13th SafePork- 26-29 August 2019 – Berlin, Germany

Development of a quantitative PCR method coupled with PMA to quantify viable *Salmonella spp.* cells in the pork supply chain

S. Jeuge(1), M. Denis(2), F. Niveau(1), C. Houdayer(2), E. Boix(1), F. Souchaud(2), B. Frémaux(1)

(1) Ifip-Institut du porc, Fresh and processed meat department, Maisons-Alfort - (2) Anses, unité HQPAP, Ploufragan

Contact: sabine.jeuge@ifip.asso.fr

In 2017, Salmonella spp. was implied in 30% of foodborne diseases in France (SPF, 2019). Few data on the contamination levels of Salmonella spp. are available along the pork supply chain. The protocol of the standard method (ISO/TS 6579-2:2012) is time-consuming and culture-based methods using chromogenic media are less efficient for matrices with high levels of background flora, and for recovering stressed cells. Along the food chain, the cells may be impacted by various stresses (e.g. chemical or thermal), which may lead to physiological changes and the emergence of viable but non-culturable cells (VBNCs).

Material and Methods

This study aims to develop a protocol for the quantification of viable *Salmonella spp.* cells from pork carcasses and faeces based on a quantitative TaqMan® PCR (qPCR) method combined with propidium monoazide (PMA) treatment to exclude DNA from dead cells. Performances of the PMA-qPCR method were assayed using different ratios of viable (including heat-stressed cells) and non-viable (heat-inactivated) *Salmonella* cells from pure culture in nutrient broth, and artificially contaminated samples of faeces and pork back fat. Different PMA concentrations and light exposure conditions were tested.

For each sample analysis, the concentrations of total, viable and cultivable fractions of *Salmonella* cells were determined by using qPCR, PMA-qPCR and culture-dependent approaches (the standard miniaturized MPN for faeces or chromogenic *Salmonella* plating media for pork back fat), respectively.

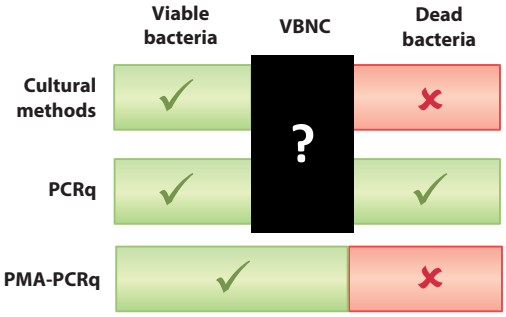

Figure 1: Viability influence on bacterial quantification

Results

Faeces

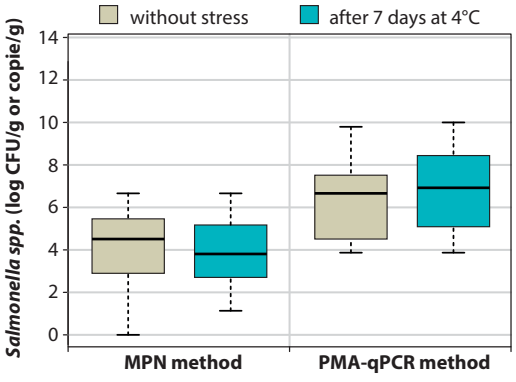

Figure 2: PMA-qPCR method vs standard method (MPN) with and without cold stress (7 days at 4°C) on artificially contaminated faeces

Back fat

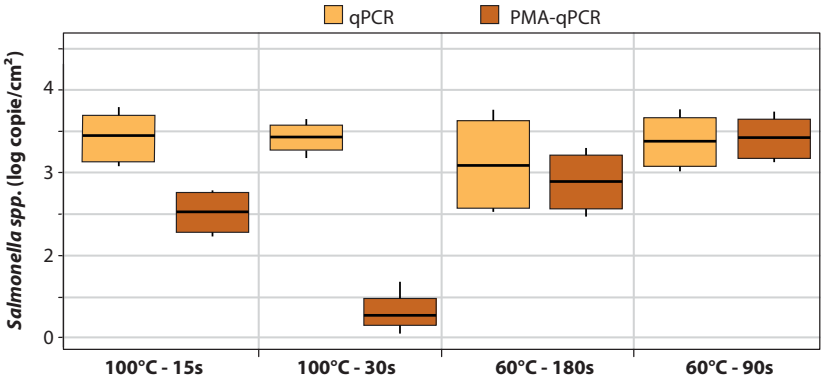

Figure 3: PMA-qPCR vs qPCR method with and without heat stress (60°C and 100°C) on artificially contaminated pork back fat

- The PMA-qPCR reaction developed in the present study exhibited a 100% inclusivity and 100% exclusivity for *Salmonella spp.*
- Quantification of VBNC cells of *Salmonella spp.* even in the presence of dead cells.
- Limits of quantification of the PMA-PCRq set from artificially contaminated faeces: 5.10^2 genome copies/g, and from artificially contaminated pork back fat: $1,6.10^2$ genome copies/cm^2

Conclusion

The PMA-qPCR method was effective to determine the impact of thermal stresses on the behaviour of *Salmonella spp.* cells in artificially contaminated samples of faeces (4°C) and pork back fat (60°C and 100°C). This method will be useful to identify farming practices related to high/low level of *Salmonella* contamination in pigs. Quantitative data on carcasses and pork cuts are also of interest to qualify slaughtering procedures and their impact on the contamination of meat with *Salmonella spp.*. A development of the method on pork cuts is planned.

This project was supported by funds from FranceAgriMer