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

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

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

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

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

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

*To whom correspondence should be sent: Instituto de Ciencias Biomédicas. Departamento de Producción Animal, Sanidad Animal, Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad CEU-Cardenal Herrera, 46115 Alfara del Patriarca, Valencia, Spain. Tel. +34-961369000. E-mail: clara.marin@uchceu.es.

Abstract

Pork is considered a major source of *Salmonella* Typhimurium infection in humans in the EU, including monophasic strains. Widespread distribution of virulent serotypes such as monophasic variants of *S.* Typhimurium have emerged as a public health threat. 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 aims of this study were: to examine the presence of *Salmonella* in the swine production system from arrival at the slaughterhouse until the end of processing, and investigate the genetic relationship among the *Salmonella* serovars isolated. During the study, a total of 21 pig herds were intensively sampled during processing at the slaughterhouse. ERIC-PCR was performed among isolates recovered at the different steps in the slaughterhouse to assess the genetic relationship. Then, PFGE was done to study the pulsotypes among the different *Salmonella* serovars isolated. The results showed a high level of *Salmonella* pork batch contamination upon arrival at the slaughterhouse (71.4%) and at the end of the slaughtering process (66.7%), with 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 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. In conclusion, there is a high level of *Salmonella* swine batch contamination upon arrival at the slaughterhouse and at the end of the slaughtering process, mST being the most frequently isolated serovar. Moreover, a strong genetic relationship has been observed between *Salmonella* strains isolated from the batch on arrival at the slaughterhouse, the processing environment and pork carcass contamination. In this sense, it would be necessary to implement a control programme to reduce the bacterium from pork farms and raise the awareness of biosecurity measures.

Keywords: Pork, mST, PFGE, ERIC-PCR, slaughterhouse

54

55

56

57

1. Introduction

According to the 2018 EFSA summary report on zoonoses, zoonotic agents and food-borne outbreaks, *Salmonella* was responsible for 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 (mST, *S*. 1,4,[5],12:i- and *S*. 1,4,12:i-) being frequently implicated (Andres and Davies, 2015; Davies et al., 2016, Campos et al., 2019). Nonetheless, no outbreak data have been reported by Spain, as the notification of non-typhoidal salmonellosis in humans is voluntary (EFSA, 2016). This is striking, as 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, as 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, there is no mandatory programme within the EU 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 swine remains a challenge (Davies et al., 2016; Dang-Xuan et al, 2019). The risk of *Salmonella* contamination is known to increase across the production chain, at farm level and transport from the farm to the slaughterhouse, reaching its maximum at the slaughterhouse and in subsequent processing (Arguello et al.,2013a,b; Duggan et al., 2010; Visscher et al., 2011; Colello et al., 2019). At the moment, the slaughterhouse remains the most appropriate stage of the food chain for 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 the slaughterhouse (Botteldoorn et al., 2003; Pesciaroli et al., 2016). *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., 2010a). 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 commercialisation have their origin in the same batches on the farm, so that *Salmonella* enters the slaughterhouse mainly along with the live animals. Thus, a longitudinal study was conducted to investigate the 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.

2.1 Study design

This study was conducted from September 2015 to September 2016 in 8 slaughterhouses from the Valencian Region, Eastern Spain. The processing plants selected slaughters 90% of the 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 pigs at an average live weight of 160 kg.

2.2 Sample collection

At each sampling visit, pooled faecal material was collected from lairage pens at the

slaughterhouse. 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; the faeces collected were thus linked to an individual batch. Overall, 21 batches were studied. From each batch, five animals were randomly selected and followed along the processing line. Then, the caecum from each individual animal was aseptically collected and placed into a sterile bag. Caeca were incised with a sterile scalpel blade and approximately 50 mL of the contents were 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 vertically and horizontally (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.

2.3 *Salmonella* isolation

Samples were collected directly into sterile sample jars and analysed 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–Deoxycholate (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, five 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

142 Laboratori Agroalimentari (Cabrils, Spain) of the Departament d'Agricultura, Ramaderia, Pesca i
143 Alimentació.
144
145 2.4 Molecular typing of *Salmonella* isolates
146 Two different subtyping methods were carried out for genotyping *Salmonella* isolates. All
147 isolates were first genotyped by enterobacterial repetitive intergenic consensus (ERIC)-PCR, as
148 previously described (Moré et al., 2017). Representative isolates from the different *Salmonella* ERIC-
149 PCR patterns identified per sample were further analysed by pulsed-field gel electrophoresis
150 (PFGE).
151 PFGE was performed according to the PulseNet standardised protocol "Standard Operating
152 Procedure for PulseNet PFGE of *Escherichia coli* O157:H7, *Escherichia coli* non-O157 (STEC),
153 *Salmonella* serotypes, *Shigella sonnei* and *Shigella flexneri*" (www.pulsenetinternational.org).
154 Restriction endonuclease digestion was carried out using Xbal (Roche Applied Science,
155 Indianapolis, IN, USA).
156 ERIC and PFGE band patterns were analysed using Fingerprinting II software, v3.0 (Bio-
157 Rad, Hercules, CA, USA). Similarity matrices were calculated with the Dice coefficient and cluster
158 analysis was performed by the unweighted-pair group method with arithmetic mean (UPGMA). The
159 isolates with a minimum level of similarity of 90% were considered genetically similar or identical
160 and were assigned the same pulsotype.
161
162 2.5 Statistical analysis
163 A generalised linear model (GLM), which assumed a binomial distribution for *Salmonella*
164 presence, was fitted to the data to determine whether there was an association between sample
165 type collected (faeces, caeca, carcass, whips, operator and knives) and *Salmonella* status of the
166 batch. A batch was considered infected upon arrival at the slaughterhouse, if at least one of the five
167 samples collected from caeca was positive. A batch was considered positive at the end of the
168 processing, if at least one of the five samples collected from the carcasses was positive. For this
169 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 *Salmonella* or a zero if they did not. A P-value of less than 0.05 was considered to indicate a statistically significant difference. Data are presented as least squares means ± standard error of the least squares means. All statistical analyses were carried out using a commercially available software program (SPSS 21.0; SPSS Inc., Chicago, IL).

3. Results

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

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

From all samples collected at the slaughterhouse, 34.0% (107/315) were positive for *Salmonella* spp. The frequency of *Salmonella* contamination throughout the different slaughter steps according to the samples collected is summarised in Table 1.

Salmonella Typhimurium monophasic variant (mST) was the serovar most frequently isolated in that kind of samples (Table 1), most frequently being contaminated with *Salmonella* (faeces and caeca). Carcass samples showed significantly reduced frequency of positives (P=0.000), but a similar rate of mST serovar (P=0.523), compared with faeces and caecal samples. For environmental samples, no significant differences were observed for operator and knife samples, which showed a low proportion of positives (P=0.523 and P=0.523, respectively). However, a high percentage of mST was found in both samples. On the contrary, a relatively high proportion of *Salmonella*-positive samples were observed in whips, but the mST frequency was lower.

The frequency of *Salmonella* serovar isolated during the slaughter processing is summarised in Table 2. As reported above, from 107 isolates recovered, the most prevalent *Salmonella* serovar isolated during the slaughter processing was mST, followed by serovars Rissen, Reading, Albona, Derby, Kedougou and Typhimurium. From all strains isolated, 14.0% (15/107) could not be revived and, consequently, were not serotyped; the results were expressed as *Salmonella* spp.

To assess the genetic relationship among isolates recovered at the different steps of the slaughterhouse, 107 isolates were typed by ERIC-PCR. Next, 57 different ERIC-PCR profiles were further analysed by PGFE. The PFGE analysis showed a total of 18 different PFGE pulsotypes among the different serovars (Fig. 2). No PFGE pattern could be obtained from six isolates. mST and *S.* Rissen, the two most abundant serovars, also showed the highest genetic diversity, with 8 and 5 different pulsotypes, respectively (Fig. 2). In contrast, Reading, the third most frequent serovar, showed a low diversity, with all isolates grouped in a single cluster with the same pulsotype (X18). Each of the remaining serovars (Albona, Derby, Kedougou, Typhimurium) was also represented by one pulsotype (X17, X16, X15 and X14, respectively), each including 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 (Fig. 2). Notably, some of them (X4-batch 3, X8-batch 21, X17-batch 2, X18-batch 13) showed carcass strains to have the same XbaI-PFGE pattern as their own animal batch upon arrival at 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 caecal content and animal faeces isolates show several strains not to be disseminated during the carcass processing, as they were not found in carcasses or in environmental samples.

4. Discussion

This study demonstrated a high level of *Salmonella* pork batch contamination upon arrival at the slaughterhouse (71.4%) and at the end of the slaughtering process (66.7%), mST being the main serovar isolated from both sources (53.1% and 38.2%, respectively). The high level of *Salmonella* spp. detected 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). Notably, 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, as 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 swine 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 poses a potential risk for carcass contamination and is considered an important source of *Salmonella* spp. by several authors (Arguello et al., 2012; Gomes-Neves et al., 2012; Mannion et al., 2012; De Busser et al., 2013; Piras et al., 2014; Pesciaroli et al., 2016; Campos et al., 2019). 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

253 faeces). This could be explained because *Salmonella* could remain on contaminated equipment
254 and be transferred to other carcasses that are subsequently slaughtered. Moreover, *Salmonella* can
255 also be spread by workers, as the hands and tools of meat handlers can frequently be contaminated.
256 However, cross-contamination at slaughterhouse is easy to control with the implementation of
257 proper measures of hygiene and staff protocols that reduce the impact of the slaughterhouse
258 environment on carcass contamination (Bonardi, 2017; Campos et al., 2019; Dang-Xuan et al.,
259 2019). According to the current legislation, these control measures should be registered in the
260 Slaughterhouse Hazard Analysis and Critical Control Points (HACCP) (Hernández et al., 2012).
261 On the other hand, this study shows that there is a strong association between the *Salmonella*
262 status of the batch upon arrival at the slaughterhouse and pork carcass contamination, as previously
263 reported (Baptista et al., 2010b; Andres and Davies, 2015). In fact, the same strains were isolated
264 from carcasses and from their corresponding animal batch upon their arrival at the slaughterhouse,
265 with a high frequency. Thus, control measures applied in pre-harvest stage (mainly at farm level)
266 would reduce the burden on subsequent steps of the production chain, consequently leading to
267 less-contaminated pork carcasses (Andres and Davies, 2015). *Salmonella* status of the batch at
268 farm can vary depending on several factors, such as feeding practices, including the degree to
269 which the feed is ground, and the pH and type of feed, the management procedures, such as
270 continuous or all-in/all-out production systems, different types of herds (farrow-to-finish herds or
271 fattening herds), size of the herds and the level of hygiene and general health status of the pigs
272 (Vidic et al., 2015; Bonardi, 2017; Campos et al., 2019). However, despite all the investments made
273 at farm level over the last 20 years to control *Salmonella* spp. in pig production, no reduction of the
274 on-farm *Salmonella* prevalence has been shown (EFSA, 2016). This is mainly because, within the
275 EU, there is no mandatory programme for the control of *Salmonella* at primary swine production
276 level, as indicated above. For this reason, more studies are needed to develop measures for
277 *Salmonella* control at farm level.
278 Moreover, the importance of transport and the stay in the lairage pens must be studied in
279 depth, as these stages play a double role. In one way, some authors demonstrate the animal
280 transport to the processing plant or long stays in lairage pens increases *Salmonella* prevalence in

faeces (Bonardi, 2017, Campos et al., 2019; Dang-Xuan et al., 2019). This fact could be explained because a stressful situation could induce the carrier batch to shed *Salmonella* at higher rates due to a disturbance in intestinal functions that may increase the spread of intestinal bacteria in livestock (Mulder, 1995; Marin and Lainez, 2009; Casanova-Higes et al., 2019). 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).

Moreover, some authors highlight that transport to the slaughterhouse in contaminated trucks or long stays in lairage contaminated pens are of great concern, as *Salmonella* may be introduced into a *Salmonella*-free batch (Hurd et al., 2002; Bonardi, 2017; Schut et al., 2019). Although it is difficult to avoid animal stress in pig production during transport and lairage stay, the role of contaminated trucks and lairage pens can easily be controlled. This can be achieved with proper cleansing and disinfection of the truck and the pens between batches, according to the current standard implemented in European slaughterhouses (HAAPC), as reported above. The controls set out by slaughterhouses that took part in this study certified that the cleaning and disinfection of the trucks and lairage pens were accurate and sufficient to remove the bacteria between different batches.

It has been argued that biosecurity plays a very important role in avoiding the introduction of *Salmonella* and other pathogens and also in limiting its spread once it has entered the production chain (Andres and Davies, 2015). However, there is no universal biosecurity protocol that all farms can put into place to minimise 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; Colello et al., 2019). To this end, it is important to follow the example applied in *Salmonella* control in poultry, which has obtained excellent results at primary production stage, and subsequently in poultry meat. It is important to emphasise that, unlike

poultry production, which is much more homogeneous and integrated in few companies, the swine production system is not generally integrated and each farm has its own particularities, making it more difficult to apply proper and standardised biosecurity plans to control the bacteria.

5. Conclusion

In conclusion, there is a high level of *Salmonella* swine batch contamination upon arrival at the slaughterhouse and at the end of the slaughtering process, mST being the most frequently serovar isolated. Moreover, a strong genetic relationship has been observed between *Salmonella* strains isolated from the batch on arrival at the slaughterhouse, the processing environment and pork carcass contamination. In this sense, it would be necessary to implement a control programme to reduce the bacterium from pork farms and raise awareness of biosecurity measures.

Acknowledgements

We wish to thank the slaughterhouse staff for their collaboration in the study and their technical support. In addition, we want to thank University CEU-UCH for the financial support (Consolidación de Indicadores INDI 18/19 and IDOC 18/12). CERCA Programme from the Generalitat de Catalunya is also acknowledged. The English text version was revised by N. Macowan English Language Service.

References

Amass, S.F, 2005a. Biosecurity: stopping the bugs from getting in. Pig J. 55, 104-14. Available online in: https://thepigsite.com/articles/biosecurity-stopping-the-bugs-from-getting-in-part-1-of-2. Accessed: 17th March, 2017.

Amass, S.F, 2005b. Biosecurity: reducing the spread. Pig J. 56, 78-87.

Andres, V.M., Davies, R.H., 2015. Biosecurity Measures to Control *Salmonella* and Other Infectious Agents in Pig Farms: A Review. Compr. Rev. Food Sci. Food Saf. 14, 317–335. https://doi.org/10.1111/1541-4337.12137.

Arguello, H., Alvarez-Ordoñez, A., Carvajal, A., Rubio, P., Prieto, M., 2013a. Role of Slaughtering in *Salmonella* Spreading and Control in Pork Production. J. Food Prot. 76, 899–911. https://doi.org/10.4315/0362-028x.jfp-12-404.

Arguello, H., Carvajal, A., Collazos, J.A., García-Feliz, C., Rubio, P., 2012. Prevalence and serovars of *Salmonella* enterica on pig carcasses, slaughtered pigs and the environment of four Spanish slaughterhouses. Food Research International 45, 905–912. https://doi.org/10.1016/j.foodres.2011.04.017.

Arguello, H., Carvajal, A., Naharro, G., Arcos, M., Rodicio, M.R., Martin, M.C., Rubio, P, 2013b. Sero- and genotyping of *Salmonella* in slaughter pigs, from farm to cutting plant, with a focus on the slaughter process. Int. J. Food Microbiol. 161, 44–52. https://doi.org/10.1016/j.ijfoodmicro.2012.11.013.

Baptista FM, Alban L, Nielsen LR, Domingos I, Pomba C, Almeida V., 2010a. Use of herd information for predicting *Salmonella* status in pig herds. Zoonoses Public Health. 57, 49–59. https://doi.org/10.1111/j.1863-2378.2010.01354.x.

Baptista, F.M., Dahl, J., Nielsen, L.R., 2010b. Factors influencing *Salmonella* carcass prevalence in Danish pig abattoirs. Prev. Vet. Med. 95, 231–238. https://doi.org/10.1016/j.prevetmed.2010.04.007.

Bonardi, S. 2017. *Salmonella* in the pork production chain and its impact on human health in the European Union. Epidemiol. Infect. 28:1–14. https://doi.org/10.1017/S095026881700036X.

Bonardi, S., Bassi, L., Brindani, F., D'Incau, M., Barco, L., Carra, E., Pongolini, S., 2013. Prevalence, characterization and antimicrobial susceptibility of *Salmonella* enterica and Yersinia enterocolitica in pigs at slaughter in Italy. Int. J. of Food Microbiol. 163, 248–257. https://doi.org/10.1016/j.ijfoodmicro.2013.02.012.

Bonardi, S., Bruini, I., Magnani, R., Cannistrà, N., Brindani, F., 2017. Low Prevalence of *Salmonella* Enterica in Cull Dairy Cattle at Slaughter in Northern Italy. Ital. J. Food Saf. 24, 61–72. https://doi.org/10.4081/ijfs.2017.6172.

Botteldoorn, N., Heyndrickx, M., Rijpens, N., Grijspeerdt, K., Herman, L., 2003. *Salmonella* on pig carcasses: Positive pigs and cross contamination in the slaughterhouse. J. Appl. Microbiol. 95, 891–903. https://doi.org/10.1046/j.1365-2672.2003.02042.x.

Campos, J., Mourão, J., Peixe, L., Antunes, P. 2019. Non-typhoidal *Salmonella* in the Pig Production Chain: A Comprehensive Analysis of Its Impact on Human Health. Pathogens. 8, 19. https://doi.org/10.3390/pathogens8010019.

Casanova-Higes, A., Marín-Alcalá, C.M., Andrés-Barranco, S., Cebollada-Solanas, A., Alvarez, J., Mainar-Jaime, R.C. 2019. Weaned piglets: another factor to be considered for the control of *Salmonella* infection in breeding pig farms. Vet. Res. 50, 45. https://doi.org/10.1186/s13567-019-0666-7.

Colello, R., Ruiz, M.J., Padín, V.M., Rogé, A.D., Leotta, G., Padola, N.L., Etcheverría, A.I. 2018. Detection and Characterization of *Salmonella* Serotypes in the Production Chain of Two Pig Farms in Buenos Aires Province, Argentina. Front. Microbiol. 9, 1370. https://doi.org/10.3389/fmicb.2018.01370.

Dang-Xuan, S., Nguyen-Viet, H., Pham-Duc, P., Unger, F., Tran-Thi, N., Grace, D., Makita, K. 2019. Risk factors associated with *Salmonella* spp. prevalence along smallholder pig value chains in Vietnam. Int. J. Food Microbiol. 290, 105–115. https://doi.org/10.1016/j.ijfoodmicro.2018.09.030.

Davies, R., Gosling, R.J., Wales, A.D., Smith, R.P., 2016. Use of an attenuated live *Salmonella* Typhimurium vaccine on three breeding pig units: A longitudinal observational field study. Comp. Immunol. Microbiol. Infect. Dis. 46, 7–15. https://doi.org/10.1016/j.cimid.2016.03.005.

De Busser, E.V., De Zutter, L., Dewulf, J., Houf, K., Maes, D., 2013. *Salmonella* control in live pigs and at slaughter. Vet. J. 196, 20–27. https://doi.org/10.1016/j.tvjl.2013.01.002.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010. Official Journal of European Union (DOI 20.10.2010). L276/33-79.

Duggan, S.J., Mannion, C., Prendergast,D.M., Leonard, N., Fanning, S., Gonzales-Barron, U., Egan, J., Butler, F., Duffy, G., 2010. Tracking the *Salmonella* Status of Pigs and Pork from

411 Lairage through the Slaughter Process in the Republic of Ireland. J. Food Prot. 73, 2148–2160.
412 https://doi.org/ 10.4315/0362-028x-73.12.2148.
413
414 EFSA (European Food Safety Authority), 2008. Report of the Task Force on Zoonoses Data
415 Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in slaughter
416 pigs, in the EU, 2006-2007 - Part A: *Salmonella* prevalence estimates, 2008. EFSA J. 6, 135r.
417 https://doi.org/10.2903/j.efsa.2008.135r.
418
419 EFSA (European Food Safety Authority), 2016. The European Union summary report on trends
420 and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. EFSA J. 14, 4634.
421 https://doi.org/10.2903/j.efsa.2016.4634
422
423 EFSA (European Food Safety Authority), 2018. The European Union summary report on trends
424 and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA J. 16, 5500.
425 https://doi.org/10.2903/j.efsa.2017.5077
426
427 Gomes-Neves, E., Antunes, P., Tavares, A., Themudo, P., Fonseca-Cardoso, M., Gärtner, F-,
428 Costa, J.M., Peixe, L., 2012. *Salmonella* cross-contamination in swine abattoirs in Portugal:
429 Carcasses, meat and meat handlers. Int. J. Food Microbiol. 157, 82–87.
430 https://doi.org/10.1016/j.ijfoodmicro.2012.04.015.
431
432 Grimont, P., Weill, F.X., 2007. Antigenic formulae of the *Salmonella* servovars. 9th Edition. WHO
433 Collaborating Center for Reference and Research on *Salmonella* (WHOCC-Salm). Institut
434 Pasteur, Paris.
435
436 Hernández, E., Rodriguez, J.L., Herrera-León, S., García, I., de Castro, V., Muniozguren, N.,
437 2012. *Salmonella* Paratyphi B var Java infections associated with exposure to turtles in Bizkaia,

438 Spain, September 2010 to October 2011. Euro Surveill. 17, 20–201.
439 https://doi.org/10.2807/ese.17.25.20201-en.
440
441 Hurd, H.S., McKean, J.D., Griffith, R.W., Wesley, I.V., Rostagno, M.H., 2002. *Salmonella*
442 enterica infections in market swine with and without transport and holding. Appl. Environ.
443 Microbiol. 68, 2376–2381. https://doi.org/10.1128/AEM.68.5.2376-2381.2002.

444 ISO 6579:2002 (Anexo D), 2002. Microbiology of food and animal feeding stuffs. Horizontal
445 method for the detection of *Salmonella* spp. International Organization for Standardization,
446 Geneve, Switzerland.

447 MAGRAMA (Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente).
448 2016.http://www.mapama.gob.es/es/ministerio/servicios/informacion/plataforma-de-
449 conocimiento-para-el-medio-rural-y-pesquero/observatorio-de-tecnologias-probadas/sistemas-
450 prodnut-animal/ganado-porcino.aspx. Accessed: 28th December, 2016.
451
452 Mannion, C., Fanning, J., McLernon, J., Lendrum, L., Gutierrez, M., Sharon Duggan, M.,
453 Egan, J., 2012. The role of transport, lairage and slaughter processes in the dissemination of
454 *Salmonella* spp. in pigs in Ireland. Food Res. Int. 45, 871–879.
455 https://doi.org/10.1016/j.foodres.2011.02.001.
456
457 Marin, C., Lainez, M., 2009. *Salmonella* detection in feces during broiler rearing and after live
458 transport to the slaughterhouse. Poult. Sci. 88, 1999–2005. https://doi.org/10.3382/ps.2009-
459 00040.
460
461 Marquer, P., Rabade, T., Forti, R., 2014. Pig farming in the European Union, considerable
462 variations from one Member State to another. Eurostat Stat. Focus. 15, 1–12.
463 http://ec.europa.eu/eurostat/statisticsexplained/index.php/Pig_farming_sector_-
464 _statistical_portrait_2014. Accessed: 1st March, 2017.

Moré, E., Ayats, T., Ryan, P.G., Naicker, P.R., Keddy, K.H., Gaglio, D., Witteveen, M., Cerdà-Cuellar, M., 2017. Seabirds (Laridae) as a source of Campylobacter spp., *Salmonella* spp. and antimicrobial resistance in South Africa. Environ. Microbiol. 19, 4164-4176. https://doi.org/10.1111/1462-2920.13874.

Mulder, R.W.A.W, 1995. Impact of transport and related stresses on the incidence and extent of human pathogens in pig meat and poultry. J. Food Saf. 15, 239–246. https://doi.org/10.1111/j.1745-4565.1995.tb00136.x.

Pesciaroli, M., Cucco, L., De Luca, S., Massacci, F.R., Maresca, C., Medici, L., Paniccià, M., Scoccia, E., Staffolani, M., Pezzotti, G., Magistrali, C.F. 2017. Association between pigs with high caecal *Salmonella* loads and carcass contamination. Int J Food Microbiol. 242, 82–86. https://doi.org/10.1016/j.ijfoodmicro.2016.11.021.

Piras, F., Fois, F., Mazza, R., Putzolu, M., Delogu, M.L., Lochi, P.G., Pani, S.P., Mazzette, R. 2014. *Salmonella* Prevalence and Microbiological Contamination of Pig Carcasses and Slaughterhouse Environment. Ital J Food Saf. 3, 4581. https://doi.org/10.4081/ijfs.2014.4581.

Schut, C.H., Farzan, A., Ainslie-Garcia, M.H., Friendship, R.M., Lillie, B.N. 2019. Antibody Responses to *Salmonella* in Pigs from Weaning Up to Marketing and Presence of *Salmonella* at Slaughter. Foodborne Pathog Dis. 16, 187–194. https://doi.org/10.1089/fpd.2018.2454.

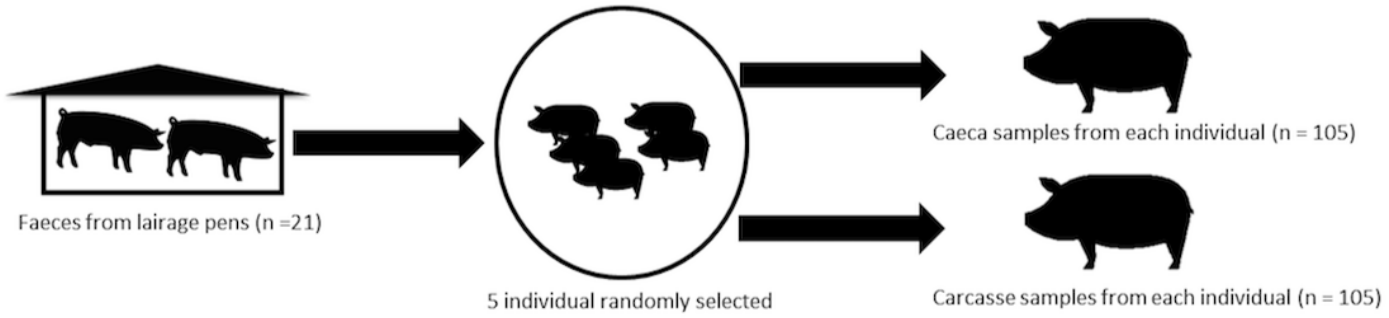
Vidić, B., Savic, S., Prica, N.B., Suvajdžić, L. 2015. Epizootiology and Control Measures for *Salmonella* in Pigs. Procedia Food Sci. 5, 312–315. https://doi.org/10.1016/j.profoo.2015.09.067.

Visscher, C.F., Klein, G., Verspohl, J., Beyerbach, M., Stratmann-Selke, J., Kamphues, J., 2011. Serodiversity and serological as well as cultural distribution of *Salmonella* on farms and in abattoirs in Lower Saxony, Germany. Int. J. Food Microbiol. 146, 44–51. https://doi.org/10.1016/j.ijfoodmicro.2011.01.038.

Figure Legends

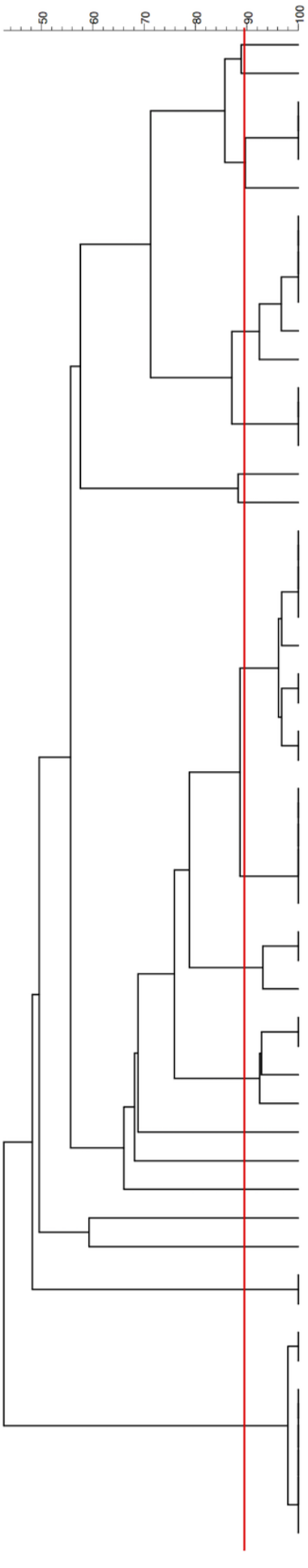
Fig 1. Samples taken during the study.

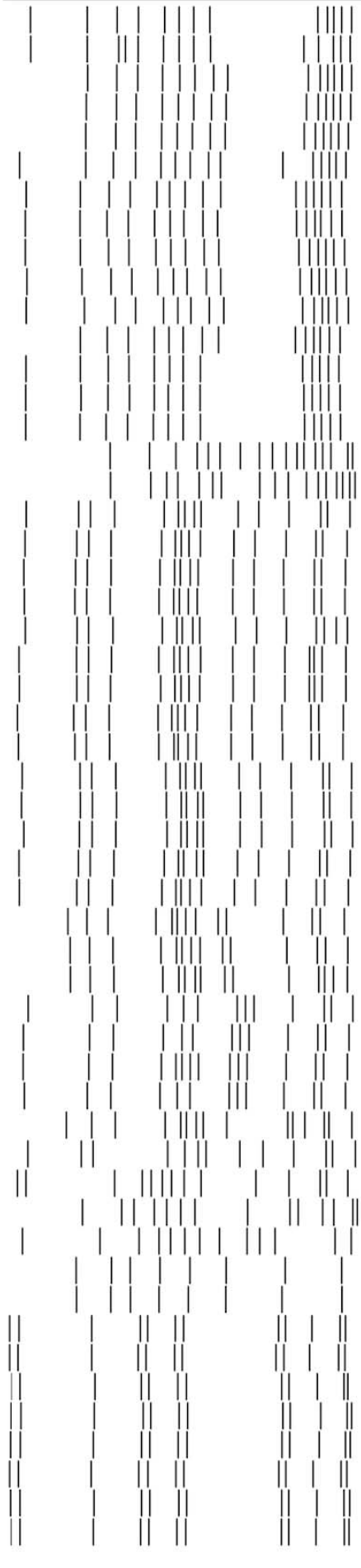
Fig 2. PFGE dendrogram of XbaI profiles of *Salmonella* spp. isolates. The similarity matrices were calculated using the Dice coefficient and UPGMA clustering method. Profiles with a similarity ≥ 90% were considered the same pulsotype. X: pulsotypes.



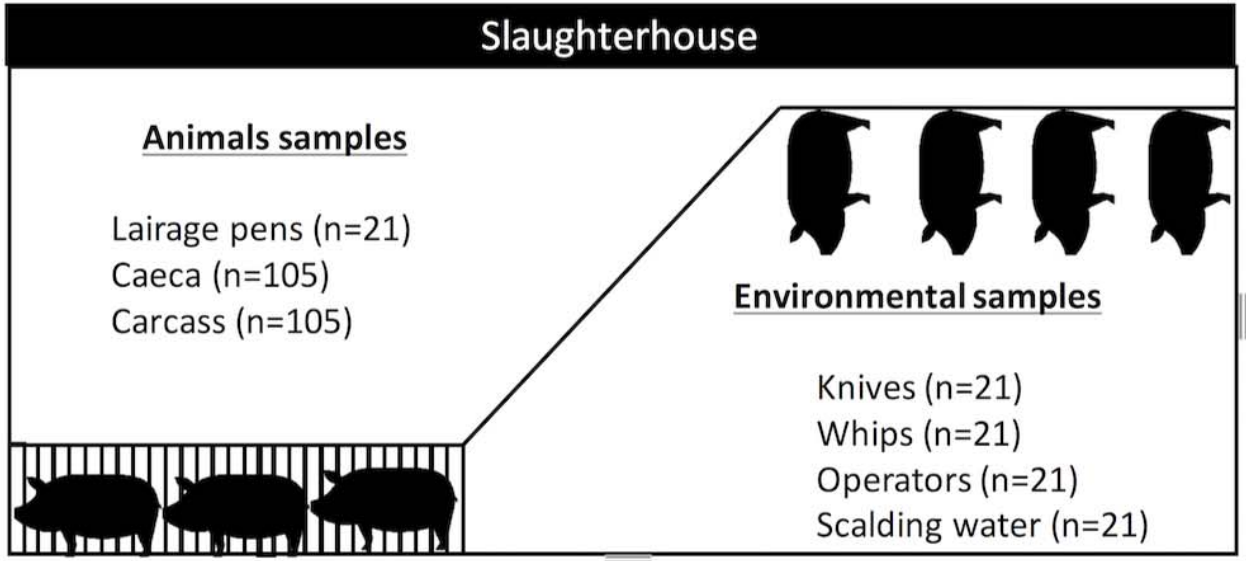
PIG SAMPLES

ENVIRONMENTAL SAMPLES





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



Sample type	n	*Salmonella* (%)	
		All serovars	Typhimurium monophasic variant
Faeces	21	52.4±10.9^{a}	45.4±15.0^{abc}
Caeca	105	46.7±4.9^{a}	53.1±7.2^{b}
Carcass	105	32.4±4.6^{b}	38.2±8.2^{abc}
Whips	21	38.1±10.6^{ab}	12.5±10.9^{c}
Operator	21	14.3±7.6^{b}	66.7±36.5^{a}
Knives	21	9.5±6.4^{b}	50.0±27.0^{abc}

^{a,b,c} superscript: Data in the same column with uncommon letters are different (P <0.05)

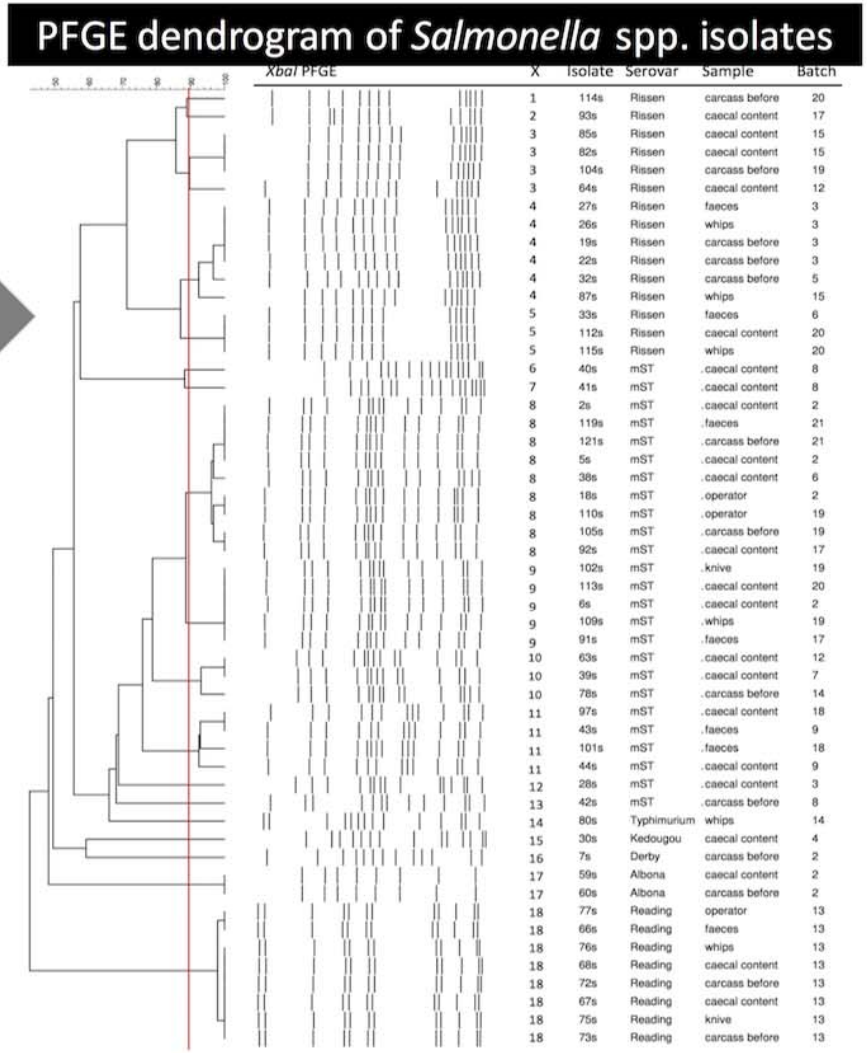


Table 1.
Salmonella spp. isolated according to the sample type collected and the relationship with monophasic *Salmonella* Typhimurium, the most prevalent serovar isolated. Data are presented as least squares means ± standard error of the least squares means.

Sample type		n	All *Salmonella* serovars (%)	mST (%)
Animal samples	**Faeces**	21	52.4±10.9^{a}	45.4±15.0^{abc}
	Caeca	105	46.7±4.9^{a}	53.1±7.2^{b}
	Carcass	105	32.4±4.6^{b}	38.2±8.2^{abc}
Environmental samples	**Whips**	21	38.1±10.6^{ab}	12.5±10.9^{c}
	Operator	21	14.3±7.6^{b}	66.7±6.5^{a}
	Knives	21	9.5±6.4^{b}	50.0±27.0^{abc}

n: total samples collected, mST: *Salmonella* Typhimurium monophasic variant. ^{a,b,c} superscript: Data in the same column with uncommon letters are different ($P < 0.05$).

Table 2.

Percentage of each *Salmonella* serovar isolated by sample type.

***Salmonella* serovars**	**n**	**Total (%)**	**Sample type (%)**					
			Animal samples			**Environmental samples**		
			Faeces	**Caeca**	**Carcass**	**Whips**	**Operator**	**Knives**
mST	48	44.9						
Rissen	23	21.5	8.7	39.1	39.1	13.0	-	-
Reading	12	11.2	8.3	41.7	25.0	8.3	8.3	8.3
Albona	5	4.7	-	40	60	-	-	-
Derby	2	1.9	-	-	100	-	-	-
Kedougou	1	0.9	-	100	-	-	-	-
Typhimurium	1	0.9	-	-	-	100	-	-
NA	15	14.0	20.0	40.0	26.7	13.3	-	-
All *Salmonella* spp.	107	34.0						

n= number of isolates from each serovar. NA: isolates not serotyped.