

What is the level of *Salmonella* shedding by pigs at farms and the impact of quaternary ammonium compound-based disinfectants on antibiotic resistance in *Escherichia coli*?

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Abstract

The objectives of this study were to quantify *Salmonella* shedding in naturally infected pigs and the potential impact of biocides used as disinfectants on the evolution of antibiotic resistant in *Escherichia coli* (*E. coli*) strains. Faecal swabs were collected from lactating sows and finishing pigs in 4 farms visited three times. *Salmonella* enumeration and detection, serotyping and genotyping were done using standards methods. On three farms, the pen partitions and floor surfaces of 12 farrowing pens were swabbed before and after cleaning and disinfection (C&D) procedures. Total *E. coli* and *E. coli* resistant to antibiotics were counted before and after C&D. *Salmonella*-positive samples (52/240) were only found in finishing pigs. Almost two thirds of the countable samples (28/44) contained a low amount of *Salmonella* (<10 Most Probable Number of *Salmonella*/g). C&D procedures were efficient by reducing by 100 to 1000-fold *E. coli* and total bacteria counts on these herds. Moreover, antibiotic susceptibility profile had not evolved between *E. coli* strains before and after C&D during this study. These quantitative data on the level of *Salmonella* excretion in pigs naturally infected are important to enable better control of the risk associated with *Salmonella* contamination of food.

Introduction

The control of contamination of food by *Salmonella* and the reduction of antibiotic resistance are two major public health issues. *Salmonella* is the second foodborne pathogen responsible for gastroenteritis in the European Union and pork would be involved in 10 to 20% of human salmonellosis cases (EFSA and ECDC, 2021). To assess this risk along the food chain, data on *Salmonella* contamination are scarce and are needed in the different steps of the pork chain production (Anses opinion 2016-SA-037); particularly the level of *Salmonella* shedding in pigs at the farm level. *Salmonella* resistant to multi-antibiotics may also be spread to humans and responsible of therapeutic treatment failures. This multi-resistance can be acquired by the transfer of antibiotic resistance genes from *Escherichia coli* (*E. coli*) largely present on farms. Bacteria contamination can be controlled by the implementation of biosecurity measures including chemical cleaning and disinfection procedures all along the food chain production. Nevertheless, bacteria have the ability to adapt and to survive to repeated exposure to disinfectants. Controversial data are available in the literature about the impact of biocides on the cross-resistance to antimicrobials. They mainly come from experimental trials and very little from the field (Anses opinion 2016-SA-238).

This study therefore aimed at i/acquiring data on the level of *Salmonella* shedding by pigs on infected farms for a better quantitative assessment of the risk at this first stage of the food chain and ii/assessing the impact of disinfectants used on these farms on the evolution of antibiotic resistance in *Escherichia coli* strains.

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Materials and Methods

Four farrow-to-finish pig farms were visited three times. At each visit, individual faeces from 10 lactating sows located in the same farrowing room and individual faeces from 20 finishing pigs belonging to the same batch were collected. *Salmonella* detection and enumeration were performed on fecal samples using an adapted method from NF U47-102 and ISO/TS 6579-2:2012. Isolates were typed by Pulsed-Field-Gel-Electrophoresis (PFGE) and their antibiotic resistance tested by Minimum Inhibitory Concentration (MIC). Isolates were serotyped by agglutination according to the Kauffmann-White scheme and ISO/TR 6579-3:2014 method. On three farms, for 3 batches, the pen partitions and floor surfaces of 4 pens of the farrowing room were swabbed before and after cleaning and disinfection (C&D) procedures. Quaternary ammonium compounds (QAC) based disinfectants were used for disinfection in the three herds. A total of 72 samples were collected. Total *E. coli* and total *E. coli* resistant to four antibiotics (tetracycline, ampicillin, florfenicol, ciprofloxacin) were respectively enumerated on Petrifilm™ Select *E. coli* and on non-selective media.

Results

No sow faecal sample tested positive for *Salmonella*. For finishing pigs, 52 of the 240 faecal samples (21.6%) were positive and 44 were countable, of which 63.5% were below 10 MPN/g. Seven faecal samples contained more than 100 MPN/g (Figure 1).

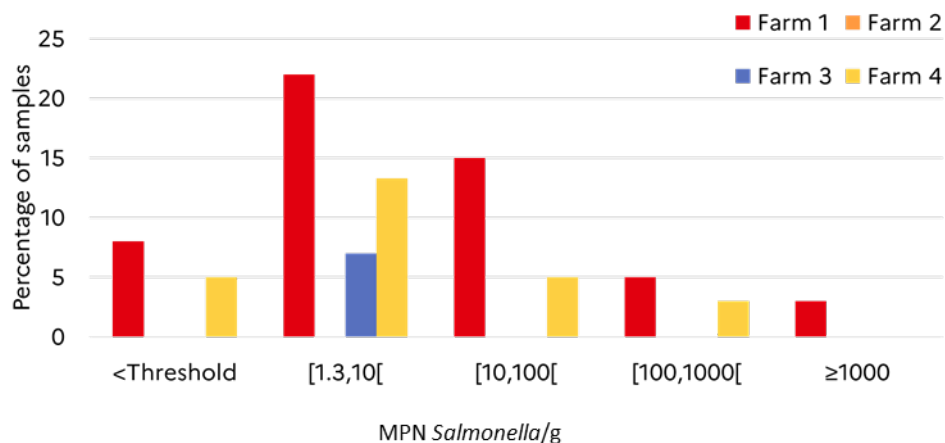


Figure 1: Distribution of the 52 positives samples according to the excretion level (MPN *Salmonella*/g) and the farms

Of the 97 *Salmonella* strains isolated, 89 strains were the monophasic variant of *Salmonella* Typhimurium (mVST) and the others *Salmonella* Derby (Table 1). All the *Salmonella* Derby strains highlighted the same PFGE profile and the same resistance to 2 antibiotics. The mVSTs were divided into 6 PFGE profiles and linked to a resistance to 3 or 5 antibiotics. Ten of 36 samples after C&D were positive for *E. coli*. Cleaning and disinfection procedures reduced by 3 to 4 Log₁₀ *E. coli* and total bacteria counts. *E. coli* resistant to antibiotics were reduced after C&D procedures.

Serotype	PFGE profile (Xbal)	Resistance profile	Farm 1			Farm 3	Farm 4		
			B1	B2	B3	B3	B1	B2	B3
mvST	mvSTX1	ACTTmSu	9		8				
mvST	mvSTX2	ACTTmSu	1	6	1				
mvST	mvSTX3	ATSu				1	4	5	3
mvST	mvSTX4	ATSu					2		
mvST	mvSTX5	ATSu					1		
mvST	mvSTX6	ATSu				1			
Derby	DX1	TSu	6			2			

Table 1: Distribution of strains (one strain per serotype and per sample) according to their PFGE profiles and antibiotics resistance profile, for each farm and each batch (B). A: ampicillin, C: chloramphenicol, T: tetracycline, Tm: thrimetroprim, Su: sulfamethoxazole.

Discussion and conclusion

This single exploratory study allows to collect for the first time in France quantitative data on the level of *Salmonella* excretion in pigs naturally infected under field conditions. These quantitative data are important to feed risk assessment models and ultimately to enable better control of the risk associated with *Salmonella* contamination of food. In our study, *Salmonella* was excreted only by finishing pigs. This excretion is mainly weak with less than 10 MPN *Salmonella*/g. These data were in agreement with those obtained from 118 fecal swabs collected at a Danish slaughterhouse (van Hoek et al., 2012) where positive samples had $6,55 \pm 4,13$ MPN *Salmonella*/g. Due to limited number of *Salmonella* samples in our work, it would be needed to carry out further studies to confirm our results. The main serotypes identified in this study were mVST and S. Derby, which are currently the most reported serotypes in Europe in the pig industry (Bonardi, 2017).

The effect of QAC-disinfectants use on antibiotic and disinfectant susceptibility of *E. coli* strains isolated from surfaces samples in farrowing rooms was investigated in the current study. QAC-disinfectants were effective to reduce from 100 to 1000 total bacteria and *E. coli*. Moreover, our results show a trend towards decreasing antibiotic resistance in *E. coli* after C&D procedures. Consequently, these disinfectants had not selected *E. coli* strains resistant to antibiotics during this study. These results are in line with other field studies for *Salmonella* (Gantzorn et al. 2014), and *E. coli* strains (Maertens et al. 2019). Selection of bacteria resistant to antibiotics after QAC-based disinfectant exposure *in vitro* is reported in several studies, this selection seems not be so easy under practical conditions. Further studies including a higher number of herds are needed to better assess this potential selection on the long run.

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