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Pigs infected experimentally with the same dose of monophasic variant of *Salmonella* Typhimurium exhibit different shedding levels

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Introduction

Salmonellosis remains the most frequent foodborne zoonosis after campylobacteriosis (EFSA and ECDC 2017). The most frequent sources of human infection are food products of animal origin. Pork meat has been considered as one of the major sources (Bonardi 2017). Pigs colonized with *Salmonella* are usually asymptomatic healthy carriers (Rostagno and Callaway 2012) with varied levels and durations of fecal shedding (Ivanek *et al.*, 2012). Thus, understand the mechanisms that result in more or less shedding may provide tools for control. Indeed, it has been demonstrated in other species that a minority of the infected individuals (super-shedders) are responsible of most of the transmission (Gopinath *et al.*, 2014). In the frame of MoMIRPPC (EJP One Health), we wanted to evaluate the apparition of different shedding patterns among a pig population. Then, immune and microbiota analyses will be performed in order to identify markers link to the shedding status.

Material and Methods

An experimental trial was conducted with a total of 45 piglets divided into five groups: one group with five piglets as control and four groups each with 10 piglets (n=40) as inoculated pigs. The piglets came from 5 sows and were distributed in such a way as to avoid a maternal effect between the groups. At 7 weeks of age, the inoculated piglets received orally 10 ml of suspension of 10⁸ CFU/ml of a monophasic variant of *Salmonella* Typhimurium strain. Pigs were followed during 3 weeks after inoculation before being necropsied. Twice a week, individual feces were sampled in order to quantify the level of *Salmonella* excretion during the trial. At necropsies, level of *Salmonella* was determined in tonsils, mesenteric lymph nodes (MLN), as well as in ileum and caecum contents from each pig. To facilitate the numeration, the strain inoculated was transformed to be resistant to rifampicine. Samples analyzed were diluted and directly plating on XLD agar plate supplemented with rifampicine.

All statistical analysis have been performed using R software version 3.5.2. The total level of excretion of each pig during the 3 weeks was determined by calculating, with a specific R script, the AULC (Air Under the Log Curve). Feces and intestinal contents have been frozen for future microbiota analyses. Blood was also sampled twice a week, to realize later on total blood count (TBC), serological and transcriptomic analyses.

Results

All control pigs remained negative for *Salmonella* throughout the course of the study while all the inoculated pigs were quantitatively positive for *Salmonella* shedding during all the study. *Salmonella* shedding varied according pigs and days between 1.48 to 9.09 Log₁₀CFU/g of feces. The excretion pic was observed at Day 2 post inoculation, with 6.77 ± 1.79 Log₁₀CFU/g in mean. The AULC calculation allowed us to identify three significantly different classes (p< 0,01). The three classes gathered 13, 16 and 11, high, intermediate and low shedders pigs respectively (Fig 1).

No difference were observed for the AULC value according mother (p = 0.42). Indeed, for each sow, among the 9 piglets of a same sow, piglets were distributed in the 3 class, low, intermediate and high shedders. However, AULC values according pens were significantly different (p< 0,05). The presence of a high shedder pig in a pen would maintain a high contamination pressure in the pen, and therefore a high excretion of several pigs in the pen all along the assay.

After necropsies, for all the pigs, tonsils, caecum and ileum contents were highly contaminated (in mean, 5.6, 3.7 and 3.5 Log₁₀UFC/g, respectively) unlike MLN (in mean, 0.85 Log₁₀UFC/g). We observed that for the group of high shedders, levels of contamination was significantly higher for MLN, ileum and caecum contents than for the group of low shedders (p< 0.01) (Table 1).

Discussion and Conclusion

Pigs infected experimentally with a same dose of monophasic variant of *Salmonella* Typhimurium exhibited different shedding levels. This was also described after *S. Typhimurium* infection (Knetter *et al.*, 2015). We also demonstrate that, in experimental conditions, these different shedding patterns are not linked to the mother. Indeed, high and low shedders pigs can originate from a same mother. In addition, in this study, when pigs are high shedders they also contain a significantly higher level of *Salmonella* in mesenteric lymph nodes, ileum and caecum. However, the presence of a high shedder pig could be responsible of a high global excretion in a pen,

causing a high contamination pressure for other pigs. This result confirm the importance to focus intervention strategies specifically on animals able to shed high level of *Salmonella*. To lead these interventions, we need to improve our knowledge on markers in microbiota (Kim and Isaacson 2017) and/or in immune response (Huang *et al.*, 2011; Knetter *et al.*, 2015; Uthe *et al.*, 2009) that could promote the high excretion in pigs.

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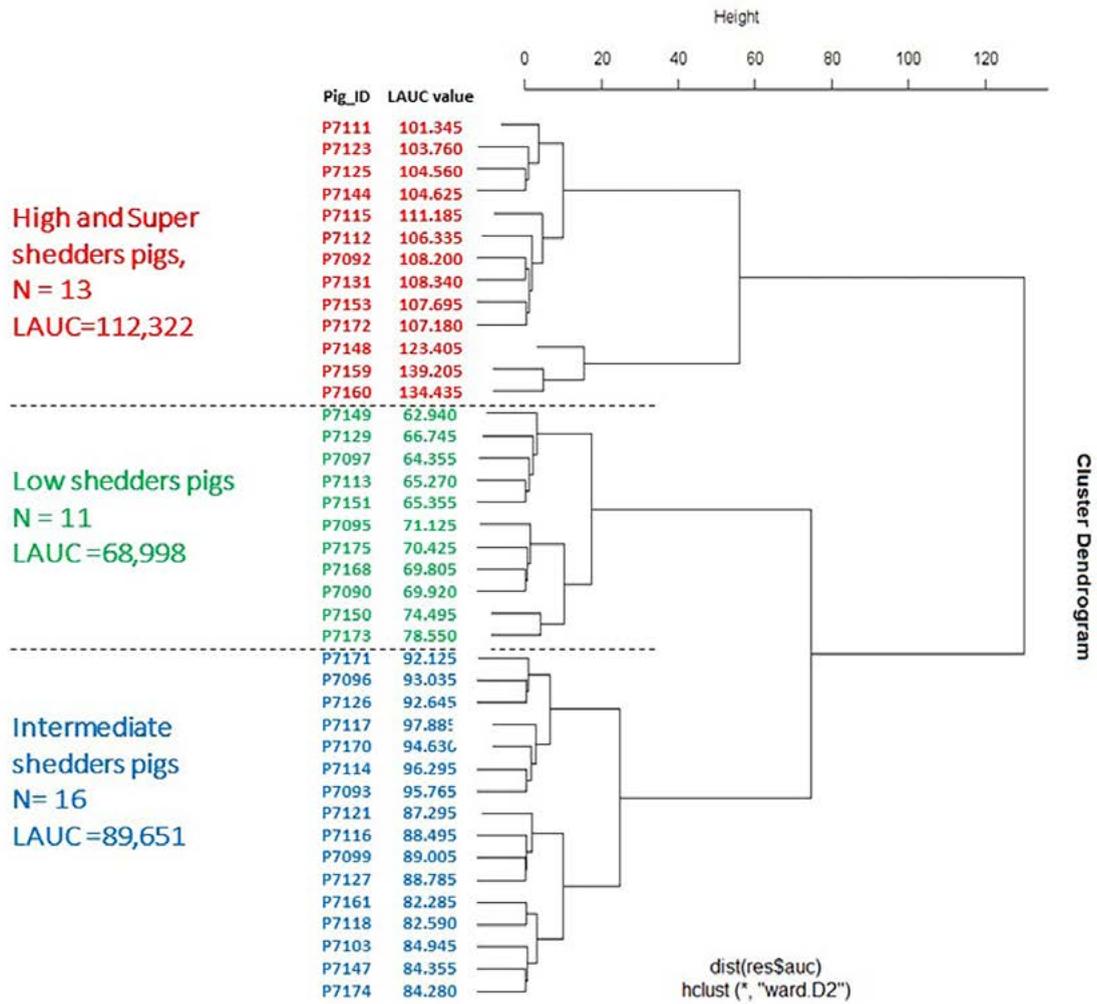


Figure 1: Hierarchical classification of pigs according the AULC calculated from the numeration values

Table 1: Salmonella positive samples and contamination levels in samples at necropsy, in log10 CFU/g

Samples	Tonsil		Mesenteric lymph nodes		Ileum content		Caecum content	
	N° of positives (%)	Mean ± SD	N° of positives (%)	Mean ± SD	N° of positives (%)	Mean ± SD	N° of positives (%)	Mean ± SD
High	13 (100%)	5.56 ± 0.28	12 (92,3%)	1.26 ± 0.60	13 (100%)	4.60 ± 0.96	13 (100%)	4.56 ± 1.47
Intermediate	16 (100%)	5.71 ± 0.42	15 (93,7%)	0.89 ± 0.69	16 (100%)	3.40 ± 0.84	16 (100%)	3.20 ± 1.35
Low	11 (100%)	5.49 ± 0.38	9 (81,8%)	0.35 ± 0.39	11 (100%)	3.10 ± 0.77	11 (100%)	2.45 ± 1.66
Total	40 (100%)	5.60 ± 0.37	36 (90%)	0.8 ± 0.68	40 (100%)	3.71 ± 1.06	40 (100%)	3.46 ± 1.66

SD: Standard deviation