Contamination of pig carcass with *Salmonella enterica* serovar Typhimurium monophasic variant 1,4 [5], 12: i:- has its origin mainly in the live animals

Clara Marín[a*], Mª Carmen Chinillac[a], Laura Montoro-Dasí[a], Sandra Sevilla-Navarro[a,d]., Francisco Marco-Jimenez[b], Marta Cerdà-Cuéllar[c], Teresa Ayats[c], SantiagoVega[a]

[a]Instituto de Ciencias Biomédicas. Departamento de Producción Animal, Sanidad Animal y Ciencia y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad CEU-Cardenal Herrera, 46115 Alfara del Patriarca, Valencia, Spain

[b]Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, 46022, Valencia, Spain

[c]Centre de Recerca en Sanitat Animal (CReSA)-Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Campus de la Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain

[d]Centro de Calidad Avícola y Alimientación Animal de la Comunidad Valenciana, C/Nules, 16, 12539, Alquerias del NP, Castellón, Spain.

* Email: clara.marin@uchceu.es

Abstract

Pork is considered, after eggs, the major source of infection in humans in the EU, with *S*. Typhimurium, including monophasic strains (*S*. 1,4,[5],12:i- and *S*. 1,4,12:i-) being frequently implicated. Widespread distribution of virulent serotypes such as monophasic variants of *S*. Typhimurium (1,4,[5],12:i- and 1,4,12:i-) have emerged as a public health threat, since it is the third most frequently isolated serovar from human cases of salmonellosis in Europe. Monophasic *S*. Typhimurium constitutes a high proportion of the multi-drug-resistant isolates and has been increasing in pigs since 2010. Despite the current situation, within the EU, there is no mandatory programme for the control of *Salmonella* at pork production level. In this context, the control of *Salmonella* carriage and shedding in pig remains a challenge. It is known that the risk of *Salmonella* contamination increases across the production chain, at farm level, transportation from the farm to the slaughterhouse and reaches its maximum at the slaughterhouse and further processing. In this context, the aim of this study was to investigate the relationship between *Salmonella* strains isolated from animals at the slaughterhouse and those isolated from carcass before chilling. During the study a total of 21 pig herds were intensively sampled along processing at the slaughterhouse for *Salmonella* detection. ERIC-PCR was performed among isolates recovered along the different steps of the slaughterhouse to assess the genetic relationship. Then, PFGE was done to study the pulsotypes among the different *Salmonella* serovars isolated. The results of this study showed a high level of *Salmonella* pork batch contamination at the arrival to the slaughterhouse (71.4%) and at the end of the slaughtering process (66.7%), being mST the main serovar isolated from both origins (53.1% and 38.2%, respectively). The slaughter environment poses a potential risk for carcass contamination and it is considered by several authors an important source of *Salmonella* spp. Similarly, this study shows that 14.3% of the strains isolated from carcasses have the same XbaI-PFGE profile as those previously recovered in the slaughterhouse environment, but not in the live animals from that same batch. Moreover, this study demonstrates there is a strong association between the *Salmonella* status of the batch upon arrival to the slaughterhouse and pork carcass contamination. These results highlight the importance of *Salmonella* control during pork production in spite of the lack of a European mandatory programme to control the bacteria.

Key words: pig, *Salmonella*, mST, PFGE, ERIC-PCR, Slaughterhouse

1. Introduction

According to the 2018 EFSA summary report on zoonoses, zoonotic agents and food-borne outbreaks, *Salmonella* was responsible of 24.4% (91,662) of food-borne outbreaks in the European Union (EU) (EFSA, 2018). It is estimated that 4.5% of outbreaks are associated with pig meat and products thereof (EFSA, 2016). Pork is considered, after eggs, the major source of infection in humans in the EU, with *S*. Typhimurium, including monophasic strains (*S*. 1,4,[5],12:i- and *S*. 1,4,12:i-) being frequently implicated (Andres and Davies, 2015; Davies et al., 2016). Nonetheless, no outbreak data has been reported by Spain since the notification of non-typhoidal salmonellosis in humans is voluntary (EFSA, 2016). This is striking since Spain is the second largest swine producer in the EU and fourth worldwide (Marquer et al., 2014). In fact, Spain is among the countries with the highest *Salmonella* prevalence, 36.2% at slaughterhouse, with 31.3% prevalence of monophasic strains of *S*. Typhimurium (EFSA, 2016). Widespread distribution of virulent serotypes such as monophasic variants of *S*. Typhimurium (1,4,[5],12:i- and 1,4,12:i-) have emerged as a public health threat, since it is the third most frequently isolated serovar from human cases of salmonellosis in Europe, representing 8.3% of confirmed human cases in 2015 (EFSA, 2016). Monophasic *S*. Typhimurium constitutes a high proportion of the multi-drug-resistant isolates and has been increasing in pigs since 2010 (EFSA, 2016). Despite the current situation, within the EU, there is no mandatory programme for the control of *Salmonella* at pork production level. In fact, each member state has to consider whether interventions should be set at farm and/or slaughterhouse level (De Busser et al., 2013).

The control of *Salmonella* carriage and shedding in pig remains a challenge (Davies et al., 2016). It is known that the risk of *Salmonella* contamination increases across the production chain, at farm level, transportation from the farm to the slaughterhouse and reaches its maximum at the slaughterhouse and further processing (Duggan et al., 2010; Argüello et al.,2013a,b; Visscher et al., 2011). At the moment, the slaughterhouse remains the most appropriate stage of the food chain for the evaluation of the carriage of *Salmonella* and other zoonotic agents by farm animals, particularly in swine (Bonardi et al., 2013). When animals and the carcass are processed, contamination of pig carcass can result from the skin or intestinal contents from the pig itself, but also due to cross-contamination from other carcasses or surfaces at slaughterhouse (Botteldoorn et al., 2003). *Salmonella* serovars present on pig carcass can be different from those detected in the same batches from the farm (Bonardi et al., 2017). However, many studies have shown that good hygienic practices at slaughter are more effective in reducing the prevalence of *Salmonella* than on-farm interventions (Baptista et al., 2010). Despite all the efforts made during the last 20 years in the control of *Salmonella* in pig production (Andres and Davies, 2015), our driving hypothesis was that the vast majority of *Salmonella* serovars present on pig carcass ready for commercialization have their origin in the same batches on the farm and therefore *Salmonella*

enters to the slaughterhouse mainly with the live animals. Thus, a longitudinal study was conducted to investigate possible relationship between *Salmonella* strains isolated from animals at the slaughterhouse and those isolated from carcass before chilling.

2. Material and methods

All the procedures used in this study were performed in accordance with Directive 2010/63/EU EEC for animal experiments.

Study design

This study was conducted from September 2015 to September 2016 in 8 slaughterhouses from Valencian Region, Eastern Spain. The processing plants selected slaughters the 90% of pork production in the Valencia Region (MAGRAMA, 2016). Samples were collected during 21 sampling visits from 21 batches of pigs. The batch definition used was a group of pigs coming from a single farm in a given day. All farms were finishing farms, with minimum nine-month old with an average live weight of 160 kg.

Sample collection

Upon each sampling visit, pooled faecal material was collected at lairage pens at the slaughterhouse. Thus, faeces samples (≥500 g) were taken aseptically into a sterile jar from five different points distributed all over the pen. Pens were washed and disinfected between batches; therefore, the faeces collected were linked to an individual batch. Overall, 21 batches were studied. From each batch, 5 animals were randomly selected and followed along the processing line. Then, caeca from each individual animal was aseptically collected and placed into a sterile bag. Caeca was incised with a sterile scalpel blade and approximately 50 mL of the contents was placed in a 500 mL sterile jar. Finally, carcass swabs from individual animals were collected at the end of the processing line by swabbing a 100 cm² area at each of the four sampling sites (ham, belly, rump and jowl) rubbing the sterile swab (bioMerieux, Madrid, Spain)10 times in the vertical and the horizontal directions (Mannion et al., 2012).

At the same time, immediately after each individual was processed environmental swabs of the slaughtering staff were collected from three sites (knives, whips and operators) by vigorous swabbing of the surface, using sterile wet swabs (bioMerieux, Madrid, Spain). Moreover, 1 L of scalding water was collected directly into a sterile jar.

Salmonella isolation

Samples were collected directly into sterile sample jars and analyzed according to ISO 6579:2002 (Annex D). Firstly, samples were pre-enriched in 1:10 vol/vol Buffered Peptone Water 2.5% (BPW, Scharlau®, Barcelona, Spain) and then incubated at 37±1 ºC for 18±2 h. The pre-enriched samples were transferred onto Semi-Solid Modified Rappaport Vassiliadis (MSRV, Difco® , Valencia, Spain) agar plates and incubated at 41.5±1 ºC for 24-48 h. Plates showing the typical haze around the inoculation spot on the MSRV plates were subcultured onto Xylose–Lysine–Desoxycholate (XLD, Liofilchem® , Valencia, Spain) and ASAP (Chromogenic *Salmonella* spp. agar plate, bioMerieux, Madrid, Spain) and incubated at 37±1 ºC for 24-48 h. After incubation, 5 presumptive *Salmonella* colonies were streaked onto nutrient agar plates (Scharlab®, Barcelona, Spain) and incubated at 37±1 ºC for 24±3 h. Then, a biochemical test (API-20®, bioMerieux, Madrid, Spain) was performed to confirm *Salmonella* spp. Confirmed *Salmonella* strains were serotyped in accordance with the Kauffman–White–Le–Minor technique (Grimont and Weill, 2007) at the Laboratori Agroalimentari (Cabrils, Spain) of the Departament d'Agricultura,Ramaderia, Pesca i Alimentació.

Molecular typing of *Salmonella* isolates

Two different subtyping methods were carried out for genotyping *Salmonella* isolates. All isolates were first genotyped by enterobacterial repetitive intergenic consensus (ERIC)-PCR, as previously described (Moré et al., 2017). Representative isolates from the different *Salmonella* ERIC-PCR patterns identified per sample were further analysed by pulsed-field gel electrophoresis (PFGE).

PFGE was performed according to the PulseNet standardised protocol “Standard Operating Procedure for PulseNet PFGE of *Escherichia coli* O157:H7, *Escherichia coli* non-O157 (STEC), *Salmonella* serotypes, *Shigella sonnei* and *Shigella flexneri*” (www.pulsenetinternational.org). Restriction endonuclease digestion was carried out using Xbal (Roche Applied Science, Indianapolis, IN, USA).

ERIC and PFGE band patterns were analysed using Fingerprinting II software, v3.0 (Bio-Rad, Hercules, CA, USA). Similarity matrices were calculated with the Dice coefficient and cluster analysis was performed by the unweighted-pair group method with arithmetic mean (UPGMA). The isolates with a minimum level of similarity of 90% were considered genetically similar or identical.

Statistical analysis

A generalized linear model (GLM), which assumed a binomial distribution for *Salmonella* presence, was fitted to the data to determine whether there was an association between sample type

collected (faeces, caeca, carcass, whips, operator and knives) and *Salmonella* status of the batch. A batch was considered infected at their arrival to the slaughterhouse, if at least one of the 5 samples collected from caeca was positive. A batch was consired positive at the end of the processing, if at least one of the 5 samples collected from the carcassess was positive. For this analysis, the error was designated as having a binomial distribution, and the probit link function was used. Binomial data for each sample were assigned a one if they had *Samonella* or a zero if they did not. Moreover, a chi-squared test was ussed to analyse de relationship between sample collected and *Salmonella* serovar isolated. A P-value of less than 0.05 was considered to indicate a statistically significant difference. Data are presented as least squares means ± SE of the least squares means. All statistical analyses were carried out using a commercially available software program (SPSS 16.0; SPSS Inc., Chicago, IL).

3. Results

During this study, a total of 315 samples were collected from different points of the slaughterhouse (Image 1). Samples were collected from the lairage pens (faeces, n=21), scalding water (n=21), whips surfaces (n=21), operators (n=21), working knives (n=21), caeca content (n=105) and carcasses after processing (n=105).

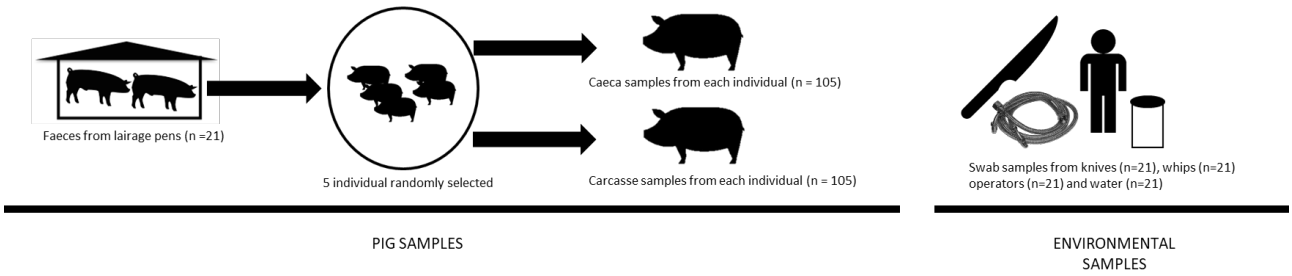

Image 1. Samples taken during the study.

According to the different batches sampled (n=21), 71.4% (n=15) arrived at the slaughterhouse colonized by *Salmonella* spp (caecal content) and the 66.7% (14/21) of carcasses were also contaminated with *Salmonella* spp at the end of processing.

The frequency of *Salmonella* contamination along the different slaughter steps according to the samples collected are summarized in Table 1. From all samples collected at the slaughterhouse 34.0% (107/315) were positive to *Salmonella* spp. The higher prevalence was found in faeces from lairage pens and caecal content (52.4% and 46.7, respectively), followed by whips (38.1%), carcass (32.4%), operator (14.3%) and knives (9.5%).

Table 1. *Salmonella* spp isolated according to the sample type collected and the relationship with monophasic *Salmonella* Typhimurium, the most prevalent serovar isolated

			All *Salmonella* serovars		mST	
Sample type	nt	np	%	SE	%	SE
Individual samples						
Faeces	21	11	52.4[a]	±10.9	45.4 [abc]	±15.0
Caeca	105	49	46.7[a]	±4.9	53.1 [b]	±7.2
Carcass	105	34	32.4[b]	±4.6	38.2 [abc]	±8.2
Environmental samples						
Whips	21	8	38.1 [ab]	±10.6	12.5 [c]	±10.9
Operator	21	3	14.3 [b]	±7.6	66.7 [a]	±36.5
Knives	21	2	9.5 [b]	±6.4	50.0 [abc]	±27.0
Total	**315**	**107**	**34.0**		**44.9**	

nt: total samples collected, np: *Salmonella* spp. positive samples, mST: *Salmonella* Typhimurium monophasic variant, %: percentage of positive samples, SE: Standard error. [a,b,c] Means with different superscripts are statistically different ($p < 0.05$).

Salmonella Typhimurium monophasic variant (mST) was the serovar more frequently isolated in those kind of samples being most frequently contaminated with *Salmonella* (faeces and caeca), (45.2±15.0% and 16.7±7.2%, respectively) (Table 1). Carcass samples showed significantly reduced frequency of positives (32.4±4.6%, P=0.000), but similar rate of mST serovar (38.2±8.2%, P=0.523), compared with faeces and caeca samples. For environmental samples, no significant differences were observed for operator and knife samples, which showed a low proportion of positives (14.3%, P=0.523 and 9.5%, P=0.523, respectively). However, a high percentage of mST was found in both samples (66.7.0±36.5% and 50.0±27.0%, respectively). On the contrary, a relatively high proportion of *Salmonella*-positive samples was observed in whips (38.1±10.6%), but mST frequency was lower (12.5±10.9%).

As reported above, from 107 isolates recovered, the most prevalent *Salmonella* serovar isolated along the slaughter processing was mST (44.9%), followed by serovars Rissen (21.5%), Reading (11.2%), Albona (4.7%), Derby (1.9%), Kedougou and Typhimurium (0.9%) (Table 2). From all strains isolated, 14.0% (15/107) couldn´t be revived and, consequently, weren´t serotyped, the results were expressed as *Salmonella* spp. The results obtained from different serovars related to the sample collected were represented in Table 2.

Table 2. Percentage of each *Salmonella* serovar isolated by sample (excluding mST).

Serovars	nt	%t	Faeces (%)	Caeca (%)	Carcass (%)	Whips (%)	Operator
S. Rissen	23	21.5	8.7	39.1	39.1	13.0	-
S. Reading	12	11.2	8.3	41.7	25.0	8.3	8.3
S. Albona	5	4.7	-	40	60	-	-
S. Derby	2	1.9	-	-	100	-	-
S. Kedougou	1	0.9	-	100	-	-	-
*S*T	1	0.9	-	-	-	100	-
S. spp	15	14.0	20.0	40.0	26.7	13.3	-

S: *Salmonella*. ST: nt= number of isolates from each serovar. %t: Total percentage. *S.* spp: *Salmonella* isolates not serotyped.

To assess the genetic relationship among isolates recovered along the different steps of the slaughterhouse, 107 isolates were typed by ERIC-PCR. Next, 57 different ERIC-PCR profiles were further analyzed by PGFE. The PFGE analysis showed a total of 18 different PFGE pulsotypes among the different serovars (Figure 2). No PFGE pattern could be obtained from 6 isolates. mST and *S.* Rissen, the two most abundant serovars, showed also the highest genetic diversity, with 8 and 5 different pulsotypes, respectively (Figure 2). On the contrary, serovar Reading, the third most frequent serovar, showed a low diversity, with all isolates grouped in a single cluster with the same pulsotype. The remaining serovars (Albona, Derby, Kedougou, Typhimurium) were represented by one or two pulsotypes, including each only one or two isolates.

Isolates of carcass origin were distributed among 9 different pulsotypes, 3 for S. Rissen isolates, 3 for mST, 1 for each of the serovars Albona, Derby and Reading. Isolates of faeces were allocated in 5 different pulsotypes associated with three serovars: mST with 3 pulsotypes, Rissen with 2 and Reading with 1.

Ten pulsotypes (X3, X4, X5, X8, X9, X10, X11, X16, X17, X18) included isolates of faeces, caecal content and/or carcass (Figure 2). Notably, some of them (X4-batch 3, X8-batch 21, X17-batch 2, X18-batch 13) showed carcass strains to have the same Xbal-PFGE pattern as their own animal batch upon arrival to the slaughterhouse (faeces or caecal content isolates). Also, the same strain (pulsotype) was isolated from carcasses and slaughterhouse environment (knives, whips and operator) during processing (same batch), represented by pulsotypes X4, X8, X18 (batches 3, 19, 13, respectively). Similarly, the same pulsotype was found among caecal isolates and the slaughterhouse environment (whips, operator) from the same batch (X5-batch 20, X8-batch 2, X18-batch13). Finally, the same pulsotype was found in carcass isolates and the slaughterhouse environment, but different from their own animal batch. On the contrary, several PFGE patterns obtained from caeca content and animal faeces isolates show several strains not to be

disseminated during the carcass processing, since they were not found in carcasses or in environmental samples.

4. Discussion

This study demonstrated a high level of *Salmonella* pork batch contamination at the arrival to the slaughterhouse (71.4%) and at the end of the slaughtering process (66.7%), being mST the main serovar isolated from both origins (53.1% and 38.2%, respectively). The high level of *Salmonella* spp. obtained can be explained by the lack of a *Salmonella* control programme in pork in Spain (Arguello et al., 2012). Moreover, the results obtained correlate with the previously reported high prevalence of *Salmonella* infection in Spanish pig farms (EFSA, 2018). Pork is considered the second source of *Salmonella* human infection in the EU, with *S*. Typhimurium, including monophasic variants (1,4,[5],12:i- and 1,4,12:i-) being frequently implicated (EFSA, 2018). Noteworthy, mST strains were the most frequent in this study. Currently, monophasic variants of *S.* Typhimurium (1,4,[5],12:i- and 1,4,12:i) have emerged as a public health threat, since it is the third most frequently isolated serovar from human cases of salmonellosis in Europe, representing 7.9% of confirmed food-borne outbreaks. It also constitutes a high proportion of the multi-drug-resistant isolates and has been increasing in pigs since 2010. The international dissemination of 1,4,[5],12:i:- mST in pig populations is likely to be related to the selective advantage offered by multi-drug-resistant strains associated with stable genetic elements, also carrying virulence determinants within bacterial lineages that are well adapted to the porcine host and are prevalent in human infections as a result of contaminated pig meat (EFSA, 2018).

The slaughter environment pose a potential risk for carcass contamination and it is considered by several authors an important source of *Salmonella* spp (Gomes-Neves et al., 2012, Mannion et al., 2012, Arguello et al., 2012, De Busser et al., 2013). Similarly, this study shows that 14.3% of the strains isolated from carcasses have the same XbaI-PFGE profile as those previously recovered in the slaughterhouse environment, but not in the live animals from that same batch (caecal content or lairage pens faeces). This could be explained because *Salmonella* could remain on contaminated equipment and be transferred to other carcasses that are subsequently slaughtered. Moreover, *Salmonella* can also be spread by workers, since the hands and tools of meat handlers can be frequently contaminated. However, cross-contamination at slaughterhouse is easy to control with the implementation of proper measures of hygiene and staff protocols that reduce the impact of the slaughterhouse environment on the carcass contamination. According with the current legislation, these control measures should be registered on the Slaughterhouse Hazard Analysis and Critical Control Points (HACCP) (Hernandez et al., 2012).

On the other hand, this study demonstrates there is a strong association between the *Salmonella* status of the batch upon arrival to the slaughterhouse and pork carcass contamination, as previously reported (Baptista et al., 2010b, Andres and Davies, 2015). In fact, the same strains were isolated from carcasses and from their corresponding animal batch upon their arrival to the slaughterhouse, with a high frequency. Thus, control measures applied on pre-harvest stage (mainly at farm level) would reduce the burden on subsequent steps of the production chain, consequently leading to less-contaminated pork carcasses (Andres and Davies, 2015). *Salmonella* status of the batch at farm can vary depending on several factors, such as feeding practices, including the degree to which the feed is ground, and the pH and type of feed; the management procedures, such as continuous or all-in/all-out production systems; different types of herds (farrow-to-finish herds or fattening herds); size of the herds; as well the level of hygiene and general health status of the pigs (Bonardi, 2017). However, despite all the investments done at farm level during last 20 years to control *Salmonella* spp on pig production, no reduction of the on-farm *Salmonella* prevalence has been shown (EFSA, 2016). This is mainly because, within the EU, there is no mandatory programme for the control of *Salmonella* at pig primary production level as indicated above. For this reason, more studies are needed to develop measures for *Salmonella* control at farm level.

Moreover, the importance of transport and stay in the lairage pens must be studied in depth, because these stages play a double role. On one way, some authors demonstrate the animal transport to the processing plant or long stays in lairage pens increases *Salmonella* prevalence in faeces (Bonardi, 2017). This fact could be explained because a stressful situation could induce the carrier batch to shed *Salmonella* at higher rates because of a disturbance in intestinal functions that may increase the spread of intestinal bacteria in livestock (Mulder, 1995, Marin and Lainez, 2009). Thus, the assessment of *Salmonella* status of the pig batch at the slaughterhouse could be the best option to detect the bacteria and to avoid underestimating the prevalence obtained when samples are collected at farm level (EFSA, 2008, Arguello et al., 2012, EFSA 2016).

Besides, some authors highlight that transport to the slaughterhouse in contaminated trucks or long stays in lairage contaminated pens are of great concern, because *Salmonella* may be introduced into a *Salmonella*-free batch (Hurd et al., 2002, Bonardi, 2017). Despite it is difficult to avoid animal stress in pig production during transport and lairage stay, the role of contaminated trucks and lairage pens is easy to be controlled. This can be achieved with a proper cleaning and disinfection of the truck and the pens between batches according to the current normative implemented in European slaughterhouses (HAAPC), as reported above. The controls set from

slaughterhouses that took part in this study certified that the cleaning and disinfection of the trucks and lairage pens were accurate to remove the bacteria between different batches.

It has been discussed that biosecurity plays a very important role in avoiding the introduction of *Salmonella* and other pathogens and also to limit its spread once it has entered in the production chain (Andres and Davies, 2015). However, there is no universal protocol of biosecurity that all farms can put into place to minimize the risk of disease introduction. Each farm is unique in terms of location, facilities, management, host susceptibility, and other influential factors (Andres and Davies, 2015). Therefore, biosecurity should be a continuous process which assesses the risks, implements protocols according to needs and costs, evaluates the effectiveness, and modifies the procedures as critical areas of risk change (Amass, 2005ab). Thus, it is important to follow the example applied in *Salmonella* control in poultry, which have obtained excellent results at primary production stage, and subsequently, at poultry meat. It is important to emphasize that, unlike poultry production, which is much more homogeneous and integrated in few companies, pig production system is not generally integrated and each farm has its own particularities, being more difficult to apply proper and standardized biosecurity plans to control the bacteria.

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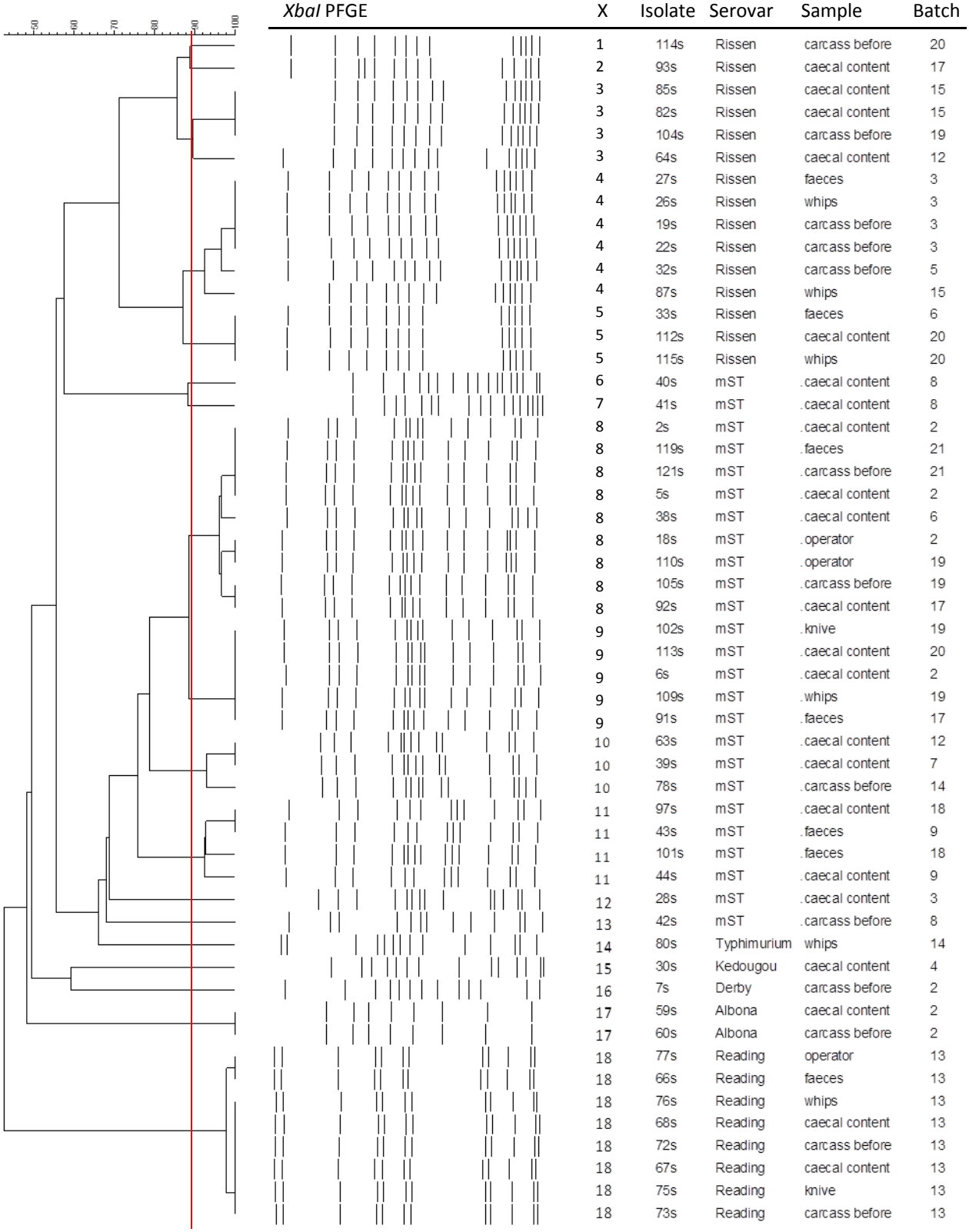
Figure 2. PFGE dendrogram of KpnI profiles of Salmonella spp. isolates. The similarity matrices were calculated using the Dice coefficient and UPGMA clustering method. Profiles with a similarity ≥ 90% were considered same pulsotype. X: pulsotypes.



XbaI PFGE	X	Isolate	Serovar	Sample	Batch
	1	114s	Rissen	carcass before	20
	2	93s	Rissen	caecal content	17
	3	85s	Rissen	caecal content	15
	3	82s	Rissen	caecal content	15
	3	104s	Rissen	carcass before	19
	3	64s	Rissen	caecal content	12
	4	27s	Rissen	faeces	3
	4	26s	Rissen	whips	3
	4	19s	Rissen	carcass before	3
	4	22s	Rissen	carcass before	3
	4	32s	Rissen	carcass before	5
	4	87s	Rissen	whips	15
	5	33s	Rissen	faeces	6
	5	112s	Rissen	caecal content	20
	5	115s	Rissen	whips	20
	6	40s	mST	caecal content	8
	7	41s	mST	caecal content	8
	8	2s	mST	caecal content	2
	8	119s	mST	faeces	21
	8	121s	mST	carcass before	21
	8	5s	mST	caecal content	2
	8	38s	mST	caecal content	6
	8	18s	mST	operator	2
	8	110s	mST	operator	19
	8	105s	mST	carcass before	19
	8	92s	mST	caecal content	17
	9	102s	mST	knive	19
	9	113s	mST	caecal content	20
	9	6s	mST	caecal content	2
	9	109s	mST	whips	19
	9	91s	mST	faeces	17
	10	63s	mST	caecal content	12
	10	39s	mST	caecal content	7
	10	78s	mST	carcass before	14
	11	97s	mST	caecal content	18
	11	43s	mST	faeces	9
	11	101s	mST	faeces	18
	11	44s	mST	caecal content	9
	12	28s	mST	caecal content	3
	13	42s	mST	carcass before	8
	14	80s	Typhimurium	whips	14
	15	30s	Kedougou	caecal content	4
	16	7s	Derby	carcass before	2
	17	59s	Albona	caecal content	2
	17	60s	Albona	carcass before	2
	18	77s	Reading	operator	13
	18	66s	Reading	faeces	13
	18	76s	Reading	whips	13
	18	68s	Reading	caecal content	13
	18	72s	Reading	carcass before	13
	18	67s	Reading	caecal content	13
	18	75s	Reading	knive	13
	18	73s	Reading	carcass before	13

Figure 2