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6.3	Characterisation of the statistical distributions of microorganisms at Critical Control Points (CCPs) and the correlation structure between food risk factors and other factors	6	DKFZ	3	PU	M24

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List of Abbrevations

AIC	Akaike Information Criterion
BIC	Bayesian Information Criterion
BIOHAZ	EFSA Scientific Panel on microbiological Hazards
cfu	colony forming units
CI	Confidence Interval
CCP	Critical Control Point
EU	European Union
EFSA	European Food Safety Authority
GEE	Generalized Estimating Equations
GPD	Generalized Poisson distribution
HACCP	Hazard Analysis and Critical Control Point approach
LoD	Level of Detection
MC	Microbiological Criterion
MSs	EU Member States
ML	Maximum Likelihood
NB	Negative Binomial
NBD	Negative Binomial Distribution
PO	Performance Objective
VIF	Variance Inflation Factor

Executive Summary

BASELINE Deliverable 6.3 presents the results of the characterisation of statistical distributions of microorganisms at Critical Control Points (CCPs) and basic results on the correlation structure of food risk factors and other factors for data reported by WP1-5 up to project month 24.

Therefore Deliverable 6.3 relates to BASELINE's Sub-task 6.2.3 "Data", whose main aims are to provide statistical distributions of microorganisms at CCPs for historical and newly generated data from WP1-5 to identify risk factors and hazards for different food matrices. Task 6.3 (Biologically-based predictive models for health risks and economic impact) will use the results coming from this sub-task to develop new and modify existing predictive models.

Deliverable 6.3 has been achieved by

- describing the relevant distributions such as the single parameter Poisson distribution, the negative binomial distribution and their zero-inflated counterparts,
- by developing an appropriate approach to goodness-of-fit tests for being applied when analysing distribution fittings and regression analyses,
- emphasizing the role of visual tests and graphical presentations when examining distribution fits, and in particular, by
- characterising work package 1-5 data through descriptive statistics, frequency distributions and distribution fits as well as regression analyses

Therefore Deliverable 6.3 presents frequency distributions of all data provided by WP1-5 by this time, which were available for the following food-risk combinations

- Campylobacter spp. on broiler carcasses
- L. monocytogenes on beef
- Salmonella enteritides on eggshell
- Salmonella enteritides on pork cuts
- L. monocytogenes on pork cuts, and
- L. monocytogenes on smoked Salmon

For these food-risk combinations Deliverable 6.3 could adress at probable CCPs of

- Slaugherhouse,
- Temperature at storage,
- Storage time,
- Retail, and
- Consumer phase

For larger datasets from those available, distribution fittings and (zero-inflated) Poisson – and negative binomial regressions were performed. It is demonstrated, that in most cases the datasets can be well described by (zero-inflated) regression models assuming a Poisson distribution of the pathogen count after log transformation.

Furthermore, the association between the the food risk factors

- Temperature,
- Storage time in days,
- Time between sampling and testing,
- Year of sampling, and
- Season of sampling

and the amount of colony forming units have been determined by computing the Spearman rank order correlation coefficient. Significant relations were observed between temperature and Salmonella enteritidis concentration on egshell, time between sampling and testing and L. monocytogenes concenteration at retail, and year of sampling and L. monocytogenes during the consumer phase. Overall, however, the correlation was weak.

The data analysis provided new insights on the characterisation of statistical distributions of microorganisms at critical control points (CCPs) and the correlation structure between food risk factors and other factors for data which are detailed in this Deliverable case by case. As an example, we show here the major finding for the revalence data of Campylobacter on broiler carcasses that has been provided by courtesy of the European Food Safety Authority (EFSA).

At country evel:

- In 55% of all cases no pathogen could be detected. Considering the countries seperately, the proportions of non-detects ranged from 2- 100%.
- The distribution of the pathogen in the different European States differs significantly from the overall distribution of the total sample (of all 16 countries included in the analysis).
- Since the minimum AIC value represents the best model fit given a set of candidate models, the results of Tables 3.6 and 3.7 suggest that a basic Poisson model with just an intercept regressor gives the worst model fit.
- The zero-inflated regression model with EU country as independent variable assuming a Poisson distribution – as the more parsimonious model compared to the negative binomial regression model - produces the smalles AIC and largest log-likelihood statistics.

- All model parameter estimates are significant.The results indicate that there is a significant difference regarding the sampling results of Campylobacter on broiler carcasses sampling results between the European countries included in this analysis.

At slaughterhouse level

- In 55% of all cases no pathogen could be detected. Considering the slaugherhouses seperately, the proportions of non-detects ranged from 1.6 - 100%.
- The distribution of the pathogen in the different slaugherhouses differed significantly from the overall distribution of the total sample (of all 16 countries included in the analysis).
- Since the minimum AIC value represents the best model fit given a set of candidate models, the results of Tables 3.10 and 3.11 suggest that a zero-inflation regression model assuming a Poisson distribution of the categorised log transformed Campylobacter count data is the most adequate model if only the factor slaugherhouse is considered.

WP 6 recommends therefore in this case

- to examine samples carefully whether they fit the Poisson or the lognormal distribution and to use, when indicated and possible, generalized families of distributions, at first those generalizing the Poisson distributions;
- to use the lognormal distribution as default but to challenge its fits by an in depth investigation of the empirical sampling distribution and an active exclusion of other distribution classes;
- the use of Q-Q plots next to the Box-plot as primary mean for examining empirical distributions from microbial samples.

Similar analyses were performed for the other food-risk combinations

- L. monocytogenes on beef,
- Salmonella enteritides on eggshell,
- Salmonella enteritides on pork cuts,
- L. monocytogenes on pork cuts, and
- L. monocytogenes on smoked salmon

using a similar reporting scheme. Conclusions and recommendations are found in the subchapters of Chapter 3 of this Deliverable, respectively.

WP6 noted that with the increase of knowledge about the growth and decay of microbial pathogens, an increased use of models may provide better opportunities to measure such factors on a large scale. With the availability of more sophisticated statistical regression techniques pure distribution fitting should be augmented by more elaborate modelling. Nevertheless, an understanding of the distribution of the primary data sampled from the food population is still an important step to understand the data.

Finally, WP6 wants to point out explicitly on the inherent connection between the three Deliverables D6.2, D6.3, and D6.4 which should be recognised and taken into account when assessing the content of each of them. We therefore urge any reader to be aware of the splitting of the topic of distribution of contaminants in food for organisational as well as scientific reasons into the three parts covering the contamination by microbial (D6.3), by chemical (D6.4) and combining microbial distributions combined with known factors (D6.2). When the project plan of BASELINE was laid out, this structure and a timely characterisation of the different types of distributions was decided to fit best as comprehensive step to proceed within the BASELINE project.

Introduction

The objectives of WP 6 consisting of the

- Review of existing and definition of new mathematical models in predictive microbiology;
- Investigation of correlations between food risk factors and traceable environmental parameters and contamination indicators;
- Consolidation of existing and new models for microbial growth as a function of intrinsic environmental factors and extrinsic parameters,

aim at supporting – within the general objective of BASELINE - the development of predictive mathematical models for biological risks and to improve sampling schemes.

Models developed to predict microbial growth are an integral tool to evaluate and control the safety of a food product, which has developed from official, prescriptive control/inspection and compliance testing to goal-orientated systems like the structured approach of the so called Hazard Analysis and Critical Control Point approach (HACCP).

According to the Scientific Panel on biological hazards (BIOHAZ) of EFSA (2007) HACCP requires producers to identify hazards and eliminate or control them at Critical Control Points (CCP), which are steps in the food production and preparation process from farm to fork at which control can be applied and which are essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level. Examples are processing steps like slaughtering, thermal processing, or cooking at a specific temperature and at a specific time (point) in order to destroy microbiological pathogens. Likewise, refrigeration of a precooked food to prevent hazardous microorganisms from multiplying, or the adjustment of a food to a pH necessary to prevent toxin formation could also be CCPs.

Incorporating HACCP in the initial stages of food product development allows for an assessment of the risks associated with the product. The formal risk analysis approach concepts include concepts like the Food Safety Objective (FSO) and Performance Objective (PO), both introduced by Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.

FSOs and POs represent microbiological limits while a microbiological criterion (MC) consists of more specific elements such as the analytical method, the sampling plan, microbiological limit(s), the specified point of the food chain where the limit(s) apply, the number of analytical units that should confirm to the limit(s) and the actions to be taken when the criterion is not met.

Actions at CCPs can be taken to reduce or eliminate the risk of producing contaminated food that may lead to foodborne diseases. The method is used at all stages of the food production and preparation process including packaging and distribution. Therefore, CCPs should be carefully developed and documented.

The aim of D6.3 is to provide information regarding the distribution of micobiological pathogens at possible CCPs for specific food products, at which control may be applied to prevent or eliminate a food safety hazard or reduce it to an acceptable level.