QUANTIFYING THE RISK OF *SALMONELLA* CONTAMINATION OF TURKEY MEAT : SPECIFIC ROLE OF PREHARVEST CONTAMINATION AND POSSIBLE CONTROL MEASURES

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Introduction

Salmonella remains the first foodborne disease in France (2) like in many countries and the contamination of poultry products by this microorganism is a major concern. Consumption of contaminated raw eggs remains the first cause of *Salmonella* outbreaks in France. Contamination of turkey raw products is most of the time located to the surface of the meat or the skin, and regarding that, easily eliminated by cooking. Nevertheless, *Salmonella* foodborne diseases could occur in humans by cross contamination with heavily contaminated raw meat. Considering that, a study was undertaken in order to evaluate i) the amount of *Salmonella* present on the surface of the turkey skin after slaughter and on the breast fillet after cutting, ii) the relationship between caecal contamination at the age of slaughter and meat surface contamination, iii) some pre-harvest control measures that may be implemented in order to lower the risk of *Salmonella* on turkey raw meat.

Material and methods

Risk assessment : On the basis of pre harvest swabs realised 15 days before slaughter, *Salmonella* positive turkey flocks were selected. For each flock entering the abattoir, cleaning and disinfection of the plant was assessed by swabbing, and then 60 caeca, 60 neck skin and 60 breast meat were sampled. *Salmonella* monitoring was done by a classical pre-enrichment, enrichment, isolation procedure, using buffered peptone water (BPW), Modified Semi Solid Rappaport Vassiliadis (MSRV) and Xylose Lysine Tergitol 4 (XLT4) agar. *Salmonella* numeration was performed with the Fravalo *et al.* (1) method based on miniaturised Most Probable Number using BPW, MSRV, and XLT4. Sensitivity of the miniaturized MPN method was 1,3 CFU/g of sample.

Evaluation of pre-harvest control measures : day old turkeys (strain BUT9) were inoculated by 10^4CFU/bird of a *Salmonella* Typhimurium rifampicine resistant suspension. The birds were raised in an isolator during 14 days. Two days after *Salmonella* inoculation, they received through drinking water or through the feed a treatment supposed to be efficient to lower *Salmonella* excretion. Ten birds were sacrified at day 6, day 10 and day 14 and were checked for caecal contamination by *Salmonella* Typhimurium rifampicine resistant with brilliant green agar containing rifampicine. The following treatments were evaluated :

- -organic acid mix
- -acetic acid 0,1%
- -lactic acid 0,1%
- -ascorbic acid 0,1%
- -nisin 7 ppm
- -essential oils mixes (4 different formulations) 0,05%, 0,2%, 0,3%
- -*Saccharomyces boulardii* 50 ppm
- -Lactobacilli 2%
- -Alpha tocopherol 2500 ppm
- -Competitive exclusion (CE) treatment 0,1%.

Results

The results of the experiments are reported in tables 1 to 4.

Risk assessment experiments :

Table 1 : Salmonella contamination of the batches coming from positive flocks

	Flock1	Flock2	Flock3	Flock4	Flock5	Flock6
Caeca	25%	25%	0%	0%	95%	72%
Neck skin	20%	37%	0%	3%	93%	100%
Breast meat	0%	3%	0%	0%	41%	12%

Table 2 : Numeration of Salmonella in 36 positive samples coming from lightly contaminated flocks (flocks 1 to 4)

	N>100/g	10/g<N<100/g	N<10/g	N<nl
Caeca	0	0	1	3
Neck skin	0	0	11	19
Breast meat	0	0	0	2

nl : numeration limit

Table 3 : Numeration of Salmonella in 216 positive samples coming from heavily contaminated flocks (flocks 5 and 6)

	N>100/g	10/g<N<100/g	N<10/g	N<nl
Caeca	38	28	43	19
Neck skin	2	26	47	11
Breast meat	0	0	1	1

nl : numeration limit

As it can be seen on tables 1 to 3, *Salmonella* contamination resulting from the slaughtering of a pre-harvest positive flock is tightly depending on the contamination level of live birds as measured by caecal contamination. When the contamination of the gut was lower than 10 CFU/g of caecal content, all the *Salmonella* positive neck skins were contaminated by less than 10 CFU/g and most of them by less than 0,1 CFU/g which was the detection limit. As a consequence, breast meat taken from such carcasses was contaminated by less than 0,1 CFU/g when positive. On the contrary, heavily contaminated guts (N> 100 CFU/g) were responsible for more heavily contaminated neck skins even if most of the samples were contaminated by less than 100 CFU/g. In the meantime, contamination of the positive breast meat samples remained low (less than 10 CFU/g).

Evaluation of pre-harvest control measures :

Table 4 : results of the most significantly efficient treatments (expressed in log10 Salmonella reduction)

Product	1st trial	2nd trial	3rd trail
α tocopherol	-3.5	-1.8	Not done
Lactobacilli	-2.7	-2.4	-2.4 -1.1
CE treament	-1.6	-1.0 ns	Not done
Essential oil PO96A	Not done	-1.7	-0.8 ns

ns : not significant

Organic acids, nisin, ascorbic acid and yeasts did not prove their efficiency during these trials. Among the 4 tested formulation of essential oils, only one was significantly efficient in one trial.
As it can be seen in table 4, some treatments able to lower *Salmonella* excretion were significantly efficient even if their efficiency could vary from a trial to the other. The most efficient treatments able to significantly lower *Salmonella* contamination in the gut were high doses of α–tocopherol and the strain of *Lactobacillus* tested. This strain of *Lactobacillus* is able to decrease the contamination of more than 2 log10.

Discussion

Using an original method for *Salmonella* numeration, we were able to quantify the risk of meat contamination linked to gut content. As it could be seen, the proportionality between caecal bacterial content and neck skin contamination existed and is relevant. These results also showed that even if contamination of the caecal content is high (more than 100 CFU/g), breast meat samples remained lightly contaminated and did not present a high risk of foodborne disease for the consumer. In order to lower the risk of neck skin and meat contamination, the potential of some "natural" molecules, probiotics or competitive exclusion treatment was evaluated. The purpose of the work was to screen some active products in experimental facilities using a one day old turkey model, and then to validate the results with field trials. The first part of the experiment enabled the authors to screen α tocopherol and a strain of lactobacillus which both proved their regular efficiency in *Salmonella* exclusion. These two products will be now evaluated in a field trial. Concerning the mechanisms that determine such an efficiency, the role of probiotics is well known and could be described as a combination of some of the following effects : direct production of *Salmonella* efficient bacteriocin, nutrient competition or acid production (3) or competition with mucosal adhesion sites. Few elements are available in this study to explain the observed effect with the *Lactobacillus* strain. It should be noted that a specific antimicrobial or non dissociated form of acid production did not explain alone the effect of the strain tested as organic acid and nisin tested separately did not have any effect. A synergistic effect between these two molecules was not tested but perhaps this particular strain had another property which is still to be investigated. α tocopherol is well known for its antioxidant properties and has recently be involved in lowering the virulence of *Listeria monocytogenes* in turkey (4). This could partly explain its efficiency against *Salmonella.* The mechanisms of the efficiency of both products have to be further investigated regarding their impact on the composition and equilibrium of the gut and caecal flora.

Conclusion

This preliminary work is reassuring as it confirms that the amount of *Salmonella* that might contaminate turkey meat are low, and the associated risk for the consumer consequently low as well, even in potential cases of cross contamination in the kitchen.
The use of molecules or probiotics able to decrease the excretion of *Salmonella* before slaughtering is also promising as the proportionality between the contamination of the gut content and those of the skin existed. If confirmed by field trials, this treatment might be used for the treatment of the turkey flocks detected positive 2 weeks before slaughter.

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