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# Experimental infection of pigs by *Salmonella* Derby, *S.* Typhimurium and monophasic variant of *S.* Typhimurium: Comparison of colonization and serology

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ABSTRACT

*Salmonella* serovars Derby, Typhimurium and the monophasic variant of *Salmonella* Typhimurium are the most frequently isolated serovars in pigs in France. To compare the excretion patterns, seroconversion to *Salmonella* and contamination of the organs of pigs inoculated with strains of all three serovars, we conducted an experimental trial with 28 SPF piglets. Four were used as a negative control, while the other 24 were divided equally into three groups. Each group was inoculated at 7 weeks of age with a different strain: *S.* Derby (SDb), *S.* Typhimurium (ST), and the monophasic variant of *S.* Typhimurium (mST). Fecal and blood samples were collected twice a week up until necropsy, on 21 days post-inoculation (DPI) for half of each group and 49 DPI for the remaining piglets. During necropsy, the tonsils, mesenteric lymph nodes and various intestinal contents were collected from each pig. *Salmonella* bacteria were quantified in CFU/g by a bacteriological method, and levels of *Salmonella* antibodies were measured using an ELISA Kit. Piglets inoculated with mST continuously excreted *Salmonella* in their feces throughout the trial. For each of the other serovars, one piglet was *Salmonella*-negative on one DPI. The quantity of *Salmonella* excreted was statistically different between the group inoculated with ST and mST ($p < 0.05$), but no differences were found between the other serovars. The tonsils, cecum and jejunum were the most contaminated organs in all groups. Seroconversion for all the piglets was completed by different DPI: 28 for ST, 31 for mST and 38 for SDb. No major differences were found in terms of excretion and colonization among the studied serovars.

## 1. Introduction

*Salmonella* (or *S. enterica,* subspecies *enterica*) is responsible for human and/or animal salmonellosis and is one of the most broadly-distributed foodborne pathogens worldwide. In Europe, it is the second-ranked cause of zoonosis after *Campylobacter sp.*, with a notification rate of 21.2 cases per 100,000 population (EFSA and ECDC, 2016). In France, *Campylobacter* spp., *Salmonella* spp., and norovirus were responsible for 73% of all foodborne illnesses and 76% of all associated hospitalizations (Van Cauteren et al., 2017). *S.* Typhimurium is recognized as the predominant serovar isolated from humans in Europe and *S.* Derby was one of the ten serovars most frequently isolated from humans in different countries (Boyen et al., 2008; EFSA and ECDC, 2016). The monophasic variant of *Salmonella* Typhimurium is an emerging serovar in human infections around the world (Switt et al., 2009; Weaver et al., 2017). Source attribution studies have established that pork has a key role in foodborne outbreaks of human salmonellosis, because in several investigations many isolates detected in pigs were responsible for these human cases (Bonardi, 2017). Pigs can be infected by several *Salmonella* serovars and the occurrence of these serovars is also partly geographically-determined (Boyen et al., 2008; Xu et al., 2017). *S.* Typhimurium, *S.* Derby, and the monophasic variant of *S.* Typhimurium are nowadays the most frequently isolated serovars from swine and pork products in the European Union (Bonardi, 2017; EFSA and ECDC, 2016; Gosling et al., 2018). In France, *S.* Derby and *S.* Typhimurium are also the most frequently isolated serovars in pig production (Denis et al., 2013) and, in the last ten years, the French *Salmonella* Network has observed an increase in the diagnosis of

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monophasic serovars in animal feed and environmental sources (Bugarel et al., 2012b).

Differences in the excretion and immune response in pigs have been reported between *Salmonella* serovars (Ivanek et al., 2012; Osterberg et al., 2010), but these studies have never included the monophasic variant of *S.* Typhimurium.

While all three serovars are present in pork products prior to ingestion, serovar Typhimurium and its monophasic variant are more widely implicated in human salmonellosis. In addition, the infection produced by *S.* Derby seems to be less severe than that produced by *S.* Typhimurium (Ivanek et al., 2012; Matiasovic et al., 2014; Osterberg et al., 2009). To better understand the duration and dynamics of fecal shedding and seroconversion after infection with these three pig-associated serovars, we carried out an experimental trial, which was conducted with the same parameters as those previously used for an experimental trial on monophasic *S.* Typhimurium (Cevallos-Almeida et al., 2018).

## 2. Materials and methods

### 2.1. Strains

Three strains of *Salmonella* from the ANSES laboratory's private collection were used to inoculate the piglets. The strains had been isolated in pigs' fecal samples at farm level or in their mesenteric lymph nodes at the slaughterhouse (Table 1). To ensure that strains could be easily enumerated during the trial, they were made resistant to Rifampicin (Rif) after several passages on XLD agar (Biokar, France) supplemented with Rifampicin (100 mg/lt, Sigma, France). Rif strains were checked to confirm similarity to parental strains using conventional serotyping, PFGE typing (Ribot et al., 2006) and antimicrobial susceptibility testing (data not shown).

### 2.2. Experimental design

This in vivo trial protocol was approved by the Ethics Committee on Animal Research No. 16, of the French Ministry of National Education, Higher Education and Research (APAFiS license no. 7689-2016112311346069). The trial was conducted on 28 secondary specific-pathogen-free (SPF) Large White piglets born at ANSES Ploufragan's protected animal facilities. The secondary SPF piglets are naturally born from SPF sows controlled for presence of several pathogens (Le Devendec et al., 2018).

At 4 weeks of age, three weeks before inoculation, the piglets were weaned and placed in separate hermetic experimental animal rooms of biosecurity level 3. The 24 piglets intended to be inoculated were placed in three separate rooms, with two pens per room (eight piglets per room, four per pen). Each room was considered a group. In Groups SDb, ST, and mST, piglets were inoculated by *S.* Derby, *S.* Typhimurium and the monophasic variant of *S.* Typhimurium respectively.

Staff at the animal facility complied with all the necessary biosecurity measures. The piglets were monitored daily; their rectal temperature was taken and any clinical manifestations were recorded. Food consumption and body weight were reported weekly.

### 2.3. Experimental inoculation

Seven days before inoculation, the 28 piglets were tested and confirmed to be free of *Salmonella.* Fecal samples from each piglet were analyzed in accordance with the NF U47-102 method (AFNOR, 2008). On day 0, the 24 piglets of seven weeks of age were inoculated as previously described for an earlier experimental study (Cevallos-Almeida et al., 2018). The inoculum were prepared after an overnight culture of the three strains at 37 °C. Cultures were adjusted in Tryptone Salt Broth (SLT) (bioMérieux, France), after a DO measurement, in order to obtain an inoculum of about $10^8$ CFU/ml ($8Log_{10}$CFU/ml). After numeration, inoculum concentrations were 8.23, 8.40, 8.19 $Log_{10}$CFU/ml for SDb, STM and vmST, respectively. Then, piglets were inoculated orally, using a cannula connected to a screw syringe, with 10 ml of the inoculum. The four control piglets were given 10 ml of SLT.

### 2.4. Sampling and necropsy

Fecal samples (at least 30 g) were taken directly from the animal rectum on 1, 2 and 3 DPI in the first week, and then twice a week until 49 DPI.

To determine seroconversion, blood samples were taken on the same dates as fecal samples. The blood was then centrifuged at 3500 rpm for 5 min to recover the serum in order to determine anti-*Salmonella* antibody levels. These sera were stored at −20 °C until they were analyzed.

The three groups were necropsied at different ages: 21 DPI for 14 piglets (four in each group) and 49 DPI for the remaining piglets. The four control piglets were kept in a separate room and also slaughtered at different ages: two piglets on 21 DPI and two piglets on 49 DPI. Piglets were euthanized through intravenous injection with an overdose of tiletamine and zolazepam (Zoletil 100, Virbac, France). The subsequent necropsies primarily examined organs and tissues in the abdominal and thoracic cavities. The tonsils, mesenteric lymph nodes (MLN) and intestinal contents of the duodenum, jejunum, ileum and cecum were collected.

### 2.5. Enumeration and detection of Salmonella

All the analyses were performed as previously described (Cevallos-Almeida et al., 2018). Briefly, fecal and necropsy samples were diluted 1:10 in Buffered Peptone Water (BPW, bioMérieux, France). Serial dilution was then performed in the SLT until a dilution of $10^{-4}$ was obtained. One milliliter of the $10^{-1}$ dilution was seeded on three plates of XLD supplemented with rifampicin (XLD-Rif), and 100 µl of each of the subsequent dilutions was plated on separate plates of XLD-Rif. After incubation at 37 °C for 24 h, typical black colonies of *Salmonella* were counted to enumerate *Salmonella* in terms of CFU/g for each sample. If enumerations for *Salmonella* sp. were negative due to being below the enumeration limit (10 CFU/g), the NF U47-102 method (AFNOR, 2008) was used to enrich the sample so as to confirm the presence or absence of *Salmonella* in the sample. This method was also applied to the samples collected from control piglets throughout the assay in order to confirm the sustained absence of *Salmonella*.

**Table 1**
Information on the three *Salmonella* strains used for the experimental design.

| Serovar (Group) | Strain N° | Year of isolation | Source/Production stage | Resistance profile (MIC)[b] | MLVA Type[c] |
|---|---|---|---|---|---|
| *S.* Derby **(SDb)** | 07CR553 | 2007 | MLN[a]/fattening | **Tetracyclin/Spectinomycin** | ST-40 |
| *S.* Typhimurium **(ST)** | 07CR095 | 2007 | MLN/fattening | **None** | 2-10-5-N-112 |
| monophasic variant of *S.* Typhimurium **(mST)** | S12AK050 | 2012 | Feces/fattening | **Tetracyclin** | 3-12-8-N-211 |

[a] MLN: mesenteric lymph node.
[b] Minimal inhibitory concentrations (MIC) of antibiotics were determined for all strains using the broth dilution method with a customing Sensititre® plates (Trek diagnostic, Biocentric, France).
[c] For MLVA, the 5-loci MLVA allele profiles were determined according to the method described by Larsson et al. (2009).

### 2.6. Antibody response to Salmonella

For antibody screening, we used the IDEXX Swine *Salmonella Ab* Test® (IDEXX, France). This indirect ELISA is based on detecting the *Salmonella* lipopolysaccharide (LPS) antigen. In accordance with the manufacturer's recommendations, samples with an optical density (OD) equal to or greater than 15% were regarded as positive.

### 2.7. Data management and statistical analysis

Statistical analysis was performed using R software (R version 3.2.4). The bacterial excretion level was compared between groups for each sampling point post-inoculation with a Tukey test ($p < 0.05$). The CFU/g in feces from each sampling point was normalized logarithmically and plotted in order to calculate the cumulative area under the log curve (AULC) to determine total *Salmonella* shedding for each piglet over the course of the study. A Kruskal-Wallis test was used to compare the AULC between groups ($p < 0.05$). For the data obtained from necropsy, we compared the contamination levels of each group at the two dates of necropsy with a Kruskal-Wallis Test ($p < 0.05$).

## 3. Results

### 3.1. Clinical monitoring

No clinical signs were found in the control piglets. The clinical signs displayed by the inoculated piglets differed depending on the strain inoculated. Transitory diarrhea was found in all groups at least once: for Group SDb on 14 DPI, for Group ST on 7 DPI and 10 DPI, and for Group mST on 21 DPI. For Group mST, no fever was observed at any point during the trial. Furthermore, we found no statistical difference ($p > 0.05$) between inoculated piglets and the control group in terms of weight or feed consumption.

### 3.2. Excretion in fecal samples

The control piglets remained negative for *Salmonella* in their feces throughout the trial. In Group mST, all the piglets were positive on all sampling days, and fecal excretion was continuous. For Group SDb, one piglet was negative on the day 21 after inoculation (Fig. 2). In Group ST, two piglets were negative; for one on the day 14 after inoculation (Fig. 2) and, another one, on the day 45 after inoculation.

Mean excretion and standard deviation data for all groups are presented in Table 2.

We found a statistical difference by the Holm multiple comparison test ($p < 0.05$) between the average excretion levels obtained in the groups on 14, 17 and 21 DPI (Fig. 1). On 14 DPI, Group SDb differed from Group ST; on 17 DPI, Group SDb differed from Group mST; and on 21 DPI, Group mST differed from Groups SDb and ST.

The inoculated piglets in Groups ST and mST euthanized on 21 DPI showed an excretion peak on 1 DPI, with a variation in levels during the trial. Group SDb also showed a high excretion level on 1 DPI but the peak was on 14 DPI, linked to a case of diarrhea the same day (Fig. 2).

By comparing AULC measurements from the excretion curves of the 24 piglets over 21 DPI using the Kruskal-Wallis test, a significant difference ($p = 0.008$) was evident between Group ST and Group mST (Fig. 3).

### 3.3. Salmonella colonization in organs and intestinal contents

At each necropsy date, 100% of the cecum content and tonsil samples were colonized in every group. Between 25% and 75% of mesenteric lymph nodes were colonized depending on the serovar and the necropsy date. For all serovars, contamination levels in intestinal contents increased through the intestinal passage up until the ileum, then began to decrease in the cecum and fecal samples. For all serovars, the lowest levels of contamination were found in the duodenum (0.7–1.3 $Log_{10}$CFU/g) and mesenteric lymph nodes (0.7–2.3 $Log_{10}$CFU/g), while the tonsils were the most significantly contaminated ($p < 0.01$) on both necropsy days, 21 and 49 DPI (4.7–5.7 $Log_{10}$CFU/g) (Table 3). On the basis of the analysis performed at necropsy, we found no statistical difference between serovars, regardless of the type of sample, either on day 21 or on day 49 ($p > 0.05$).

### 3.4. Antibody responses

The OD% means per day are shown in Table 1. Piglets produced detectable antibodies to the *Salmonella* LPS antigen, indicating detectable seroconversion, on 7 DPI for piglets in Groups SDb and mST, and 10 DPI for piglets in Group ST. The seroconversion of 100% of piglets was completed by different dates depending on the serovar: all piglets in Group ST had seroconverted by 28 DPI, piglets in Group mST by 31 DPI, and piglets in Group SDb by 38 DPI.

On 1 and 3 DPI, the bacterial excretion frequencies and levels were high in each group (Fig. 4) and the frequency of seroconverting piglets started to increase in all groups from day 7. On day 49, 100% of piglets

**Table 2**
Average (Mean) and standard deviation (SD) of fecal excretion (in $log_{10}$ CFU/g) calculated for *Salmonella*-positive piglets and Average (Mean) and standard deviation (SD) of serology results (in OD%) for each sampling day and for all serovars.

| Group | | DPI | −7 | 1 | 2 | 3 | 7 | 10 | 14 | 17 | 21 | 24 | 28 | 31 | 35 | 38 | 42 | 45 | 49 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SDb | Fecal Excretion | Mean | 0.0 | 3.5 | 2.6 | 2.2 | 2.4 | 2.3 | 3.7 | 3.2 | 1.6 | 2.0 | 1.7 | 1.2 | 1.1 | 1.1 | 1.8 | 1.6 | 1.7 |
| | | SD | 0.0 | 1.1 | 1.0 | 0.6 | 0.6 | 0.4 | 0.6 | 0.5 | 0.9 | 0.5 | 0.7 | 0.4 | 0.6 | 0.6 | 0.3 | 0.4 | 0.6 |
| | | n/N | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 7/8 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 |
| | Serology | Mean | 0.1 | 0.0 | – | 0.3 | 13.9 | 23.0 | 20.5 | 13.3 | 18.9 | 40.1 | 45.5 | 41.7 | 41.6 | 53.9 | 58.1 | 63.5 | 70.3 |
| | | SD | 0.2 | 0.0 | – | 0.5 | 22.7 | 22.8 | 22.9 | 15.8 | 23.1 | 37.9 | 32.8 | 33.9 | 29.3 | 36.0 | 36.7 | 35.7 | 35.3 |
| | | N | 8 | 8 | 0 | 8 | 8 | 8 | 8 | 8 | 8 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| ST | Fecal Excretion | Mean | 0.0 | 3.7 | 3.0 | 2.7 | 1.2 | 2.4 | 1.7 | 1.7 | 1.8 | 1.8 | 1.8 | 1.6 | 1.2 | 1.1 | 1.7 | 2 | 1.3 |
| | | SD | 0.0 | 1.2 | 1.4 | 0.4 | 1.0 | 0.7 | 1.0 | 0.8 | 1.0 | 0.9 | 0.8 | 0.9 | 0.5 | 0.5 | 1.0 | 0.6 | 0.7 |
| | | n/N | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 7/8 | 8/8 | 8/8 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 3/4 | 4/4 |
| | Serology | Mean | 0.0 | 0.0 | – | 0.1 | 4.7 | 9.0 | 9.1 | 8.5 | 15.6 | 21.0 | 33.7 | 28.9 | 38.9 | 46.5 | 46.9 | 54.2 | 63.7 |
| | | S.D. | 0.0 | 0.0 | – | 0.2 | 5.9 | 10.2 | 12.8 | 5.7 | 7.6 | 11.6 | 21.9 | 11.3 | 16.6 | 12.6 | 13.2 | 15.9 | 23.0 |
| | | N | 8 | 8 | 0 | 8 | 8 | 8 | 8 | 8 | 8 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| mST | *Fecal Excretion* | Mean | 0.0 | 4.2 | 3.7 | 3.2 | 2.8 | 3.3 | 2.3 | 2.0 | 3.4 | 2.0 | 1.8 | 2.3 | 2.2 | 1.8 | 2.3 | 2.1 | 2.2 |
| | | SD | 0.0 | 1.7 | 0.8 | 0.4 | 0.8 | 0.9 | 0.6 | 0.7 | 0.9 | 0.9 | 0.4 | 0.6 | 0.3 | 0.4 | 0.3 | 0.8 | 0.3 |
| | | n/N | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 |
| | Serology | Mean | 0.0 | 0.0 | – | 0.0 | 7.3 | 10.1 | 6.0 | 12.3 | 20.5 | 12.5 | 16.7 | 29.0 | 51.8 | 73.0 | 76.8 | 84.0 | 82.4 |
| | | SD | 0.0 | 0.0 | – | 0.0 | 14.2 | 18.8 | 9.1 | 14.2 | 34.7 | 7.0 | 11.4 | 16.6 | 20.2 | 24.5 | 21.2 | 17.5 | 20.2 |
| | | N | 8 | 8 | 0 | 8 | 8 | 8 | 8 | 8 | 8 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

N: number of sampled piglets for each day of sampling (D-7 to D49); n: number of *Salmonella*-positive piglets. CFU: Colony-Forming Units.

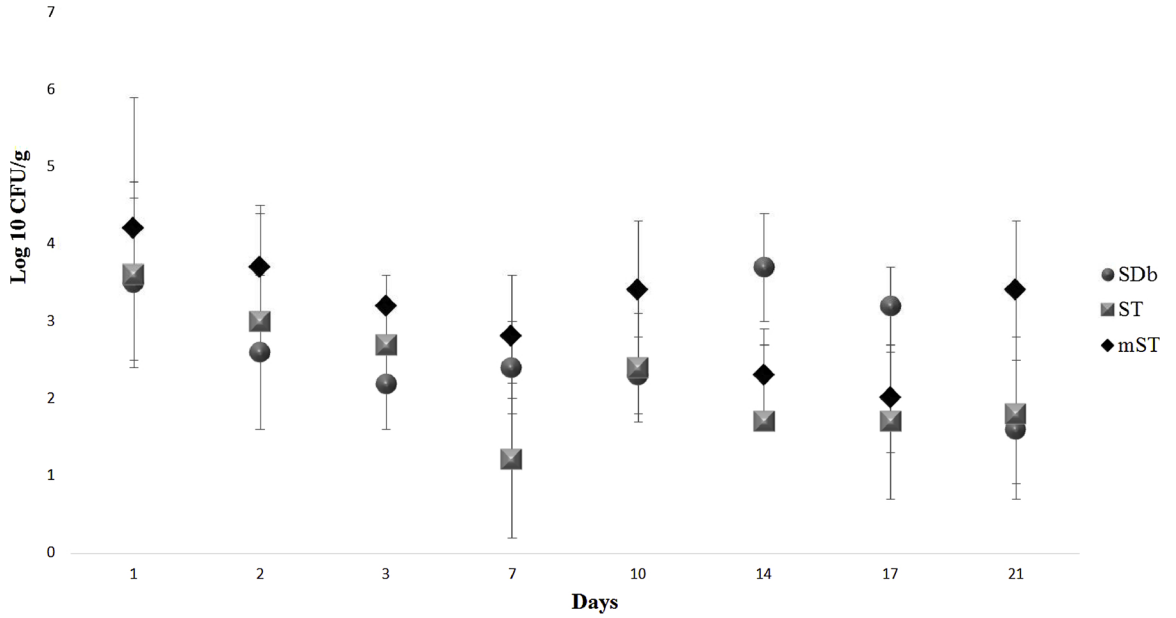


**Fig. 1.** Average fecal excretion in $Log_{10}$ CFU/g over 21 days; calculations based on the results of 8 piglets per serovar (except for days 14 and 21, when results are based on 7 piglets for ST and 7 for SD). SDb: *S.* Derby, ST: *S.* Typhimurium, mST: monophasic *S.* Typhimurium.

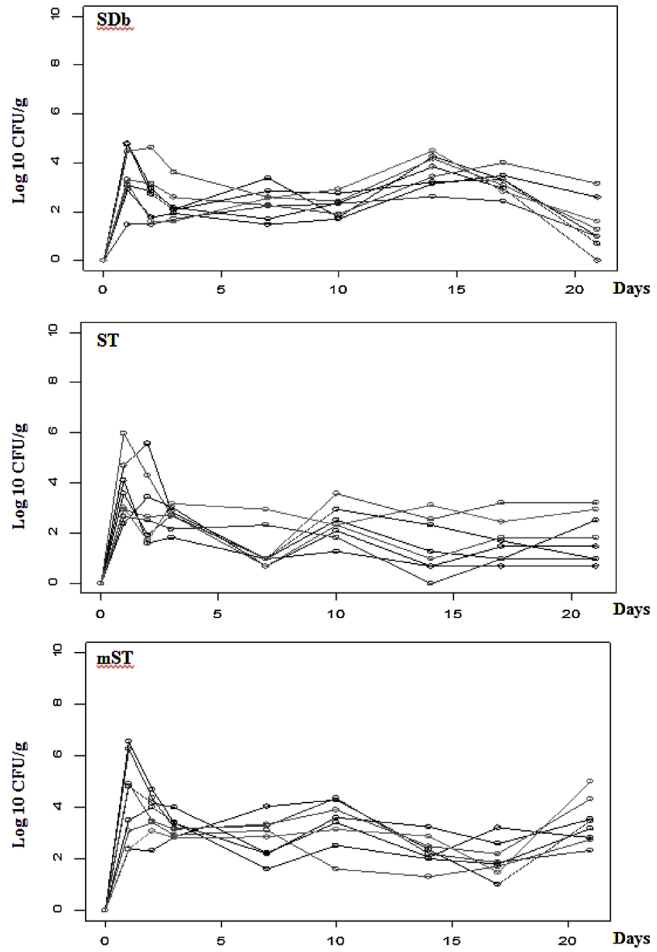


**Fig. 2.** Kinetics of *Salmonella* excretion in Log10 CFU/g for the piglets in each group (8 piglets per group), over 21 days. Total fecal shedding was calculated from these curves by calculating the cumulative area under the log curve (AULC). SDb: *S.* Derby, ST: *S.* Typhimurium, mST: monophasic *S.* Typhimurium.

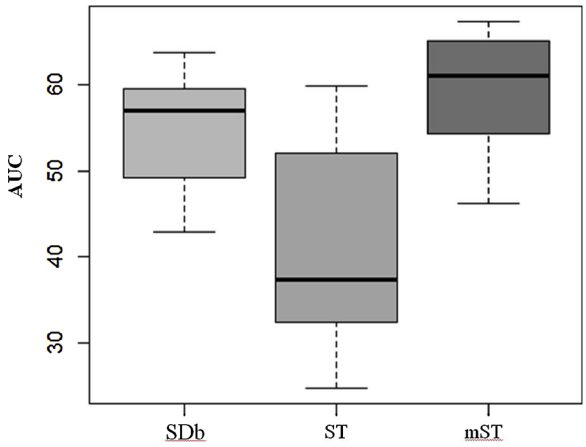


**Fig. 3.** Comparison of AULC obtained for each group (21 days). SDb: *S.* Derby, ST: *S.* Typhimurium, mST: monophasic *S.* Typhimurium.

shedding *Salmonella* were shown to have seroconverted through an ELISA.

## 4. Discussion

*Salmonella* serovars commonly associated with swine are a major human health problem. In our study we experimentally inoculated three groups of piglets with strains of three major swine serovars: *S.* Derby, *S.* Typhimurium and monophasic *S.* Typhimurium. We compared excretion patterns, seroconversion and the colonization of several organs in the three groups of animals. We found differences in some clinical signs, such as diarrhea and fever in piglets. While all groups had transient diarrhea at least once, no fever was ever recorded in the mST group. In other studies comparing either infection by *S.* Typhimurium versus *S.* Yoruba, or *S.* Derby versus *S.* Cubana, no clinical signs or diarrhea were observed (Osterberg et al., 2009; Osterberg and Wallgren, 2008). However, for *S.* Typhimurium, several clinical signs such as fever, diarrhea and anorexia were reported in animals inoculated with a dose of $10^9$ CFU (Boyen et al., 2009).

The excretion dynamics for all groups showed that the highest level

**Table 3**
*Salmonella* contamination levels in organs and intestinal contents in $\log_{10}$ CFU/g (mean ± SD) for each inoculated group, at necropsy, on day 21 p.i., on day 49 p.i., and in total.

| Group | Necropsy date | Tonsils N° of positives | Mean ± SD | MLNs N° of positives | Mean ± SD | Duodenum N° of positives | Mean ± SD | Jejunum N° of positives | Mean ± SD | Ileum N° of positives | Mean ± SD | Cecum N° of positives | Mean ± SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SD | Day 21 | 4 | 4.8 ± 0.2 | 1 | 0.7 | 3 | 0.7 ± 0.0 | 4 | 1.9 ± 1.4 | 4 | 2.8 ± 0.7 | 4 | 2.5 ± 0.7 |
| | Day 49 | 4 | 5.4 ± 0.6 | 1 | 0.7 | 3 | 0.8 ± 0.2 | 3 | 0.8 ± 0.2 | 3 | 1.4 ± 1.2 | 4 | 1.7 ± 0.7 |
| | TOTAL | 8 | 5.1 ± 0.6 | 2 | 0.7 | 6 | 0.7 ± 0.1 | 7 | 1.4 ± 1.1 | 7 | 1.9 ± 1.3 | 8 | 2.1 ± 0.8 |
| ST | Day 21 | 4 | 4.7 ± 0.7 | 1 | 2.3 | 4 | 1.3 ± 0.8 | 4 | 3.0 ± 0.7 | 4 | 2.3 ± 0.4 | 4 | 2.8 ± 1.3 |
| | Day 49 | 4 | 4.4 ± 1.1 | 3 | 1.4 ± 1.2 | 1 | 0.7 | 4 | 1.5 ± 0.2 | 4 | 2.7 ± 0.8 | 4 | 2.1 ± 0.6 |
| | TOTAL | 8 | 4.5 ± 0.9 | 4 | 1.6 ± 1.1 | 5 | 1.1 ± 0.7 | 8 | 2.2 ± 0.9 | 8 | 2.5 ± 0.6 | 8 | 2.4 ± 1.0 |
| mST | Day 21 | 4 | 4.7 ± 0.5 | 0 | – | 3 | 0.7 ± 0.0 | 3 | 1.0 ± 0.5 | 4 | 2.3 ± 1.2 | 4 | 2.4 ± 0.1 |
| | Day 49 | 4 | 5.7 ± 0.3 | 1 | 0.7 | 1 | 1.5 | 3 | 2.0 ± 1.7 | 4 | 2.6 ± 1.8 | 4 | 1.4 ± 0.9 |
| | TOTAL | 8 | 5.2 ± 0.7 | 1 | 0.7 | 4 | 0.9 ± 0.4 | 6 | 1.5 ± 1.2 | 8 | 2.4 ± 1.4 | 8 | 1.9 ± 0.8 |

MLNs: Mesenteric Lymphatic Nodes, SDb: *S.* Derby, ST: *S.* Typhimurium, mST: monophasic *S.* Typhimurium. SD: Standard deviation.

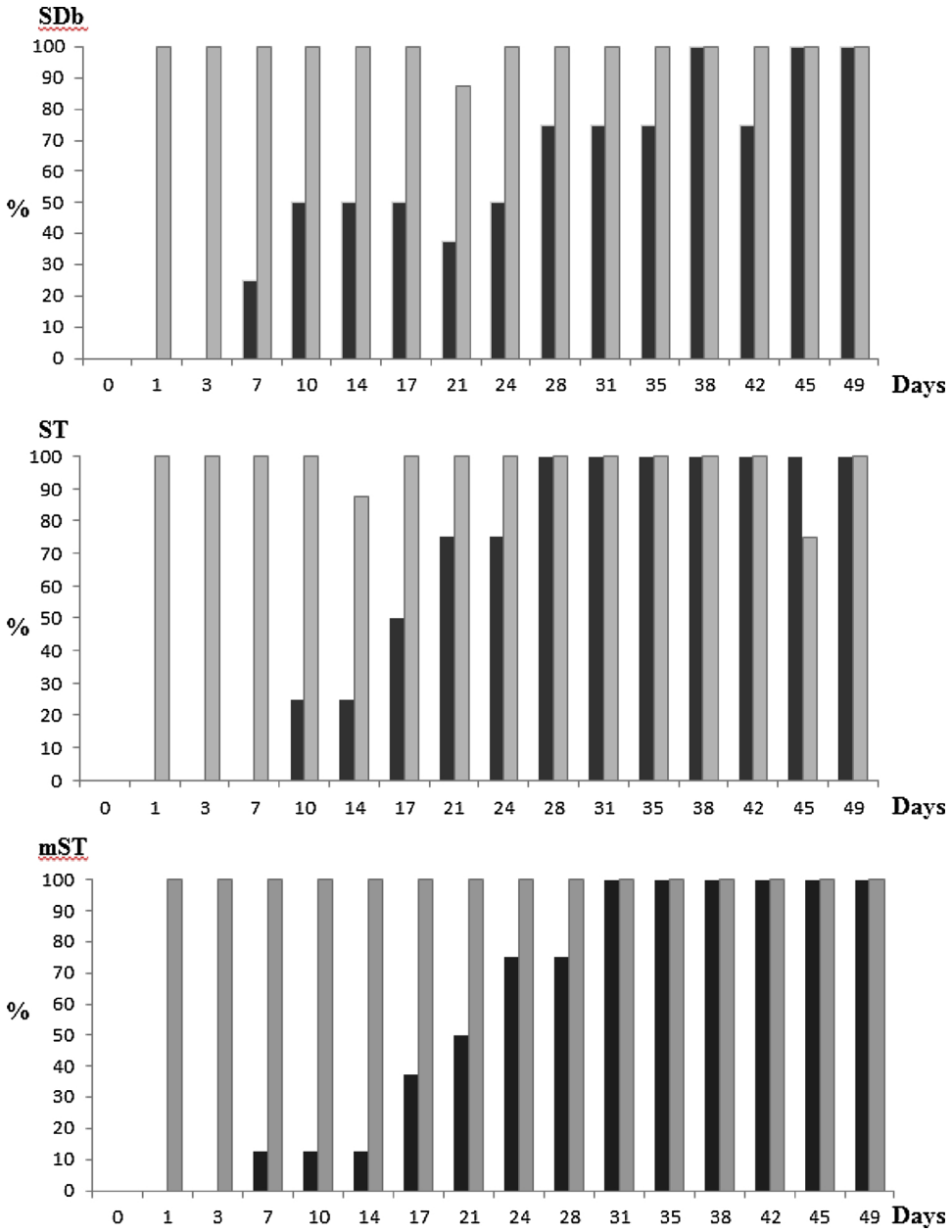


**Fig. 4.** Comparison between the percentage (%) of *Salmonella*-seroconverted pigs (in black) and *Salmonella*-shedding pigs (in gray) per day, over the 49 days of the trial. SDb: *S.* Derby, ST: *S.* Typhimurium, mST: monophasic *S.* Typhimurium.

of excretion was reached on 1 DPI, except for the group inoculated with *S*. Derby. In this group, the excretion was also very high on day 1 DPI, but the maximum amount of *Salmonella* excreted was observed on 14 DPI. In the days following inoculation, *Salmonella* excretion varied depending on the serovar inoculated. Only piglets in the mST group were shown to shed *Salmonella* continuously throughout the experimental trial. In the SDb group, one piglet was negative for *Salmonella* on one day of sampling, 21 DPI, but he was then necropsied. At necropsy, their tonsils, ileum and caecum were *Salmonella* positive. For ST group, two pigs had feces *salmonella*-free, one on 14 DPI and the other on 45 DPI. The two pigs became again *salmonella* shedders at the following sampling dates, indicating intermittent shedding.

By comparing total shedding (AULC), we found statistical differences between piglets inoculated with serovars *S*. Typhimurium and those inoculated with the monophasic variant of *S*. Typhimurium. The monophasic variant strain caused a higher excretion level than the *S*. Typhimurium strain. This study is the first comparison of colonization by *S*. Typhimurium versus that by monophasic *S*. Typhimurium. Even though the monophasic variant is genetically related to *S*. Typhimurium (Bugarel et al., 2012a; Soyer et al., 2009; Vieira-Pinto et al., 2012), further research appears necessary to establish the differences between these two serovars in their colonization of pigs.

On the other hand, no difference was observed between Sdb and ST or between Sdb and mST. In 2012, Ivanek noted that pigs inoculated with the pig-associated serovars *S*. Typhimurium and *S*. Derby tend to shed *Salmonella* in shorter periods of time (both as part of continuous and intermittent shedding) than pigs inoculated with the feed-associated serovars *S*. Yoruba and *S*. Cubana detected in Sweden (Ivanek et al., 2012). However, pigs infected with *S*. Typhimurium and *S*. Derby were more likely to (re-)enter an intermittent non-shedding state. Consequently, they showed a tendency towards longer-lasting host infection overall, with a similar or longer total duration of fecal shedding.

A recent longitudinal study in pigs naturally infected with *S*. Typhimurium indicates that this serovar may also cause high shedding rates among colonized pigs in natural conditions, particularly in younger animals (Weaver et al., 2017).

At necropsy, on 21 and 49 DPI, the tonsils and cecum of all the inoculated piglets were systematically found to be contaminated in each piglet, whatever the serovar inoculated. A previous study demonstrated that several serovars can contaminate the tonsils and cecum 3 h after inoculation (Loynachan et al., 2004). In 2004, Cote et al. also reported 100% colonization for the tonsils, cecum and MLN on 14 DPI (Cote et al., 2004). On average, the occurrence of positive samples was the lowest in MLN, then in the contents of the duodenum. The jejunum and ileum were more highly colonized, with 87.5% and 95.8% respectively. These observed trends are the same regardless of the serovar used for inoculation. Matiasovic et al. (2014) showed that after *S*. Typhimurium inoculation, only the tonsils and lymph nodes remained culture-positive up until 28 DPI and only the tonsils and cecum remained culture-positive following inoculation with *S*. Derby. In 2010, Osterberg et al. compared direct and indirect transmission of *S*. Derby and *S*. Typhimurium. For indirect transmission, pigs were necropsied after 14 days in a contaminated environment. For *S*. Derby, the cecum content, colon tissue and ileocecal lymph node were found to be contaminated in only one pig out of six (16%). For *S*. Typhimurium, four out of six of the pigs (66%) were culture-positive for cecum and/or ileocecal lymph node samples. In terms of data for the monophasic variant, in a previous study we found similar results for the colonization of tonsils and intestinal contents, but MLNs were more highly contaminated, with 100% and 62.5% of the samples positive on 21 and 49 DPI respectively (Cevallos-Almeida et al., 2018). In 2004, Loynachan detected that 75% of tonsils were culture-positive 3 h after inoculation with the monophasic variant (Loynachan et al., 2004). Tonsils are also a common site of contamination at slaughter, with about 10% contaminated with *Salmonella* (Van Damme et al., 2018; Vieira-Pinto et al., 2005), and are a major source of carcass contamination, along with the contents of the intestinal tract (Berends et al., 1997).

For all serovars, the highest level of contamination in organs and intestinal contents was found in the tonsils, cecum and jejunum. The least contaminated sites were the lymph nodes and the duodenum. In 2008, Scherer et al. found similar amounts of *Salmonella* to those we discovered in the tonsils (4.4 $Log_{10}$CFU/g) and ileum (2.7 $Log_{10}$CFU/g) after inoculation with *S*. Typhimurium (Scherer et al., 2008). In a recent study in which pigs were inoculated with $1 \times 10^9$ CFU of a multidrug-resistant monophasic *S*. Typhimurium strain, Shippy et al. made contrasting observations after 7 DPI. Specifically, tissues including the ileocecal lymph nodes were more highly contaminated than cecal content, and the tonsils were the least contaminated organ, with a mean of 1 $Log_{10}$CFU/g (Shippy et al., 2018).

In our study, piglets seroconverted at different times depending on the serovar inoculated. We found 100% of seroconversion in group mST piglets by 31 DPI. Our results are similar to those of Lynch et al., who found that 85% of pigs had seroconverted by 28 DPI (Lynch et al., 2017). For piglets in groups ST and SDb, seroconversion was 100% completed by 28 and 38 DPI. Matiasovic et al. (2014) previously described a lower immune response after infection with *S*. Derby than with *S*. Typhimurium. Another study revealed a different result, with seroconversion of 100% of pigs within two weeks of infection (Osterberg and Wallgren, 2008). For *S*. Derby our results are different to those found by Osterberg et al. (2009), with 100% seroconversion after 21 days.

## 5. Conclusion

This study revealed similar profiles of *Salmonella* excretion and colonization regardless of the serovar used for experimental inoculation. The amount of excretion, however, was different between groups inoculated with *S*. Typhimurium 07CR095 and monophasic *S*. Typhimurium S12AK50. Seroconversion had occurred by different days depending on the serovar used for inoculation. Further research is necessary to establish the virulence gene patterns of the serovars in order to understand the differences in colonization and pathogenicity among serovars colonizing pigs.

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