DISTRIBUTION OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* O157:H7 IN GROUND BEEF MIXTURE: A DEGREE OF HOMOGENEITY ASSESSMENT IN LOW AMOUNTS OF CONTAMINATION CASES

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Abstract – In order to improve the risk assessment for Shiga toxin-producing *Escherichia coli* (STEC) in ground beef, and more precisely to evaluate the probability of detection of these pathogenic bacteria at the end of ground meat processing, pilot scale experiments were conducted to study the distribution *E. coli* O157:H7 in ground beef mixtures. After several genetic transformations to lead to a non-pathogen fluorescent *E. coli* O157:H7 strain, the EDL 933 Δstx pGfp AmpR strain was inoculated on a piece of meat (to achieve a 10 cfu/g or a 100 cfu/g final concentration in the mixture), which was used to produce a 25kg ground meat mixture. At the end of a 3 steps processing, ground beef mixture was sampled (60 samples of 5g, 25g and 100g for one mixture) to detect and/or count the EDL 933 Δstx pGfp AmpR bacteria. This procedure was repeated 3 times with 100% fresh meat and 3 times with 8kg of frozen meat mixed with 17kg of fresh meat.

The results of the bacterial enumerations recovered from meat mixture were used to adjust gamma distributions and deduce the shape parameter (b) which characterize the bacterial distributions in ground meat. Distributions were characterized by b-values ranging from 1 to 2, which matches with a moderately homogeneous distribution of STEC in ground meat at the end of the processing but not a perfectly random distribution.

Key Words – Establishment of a statistical model. Risk assessment. STEC. Ground meat.

I. INTRODUCTION

Shiga toxin-producing Escherichia coli (STEC) are enteropathogens causing human infections with a broad spectrum of clinical outcomes. They represent a particularly important hazard for children health, and are the major cause of Hemolytic Uremic Syndrome (HUS). The outbreaks related to these pathogens are often due to ground beef consumption. In order to improve this product safety related to these pathogens in France, a risk assessment have been conducted by the National Agency for Food, Environment and Work Safety (ANSES) [1]. As a result, ANSES brought to light the lack of data about the STEC distribution in ground beef mixtures, in cases of low amounts of contamination, with three steps grinding process.

The aim of this study was to complete existing data by characterizing the bacterial distribution of low contamination of STEC in ground beef mixture and so enhance the accuracy of statistical models for the detection of these bacteria.

II. MATERIALS AND METHODS

Bacterial strain preparation

In order to observe the STEC distribution in ground meat, non-pathogenic easily detectable STEC strain was needed. So the natural well characterized EDL 933 strain was genetically

transformed by deletion of its stx genes [2] and insertion of a pGfp plasmid (Green fluorescent protein plasmid) containing an ampicillin resistance gene to allow the transformed cells selection and easy detection [3].
The mutant strain was grown in Luria Bertani agar (LB agar) and in buffered peptone water (BPW) before being inoculated on a piece of fresh meat.

Meat inoculation, ground beef mixture processing and sampling
Among 25kg beef meat calibrated to make a 15% fat content mixture, a 50g piece of meat was inoculated with 10µl of the bacterial suspension, in order to achieve a 10 cfu/g or a 100 cfu/g initial STEC concentration in the meat mixture.
Two hours after inoculation, the whole of the 25kg meat was coarsely ground (8 mm grain size) to make an unsophisticated mixture. This mixture was mixed using a pilot-scaled paddle mixer during 2 min, adding dry ice to keep a low temperature (-1°C to 2°C) in cases of experiments conducted with 100% fresh meat. At the end of the mixing step, the mixture was ground again, more finely, in order to obtain a marketable ground beef mixture (3 mm grain size). This three steps grinding process was applied to be as representative as possible of the industrial ground beef manufacturing process, and represents a major difference with the study conducted by Flores *et al.* [4] on *E. coli* O157:H7 distribution in ground beef.
Sixty samples were taken from the final mixture: 20 of 5g, 20 of 25g and 20 of 100g. A systematic sampling scheme was used to be representative of the whole 25kg production [5].
This experiment was conducted 6 times: 3 times with 100% fresh meat and three times with 8kg of frozen meat (1/3) and 22kg of fresh meat mixed (2/3).

Bacterial enumerations and statistical analysis
Ground meat samples were diluted in BPW supplemented with ampicillin (100 µg/ml) by Stomacher blending, making four-fold dilutions of samples. The bacterial cells of the transformed strain were counted in the resulting suspensions, by spreading 3 ml of each suspension in 9 Petri dishes filled with LB agar supplemented with ampicillin (100 µg/ml); and by using the Most Probable Number (MPN) method with BPW supplemented with ampicillin (100 µg/ml). Five tubes were inoculated with 4 ml of each initial suspension. The growing media were incubated during 24h - 48h at 37°C before being analyzed.
Bacterial counts results enabled to assess the STEC recovery rates from meat mixture. The resulting concentrations calculated from each sample were used to adjust gamma distributions and deduce the shape parameter (b) which characterize the bacterial distributions in ground meat.

III. RESULTS AND DISCUSSION

STEC recovery in ground beef mixture
Results of bacterial enumerations for each assay are presented in table 1. These results show similar patterns between assays conducted with 100% fresh meat and frozen and fresh meats mixed.

Table 1. Results of enumeration of the EDL 933 Δstx pGfp AmpR STEC strain in ground beef mixture

Assay	Meat presentation	Initial concentrations of STEC introduced in meat #	Concentrations of STEC present in meat mixtures at the time of bacterial enumeration in meat *	Number of positive samples for STEC counting	Adjusted average STEC concentrations calculated in meat mixtures (standard deviations in brackets)
1	100% fresh	13.2 ufc/g	9.2 ufc/g	39/60	0.35 ufc/g (0.47)
2	100% fresh	13.9 ufc/g	9.7 ufc/g	28/42	0.89 ufc/g (0.49)
3	100% fresh	132 ufc/g	98 ufc/g	60/60	27 ufc/g (19)
4	1/3 frozen meat, 2/3 fresh meat	124 ufc/g	97 ufc/g	60/60	18 ufc/g (19)
5	1/3 frozen meat, 2/3 fresh meat	151 ufc/g	117 ufc/g	60/60	14 ufc/g (22)
6	1/3 frozen meat, 2/3 fresh meat	103 ufc/g	47 ufc/g	60/60	4,0 ufc/g (9.8)

Calculated from control samples at the moment of meat mixture inoculation

* Calculated from control samples after transportation and dilution in BPW supplemented with ampicillin

The two first assays with fresh meat had been conducted with initial STEC concentrations introduced in meat mixtures reaching about 10 cfu/g. Obtained results showed a too low recovery rate of transformed bacteria to accurately assess their distribution among meat mixtures: only 66% of samples were over detection thresholds of enumeration methods, and bacterial recovery rates were lower than 10% (table 2). In consequence, STEC inoculated concentration had been increased to achieve 100 cfu/g for the following assays. With the increased STEC inoculation, all samples were above detection thresholds, and recovered bacteria ranged between 9 and 27% (table 2).

For all six conducted assays, it was observed that bacterial concentrations calculated from different sized samples did not depend on the sample type on the one hand; on the other standard deviations were quite significant compared to average STEC concentrations within each assay, even within each sample size of each assays (data not shown). These could indicate a relatively good STEC distribution among meat mixture but not a regular one.

Statistical assessment of STEC distribution among ground beef

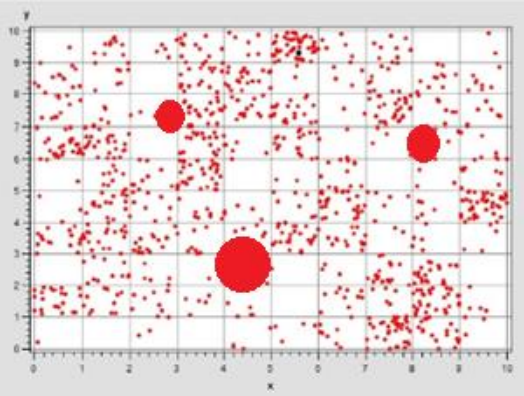
Gamma distributions were correctly adjusted to the data collected with each sample analysis from the six experimental assays (data not shown). From these adjustments, global parameters were estimated for each assay (table 2).

Table 2. Parameter estimations of adjusted gamma distributions and STEC recovery rates from ground meat mixture

Assay	Gamma distributions parameters		% recovered bacteria from ground beef mixture compared to concentrations evaluated from control samples
	Shape = b parameter	Rate	
1	1.3	3.8	4%
2	1.5	1.7	9%
3	1.1	0.04	27%
4	1.6	0.09	19%
5	1.0	0.07	12%
6	1.1	0.27	9%

The b parameters associated with STEC distribution among ground beef mixture were estimated between 1 and 1.6. These values indicate that STEC distribution among ground beef mixture was relatively homogeneous, but not enough to suppress the risk of potential bacterial aggregates presence within ground meat mixtures (figure 1).

Figure 1. Illustration of a bacterial distribution following a b value of 1, with potential bacterial aggregates



Moreover these potential aggregates could explain the low recovery rates observed in assay results (average recovery rate was about 13%).

IV. CONCLUSION

The distribution of *E. coli* O157:H7 in ground beef mixture is a moderately homogeneous distribution, but not a perfectly random one, using a three steps ground beef manufacturing process and in cases of low amount of contamination, simulating a spot contamination of meat.

The estimation of b parameter around 1 will allow risk assessors to include this data in developed statistical models to assess the biological risk represented by STEC in ground beef. From these modellings, recommendations will be provided to industrial ground meat manufacturers, in order to enhance risk management related to these pathogens and so food safety.

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