

PE5.06 Integrated traceability approach: the poultry supply chain 388.00

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Abstract— The precautionary principle advocated by the General Food Law of the European Union is bringing the food industry towards approaches of integrated traceability, in which the product flow is accompanied by information flow concerning quality and safety parameters. In the framework of the European research project CHILL-ON we have investigated the status of microbiological criteria for safety in the poultry meat supply chain and developed singleplex and duplex Real Time PCR assays for detection of main bacteria of interest. Sensitivity and reliability of the assays have been evaluated in comparison with conventional methods. The research project MENTORE has addressed the monitoring of long distance transport of livestock in connection with food safety issues, evaluating the potential impact of satellite navigation technology.

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Index Terms—Campylobacter, microbiological safety, Salmonella, satellite navigation.

I. INTRODUCTION

THE General Food Law of the European Union, 178/2002, has introduced the precautionary principle to European legislation, together with the concept of traceability along the entire food supply chain. Traceability is however limited to record keeping of suppliers and customers, allowing reconstruction of the product chain in case of need (one step back, one step forward approach).

On the other hand, the safety of food products is regulated in Europe by a comprehensive legislation (<http://europa.eu/scadplus/leg/en/s84000.htm>) covering the application of the HACCP (Hazard Analysis and Critical Control Points) system, including Directive 2002/99 and Regulations (EC) 852/04, 853/04, 854/04, 882/04, 183/05, 2073/2005. This latter Regulation concerns in particular microbiological criteria for foodstuffs, and has been formulated by taking into account opinions and documents produced by panels and

committees of EFSA (European Food Safety Agency), international guidelines, and the Codex Alimentarius guideline [1]. The Annex I of this Directive has been updated by the Regulation 1441/2007.

After the introduction of this legislation complex, food operators have been charged with the responsibility for deciding the frequency of sampling and testing in the microbiological analyses, as part of an autonomous HACCP procedure. The Regulations (EC) 2073/2005 and 1441/2007 list separate criteria for food safety and for process hygiene, associating to them specific reference methods. Food operators could also utilise alternative methods, as long as results are equivalent, and the method is validated and authorised by competent authorities.

In the most recent report on foodborne outbreaks, EFSA [2] has identified the main causative agents: *Salmonella enteritidis*, viruses, and *Campylobacter*. As in previous reports, two main causes of health problems are *Salmonella* and *Campylobacter*, and their main occurrence is recognised to be in poultry meat and products.

Salmonella is covered by Regulation 2073/2005 and 1441/2007, as food safety and process hygiene criteria, whereas *Campylobacter* is not. Recently, EFSA [3] has advocated the extensive use of molecular biology methods for microbiological typing in food safety, to improve traceability of contamination along the food chain by recognising isolates and linking them to outbreaks. A recent review explores several innovative methods for pathogen detection in food [4], recognising a constantly increasing need in the industry for rapid and sensitive tests.

A research project within the European Commission Sixth Framework Programme, CHILL-ON, is addressing the improvement of safety in the frozen and refrigerated poultry supply chain, focusing research activities also on the development of innovative and improved testing methods for bacterial contamination with molecular biology methods.

Another project financed by the European GNSS Supervisory Authority (www.gsa.europa.eu), MENTORE, and dealing with the European satellite navigation systems EGNOS and Galileo, is instead addressing the monitoring of long distance transportation of livestock and the application of satellite navigation technology

(www.gnsstracking.eu). This issue can be related to food safety because events during transportation impact the welfare and health of animals, leading eventually to increase in microbial contamination [5]. The TRACES (Trade Control and Expert System) system of the European Commission should, in future, link the information acquired during transportation to a central database for sanitary information of livestock. The New Animal Health Strategy for the European Union (2007-2013) recognises potentially heightened risks during transport, where sanitary and animal welfare rules should be tightly controlled.

These projects are examples of a new trend towards integrated traceability, in which the product flow is accompanied by information flow concerning significant properties of the product.

II. MATERIALS AND METHODS

A. Primer design for RealTime Quantitative PCR (qPCR)

Primers were designed to amplify candidate genes of *Campylobacter jejuni*, *Salmonella enterica*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Pseudomonas* spp.. Candidate genes for primer design were selected on the basis of information available in literature. In general regions showing no or low similarity with other bacteria genomes were selected, after a BLAST [6] search against the microbial DNA sequences available at NCBI (website).

Different softwares (Oligo analyzer 1.2, Fast PCR and Primer 3) were used to design primers in species-specific regions of each candidate gene. The selected oligonucleotides were analyzed for: i) the annealing temperature, chosen in the range 58-60 °C; ii) the amplicon size, 70-80 bp; iii) the primer size, of 18-22 nt and; iv) the lack of secondary structures.

B. DNA extraction

Genomic DNA isolated from certified ATCC bacterial strains was purchased from LGC Promochem (Teddington, UK). DNA was also extracted from 1 gr of chicken meat using kit Puregene (Gentra, Minneapolis, USA) after spiking with appropriate dilutions of bacterial cultures.

C. Quantitative Real Time PCR

Reference standard curves for quantification were made with the selected primers using serial dilutions of DNA of each bacteria corresponding to 100000, 10000, 1000, 100 and 1 genome copies. PCR reactions were carried out in ABI PRISM® 7000 (Applied Biosystems, Foster City, CA-USA)

using SYBR® GreenER qPCR SuperMix (Invitrogen, Carlsbad, CA), following the supplier recommendations. Three replicates for each dilution were carried out.

III. RESULTS AND DISCUSSION

Analysis of the needs of food operators

The CHILL-ON project has acquired from food operators information concerning the current status of microbiological controls in the poultry supply chain, by applying questionnaires and interviews. The distribution list involved over 50 companies in 10 countries, in Europe and abroad: Brazil, Chile, China, Germany, Greece, Iceland, Israel, Italy, the Netherlands, United Kingdom.

1-Which bacteria are currently analysed in poultry meat? Over 90% of companies perform controls for *Salmonella*, *Listeria* and *Campylobacter*. *Salmonella* is analysed by all companies interviewed. More than 60% of companies check for the presence of *Escherichia coli* and *Staphylococcus aureus*. Other microorganisms are of interest to less than 40%: *Pseudomonas*, *Clostridium*, lactic acid bacteria, *Bacillus cereus*, *Streptococcus*. Other companies indicated assays for Enterobacteriaceae, coliforms, aerobics mesophyls, enterococci, moulds and yeasts. Most of these controls are performed as routine analyses.

2-What are the legislation requirements for poultry meat? All companies cited Regulation (EC) 2073/2005, with sampling of neck skin and analysis for total bacteria at 30°C, *E. coli*, *Salmonella* spp., *L. monocytogenes*. Other acts of legislation mentioned by companies in the questionnaires were those specific for the different countries.

3-Which analytical methods are employed by companies? All companies utilise official microbiological methods, in particular ISO methods. About 25% of the companies reported the use of tests based on DNA analysis with molecular methods. These are performed by large companies in their own facilities, or by appointing external laboratories. Large distribution companies may require additional testing for their own brand poultry products. In this case, samples are taken at the producer facilities with a specified frequency, and analyses are performed either in internal or in external laboratories.

4-Is there an interest for additional testing on poultry meat? Most companies expressed a requirement for better tests to analyse bacteria which they already test with conventional methods (see also below). In 38% of companies, new tests for *Campylobacter* are required, for typing and for enumeration. Additionally, 12% of respondents

would be interested in testing for spoilage bacteria. Few companies, slaughterhouses mainly, expressed an interest for pathogens infecting birds, such as *Bordetella avium*, *Riemerella anatipestifer* and *Ornithobacterium rhinotracheale*.

5-Is there an interest for innovative methods in testing? Many companies expressed interest for rapid tests based on PCR amplification of DNA, to detect *Campylobacter*, *Salmonella*, *Listeria*, *Staphylococcus*. The requirements in particular were for rapid tests, with a response within the same day of sampling, and easiness in performing the test.

From the analysis of the questionnaires within the project CHILL-ON, it appeared that companies are willing to perform voluntarily additional tests besides those required by legislation, within the framework of HACCP procedures. In particular, *Campylobacter* detection is of great interest for poultry producers, and methods for typing and quantifying these bacteria would be appreciated. Methods currently used require enrichment of bacteria with culturing, and therefore quantification is not possible.

Development of tests for the presence of contaminant microorganisms in poultry meat

The general objective of our work is to develop appropriate tools to monitor the presence or absence of microorganisms of interest in the chilled/frozen poultry processing chain. The final goal is to provide methods and materials along with the information to start an efficient HACCP procedure and for quality certification at national and international (ISO) level. The work includes analysis of poultry samples along the supply chain and involves the comparison between existing officially prescribed methods based on microbial culture and those based on DNA extraction and PCR analysis which are still in development and have not been validated [7, 8].

On the basis of results of the survey, the research was aimed to develop qPCR assays to detect and quantify *Salmonella*, *Campylobacter*, *Listeria*, *Staphylococcus* and *Pseudomonas*. Primers designed after in silico analysis were tested and chosen based on quality of amplicons and specificity. Results with dilution of genomic bacterial DNA showed linearity down to 1-100 copies of genomes, depending on the bacteria. Experiments with spiked meat allowed a comparison among estimates of bacterial counts obtained from plating and from RealTime PCR. Results for *Salmonella* are currently submitted for publication (Figure 1).

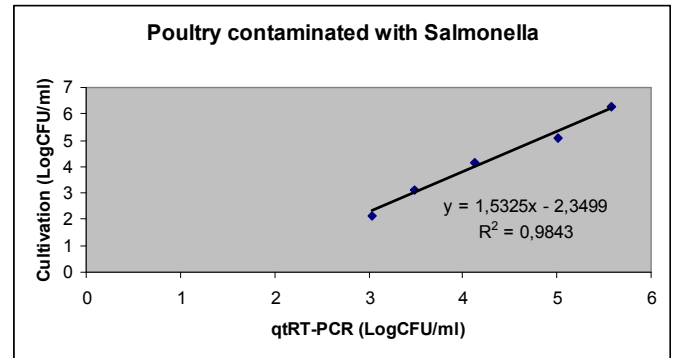


Figure 1. Linear correlation between colony count of *Salmonella* and estimation obtained through Real Time PCR. Experiments were performed with poultry meat spiked with known amounts of *Salmonella* bacteria.

In general, it is possible to establish a linear correlation among the two estimates, with RealTime PCR giving higher counts with respect to the plating system. The results will be the basis for developing Quantitative Risk Assessment in future experiments.

At the moment multiplex assays are being developed, switching from SybrGreen to TaqMan probes to allow relative quantification.

IV. CONCLUSION

Integrated approach to meat safety and traceability

Objective monitoring of safety and quality parameters along the food supply chain provides information which gives added value to the conventional traceability information [9]. Whereas traceability is usually a documentary trail accompanying movement of goods, data from objective monitoring provide additional information to be used in case of recalls, complaints, withdrawals. In the MENTORE project a specific portion of the supply chain has been addressed: long distance transportation of livestock. This sector of animal management is covered by Regulation (EC) 1/2005, advocating the use of navigation systems to track the vehicles transporting animals while at the same time acquiring data on temperature in the loading compartment. During transport, animals are accompanied by paper documentation concerning the veterinary aspects, and it would be a challenge for future developments to link the two information groups: health aspects and data on transport. Their merging would be highly beneficial in view of an integrated traceability system covering the whole animal life cycle.

The MENTORE project has addressed the use of

the European satellite navigation technology EGNOS to evaluate its value for tracking and tracing long distance transportation, in view of the future Galileo. Results have shown that economic sustainability of the system becomes interesting if the service provided combines monitoring of transportation with other service, mainly those related to food traceability.

The European legislation contains many different acts concerning food safety, quality and hygiene. Recently, several research projects have been financed by the European Commission on these topics, and results are starting to show how information attributes of food products are of high interest to consumers. More and more consumers request additional knowledge on food origin, quality parameters, safety measures. In this direction, integrated approaches to food traceability try to combine documentation of movement and logistics together with objective monitoring. Europe is now the leader in the world for research activities linked to food protection.

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