delahaut, V. Dinh Ton, B. Granier, and M. L. Scippo, 2013: Preliminary Evaluation of Antimicrobial Residue Levels in Marketed Pork and Chicken Meat in the Red River Delta Region of Vietnam. *Food and Public Health* 3, 267–276.
- Pham, D. K., J. Chu, N. T. Do, F. Brose, G. Degand, P. Delahaut, E. De Pauw, C. Douny, K. V. Nguyen, T. D. Vu, M. L. Scippo, and H. F. Wertheim, 2015: Monitoring Antibiotic Use and Residue in Freshwater Aquaculture for Domestic Use in Vietnam. *EcoHealth* 12, 480–489.
- Rabsch, W., 2007: *Salmonella* Typhimurium phage typing for pathogens. *Methods Mol. Biol.* 394, 177–211.
- Report antibiotics use, 2009: First report on antibiotic use and resistance in Vietnam hospitals in 2008–2009. Available at: http://issuu.com/cddep/docs/vn_report_web (accessed on 27 July 2015).
- Ta, Y. T., T. T. Nguyen, P. B. To, X. da Pham, H. T. Le, G. N. Thi, W. Q. Alali, I. Walls, and M. P. Doyle, 2014: Quantification, serovars, and antibiotic resistance of *Salmonella* isolated from retail raw chicken meat in Vietnam. *J. Food Prot.* 77, 57–66.
- Tabo, D. A., C. D. Diguimbaye, S. A. Granier, F. Moury, A. Brisaibois, R. Elgroud, and Y. Millemann, 2013: Prevalence and antimicrobial resistance of non-typhoidal *Salmonella* serovars isolated from laying hens and broiler chicken farms in N'Djamena, Chad. *Vet. Microbiol.* 166, 293–298.
- Tadesse, G., and E. Z. Gebremedhin, 2015: Prevalence of *Salmonella* in raw animal products in Ethiopia: a meta-analysis. *BMC Res. Notes.* 8, 163.
- Thai, T. H., T. Hirai, N. T. Lan, and R. Yamaguchi, 2012: Antibiotic resistance profiles of *Salmonella* serovars isolated from

- retail pork and chicken meat in North Vietnam. *Int. J. Food Microbiol.* 156, 147–151.
- Tran, T. P., T. L. Ly, T. T. Nguyen, M. Akiba, N. Ogasawara, D. Shinoda, T. A. Okatani, and H. Hayashidani, 2004: Prevalence of *Salmonella* spp. in pigs, chickens and ducks in the Mekong Delta, Vietnam. *J. Vet. Med. Sci.* 66, 1011–1014.
- Tran, T. H., T. T. Nguyen, Q. T. Hoang, T. T. Le, M. T. Lam, and T. H. Nguyen, 2006: Prevalence of *Salmonella* spp. in poultry in Vietnam. *Ann. N. Y. Acad. Sci.* 1081, 266–268.
- Tu, L. T., N. V. Hoang, N. V. Cuong, J. Campbell, J. E. Bryant, N. T. Hoa, B. T. Kiet, C. Thompson, D. T. Duy, V. V. Phat, V. B. Hien, G. Thwaites, S. Baker, and J. J. Carrique-Mas, 2015: High levels of contamination and antimicrobial-resistant non-typhoidal *Salmonella* serovars on pig and poultry farms in the Mekong Delta of Vietnam. *Epidemiol. Infect.* 17, 1–13.
- Van, T. T., G. Moutafis, T. Istivan, L. T. Tran, and P. J. Coloe, 2007: Detection of *Salmonella* spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. *Appl. Environ. Microbiol.* 73, 6885–6890.
- Veldman, K., L. M. Cavaco, D. Mevius, A. Battisti, A. Franco, N. Botteldoorn, M. Bruneau, A. Perrin-Guyomard, T. Cerny, C. De Frutos Escobar, B. Guerra, A. Schroeter, M. Gutierrez, K. Hopkins, A. L. Myllyniemi, M. Sunde, D. Wasyl, and F. M. Aarestrup, 2011: International collaborative study on the occurrence of plasmid-mediated quinolone resistance in *Salmonella enterica* and *Escherichia coli* isolated from animals, humans, food and the environment in 13 European countries. *J. Antimicrob. Chemother.* 66, 1278–1286.
- Vo, A. T., E. van Duijkeren, W. Gaastra, and A. C. Fluit, 2010: Antimicrobial resistance, class 1 integrons, and genomic island 1 in *Salmonella* isolates from Vietnam. *PLoS ONE* 5, e9440.
- WHO - World Health Organization. WHO - World Health Organization: critically important antimicrobials for human medicine, 3rd revision 2011. Available at: http://apps.who.int/iris/bitstream/10665/77376/1/9789241504485_eng.pdf?ua=1 (accessed on 27 July 2015).
- Yang, B., D. Qu, X. Zhang, J. Shen, S. Cui, Y. Shi, M. Xi, M. Sheng, S. Zhi, and J. Meng, 2010: Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China. *Int. J. Food Microbiol.* 141, 63–72.
- Zhang, Z., X. Meng, Y. Wang, X. Xia, X. Wang, M. Xi, J. Meng, X. Shi, D. Wang, and B. Yang, 2014: Presence of *qnr*, *aac(6')*-Ib, *qepA*, *oqxAB*, and mutations in gyrase and topoisomerase in nalidixic acid-resistant *Salmonella* isolates recovered from retail chicken carcasses. *Foodborne Pathog. Dis.* 11, 698–705.