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Modelling and magnitude estimation of cross-contamination in the kitchen for quantitative microbiological risk assessment (QMRA)

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Abstract

In the kitchen of the consumer, two main transmission routes are relevant for quantitative microbiological risk assessment (QMRA): the *cross-contamination* route, where a pathogen on a food product may evade heating by transmission via hands, kitchen utensils and other surfaces, e.g. to non-contaminated products to be consumed raw; and the *heating* route, where pathogens remain on the food product and are for the most part inactivated through heating. This project was undertaken to model and estimate the magnitude of cross-contamination in the domestic environment. Scientific information from the relevant literature was collected and analyzed, to define the cross-contamination routes, to describe the variability sources and to extract and harmonise the transfer fractions to be included as model parameters. The model was used to estimate the relative impact of the cross-contamination routes for different scenarios. In addition, the effectiveness of several interventions in reducing the risk of food-borne diseases due to cross-contamination was investigated. The outputs of the model showed that the cutting board route presents a higher impact compared to other routes and replacement of the kitchen utensils is more effective than other interventions investigated; the transfer to other surfaces and objects, which can house bacteria in the environment, is also described. Laboratory cross-contamination trials have been performed to estimate bacterial transfer via cutting, from the external surface of the meat to the cutting surfaces and to the knife. The results, obtained from the laboratory trials, show magnitudes of and differences in the bacterial transfer fraction to the knife and the cutting surface in relation to which side of the meat is contaminated. Despite the complexity of factors which influence bacterial transfer, the combination of laboratory work with mathematical modelling enhanced scientific understanding and appreciation of the uncertainty of the estimates. QMRA methodology results in magnitude estimation of cross-contamination in the kitchen and evaluation of intervention strategies.

Keywords: QMRA, cross-contamination, food-borne pathogens, risk assessment

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1. Introduction

Quantitative microbiological risk assessment (QMRA) is a methodology used to organise and analyse scientific information to both estimate the probability and severity of an adverse event as well as prioritise efforts to reduce the risk of food-borne pathogens (Habib et al., 2020). Modern food safety considerations are based on 'farm to fork' (or 'stable to table') QMRA and encompass all the steps of the agrifood chain until the food is consumed. However, when the food reaches the domestic environment, how to estimate the risk carried by the 'fork' directly into the mouth of the consumer? The European Union One Health 2018 Zoonoses Report publishes information on food-borne and waterborne outbreaks as provided by EU Member States according to Directive 2003/99/EC, and states that 40.5% of strong-evidence outbreaks occurred at home and 15.6% of the outbreak cases were due to contaminated food within the domestic environment. 'Cross-contamination' is identified as one of the contributory factors of the strong-evidence outbreaks.

The term 'cross-contamination' stands for the transfer of bacteria or viruses from a contaminated food, raw material, kitchen utensil or person, to other foods, whether it occurs directly or indirectly (Manios et al., 2015). Bloomfield and Scott (1997) state that, when assessing the risk of food-borne diseases associated with cross-contamination, the microbial contamination level on the surfaces and the probability of the transfer to the food should be taken into account. In addition to this, the estimation of the bacterial fraction transmitted to the different cross-contamination routes (i.e. hand to salad, meat to cutting board, etc.) appears of paramount importance for the correct estimation of the overall impact of cross contamination.

The available literature which describes bacterial transfer between contaminated source (e.g. meat) and recipient (e.g. cutting board) shows that the cross-contamination process is complex and affected by many factors associated with the characteristics of the pathogen, the surfaces and the contact process (e.g. pressure applied, duration of the contact) where findings might be also contradictory (e.g. the effect of the initial bacterial concentration on the transfer fraction). Quantitative data to describe the transfer of microorganisms from a contaminated source to a recipient are fundamental to carry out a risk assessment.

Mathematical modelling is a powerful tool for further investigations by including different scenarios and comparison between interventions. Data to feed the model are often not readily available and in some cases also contradictory, thus making explicit the difficulties in describing the phenomenon (Pérez-Rodríguez et al., 2008, Hoelzer et al., 2012). In addition, the variability in experimental set up used by researchers does not allow for a straightforward ascertainment of the effect of different variability sources using these cross-contamination experiments. Monte Carlo simulation can be performed to describe the uncertainty and variability associated with risk (Vose, 2008), in this case the risk of cross-contamination.

2. Description of work programme

During food preparation in the kitchen of the consumer, two main transmission routes leading to human ingestion are possible: the *cross-contamination* route, where a pathogen may evade heating by transmission via hands, taps, raw vegetables, etc., and the *heating* route, where pathogens remain on the food product (e.g. meat) and are partly inactivated through heating.

The importance of the *heating* route has been described by the research conducted at RIVM within the EU-FORA fellowship programme 2017–2018, while the investigation of the *cross-contamination* route for theoretical and practical implementation of this aspect in food safety QMRA was the purpose of the EU-FORA fellowship programme 2019–2020.

The work programme was carried out at the Centre for Zoonoses and Environmental Microbiology (Z&O) at RIVM, The Netherlands, which has an extensive experience in performing risk assessments in food, water and environment.

The work programme was structured as follows:

i) Define which cross-contamination routes (hand to tap, knife to vegetables, etc.) are potentially relevant;
ii) Define a theoretical model of cross-contamination for each transmission route, taking into account that results are presented in a rather variable way in cross-contamination literature;
iii) Define the variability that is to be included in the estimation of the intensity of cross-contamination. This concerns, e.g. differences between pathogens, whether pathogens are located only on the surface of a product or also inside, and differences in the preparation

and heating process in the kitchen of the consumer. The variability to be included is limited by the availability of variability information for the description of the realistic kitchen process;

iv) Perform a literature study on cross-contamination, during which the starting points above can be updated. The output of this literature study will be the set of quantitative data and estimates that are available at present in literature to describe cross-contamination.

v) Combine and integrate the information and estimates obtained into a framework model that describes the network of cross-contamination routes that occur in the kitchen of the consumer and estimates the final intensity of cross-contamination. This framework model will be customisable, in terms of both transmission routes and parameter values, as a function of the relevant variability aspects defined before.

vi) Estimation of the cross-contamination magnitude by simulating different scenarios.

vii) Estimation of the effect of (hygiene) interventions.

The work programme was extended with *laboratory experiments* conducted at Z&O at RIVM, The Netherlands, describing the bacterial transfer from meat spiked on the external surface to the cutting surface during the process of slicing with a knife. The laboratory trials included the setting up of the experiments, the estimation of the recovery performances of the methods used and the analysis of the original data using modelling and statistics, for future scenario analysis and integration in food safety QMRA.

2.1. Aims

The activities of the work programme were aimed at estimating the magnitude of cross-contamination in the kitchen, during the preparation of a meal, for QMRA.

For the *modelling* part of the working programme, the aim was:

i) To assess the fraction of bacteria that was in the raw food and overpassed all the steps till arriving from the same product/other products/utensils/hands to the mouth of the consumer;

   a) By calculating what is the number of colony forming units (CFU) that reach the mouth of the consumer due to cross-contamination in the kitchen, in relation to the number on the raw meat.

ii) To compare scenarios and assess the effect of interventions on the fraction of bacteria that are cross-contaminated.

For the *laboratory* part of the working programme, the aim was to investigate a specific cross-contamination step, estimating:

i) The fraction of bacteria on meat surface transferred to cutting surface during meat slicing, as these bacteria will experience a different heating regime during meat preparation compared to the bacteria that remain on the original meat surface;

ii) Transfer from meat to knife and subsequent transfer from knife to meat. This last aspect is important as bacteria remaining on the knife will be transferred also to uncontaminated pieces of meat at following cuts made by this knife.

2.2. Activities/methods

2.2.1. Modelling cross-contamination in the kitchen

In order to model and to estimate the magnitude of cross-contamination in the kitchen for QMRA, the approach was structured as described in the work programme.

i) *List of cross-contamination routes*: The starting point of the model is a contaminated food (raw meat) which can be in contact with hands ('meat to hands'), with the cutting board ('meat to cutting board') and with the knife ('meat to knife') during the preparation of a meal. After the contact with the contaminated source, hands, cutting board and knife can transfer bacteria to other products (i.e. salad) which are not exposed to any further heat treatment before reaching the mouth of the consumer; routes which are defined as 'knife to salad', 'cutting board to salad', 'hand to salad'.

In addition, the hands can transfer bacteria to any surface or other inanimate objects which will become contaminated and hold bacteria partly in the environment (such as handlers of the drawers,

kitchen utensils or kitchen counter), so called 'hand to fomite' route. The possibility of a direct contact between hand and mouth of the consumer has also been included and described by the route 'hand to lip'.

Taking into account that after the first contact the transfer of bacteria will occur also in the opposite direction (e.g. 'hand to meat'), bidirectionality of the transfer has been included.

ii) The *model* for transmission from source to recipient is the fraction (1) and the uncertainty is described by Beta-distribution.

$$\text{Transfer fraction} = \frac{\text{no. of CFU on the recipient}}{\text{no. of CFU on the source}} \quad (1)$$

iii) *Variability sources*. Bacterial transfer from one surface to another is a complex process: given the differences between the experimental set up available in investigations published in literature, the variability sources for each cross contamination steps have been investigated within the same experimental trial. This analysis allows the definition of the variability sources to be included in the mathematical model, within dedicated scenarios.

iv) Available data on bacterial transfer have been extracted from the scientific literature and collected in a *literature database*, structured to include information on the food-borne pathogen, the cross-contamination route (source, recipient, description of the step), values as reported by the authors, values expressed as mean, standard deviation, alpha and beta parameters of the beta distribution after harmonisation of the data, and relevant details on the paper. Each row of the file describes in detail one step of cross-contamination. The literature database allows to retrieve information by selecting the field of interest (for example, authors or cross-contamination step). Data from papers which describe transfer for cumulative or combined steps (i.e. from meat to salad or from meat to cutting board and knife) are presented in the literature database but not included in the mathematical model. Even though many papers describe cross-contamination events, in order to be able to include the data in the model, harmonisation of the data from the literature was necessary using consistent criteria.

v) *Mathematical model.* The model was developed in Excel and @Risk, as deterministic and probabilistic model and it is structured using 8 steps which mimic the preparation of a meal, which starts with raw meat and ends with a salad. The final output of the model is the fraction of CFU originating from the raw meat that reaches the mouth of the person preparing and eating the meal. It is also possible to describe the distribution of CFU among the different recipients included in the model, expressed as number of CFU per recipient and as a percentage of the number on the raw meat.

vi) *Scenarios.* The 'chicken-salad' scenario was defined as baseline scenario to describe a condition of surface contamination while the 'ground beef-salad' scenario was included for contamination of the surface and the interior of the raw meat. The model allows to include also the investigation of the estimation of cross-contamination in case of a 'next meal scenario', which stands for the preparation of a meal, using the same utensils after a certain timeframe. An example of the output of the Monte Carlo simulation done in @Risk is represented in Figure 1.

vii) *Interventions* included in the model are washing hands, cutting board or knife – by describing the fraction of bacteria which remains after washing – replacement of the kitchen utensils and the order of actions.

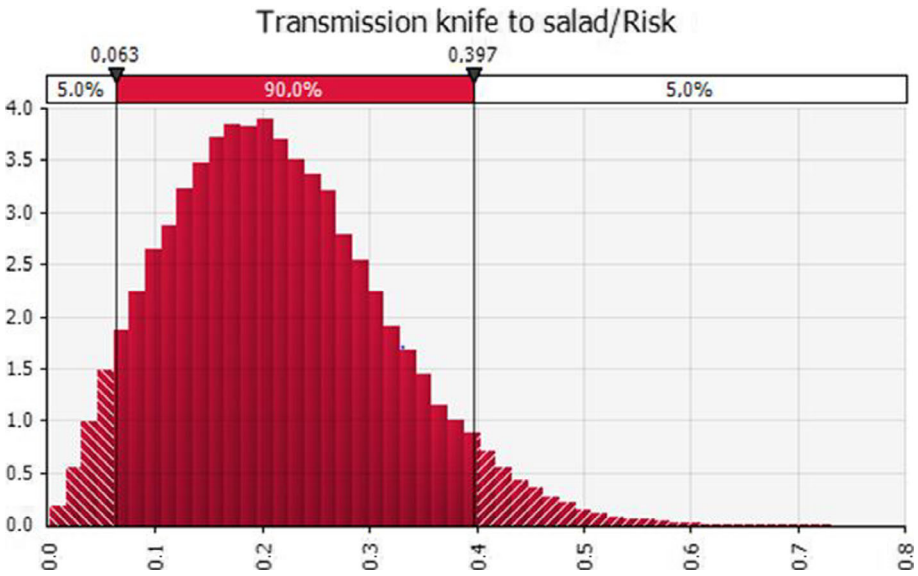

Figure 1: Probability density function describing variation of fraction of bacteria transmitted from knife to salad

2.2.2. Laboratory experiments

The presence of food-borne pathogens on food and food contact surfaces represent a concern. Many factors can influence the adhesion of microorganisms and the process of attachment can start in less than 5 seconds and can vary according to different substrates (Miranda and Schaffner, 2016). Laboratory trials available in literature describe cross-contamination and bacterial transfer with different approaches and laboratory techniques), and some authors provides insight on the bacterial transfer 'from meat (or vegetable) to knife' via the cutting process (Zhao et al., 1998; Luber et al., 2006; Ravishankar et al., 2010; Zilelidou et al., 2015; Sarjit and Dykes, 2017).

From October 2019 to July 2020, experimental trials have been conducted at RIVM laboratories focusing on the *cutting process* and estimating not only the bacterial transfer 'from meat to knife' but also 'from knife to the cutting surfaces' (Figure 2). Importantly, as a preliminary step, the performance of different recovery methods has been investigated to choose the preferable one to implement.

2.2.2.1. Recovery performance trials

Recovery of CFU from spiked meat and knife have been performed as follow: after spreading a solution with known concentration of CFU/ml of *Escherichia coli* O111:H2 on the meat, quantification of the bacterial strain was conducted by a direct contact method (i.e. agar stamp) (Figure 3) and by swabbing (using a cotton swab to recover bacteria and to release them into a physiological solution during vortexing, followed by plating). The recovery from a spiked knife was conducted by rinsing, by combining rinsing and swabbing, and by swabbing. Viable plate count (the number of CFU on the agar plates) was compared with the number of CFU on the inoculum to quantify the recovery performance of the method.

2.2.2.2. Meat slicing experiment

As mentioned in the previous section, the aim of the *meat slicing experiment* was to estimate the transfer fraction from an externally spiked meat to the cutting surfaces, taking into account also the intermediate transfer step to the knife.

After the inoculum of a known concentration of CFU/ml of *E. coli* O111:H2 was spread on the surface of beef meat and overnight storage at refrigeration temperature, the meat was cut to obtain two symmetrical slices, exposing two cutting surfaces (A and B); the four sides of the meat (top side, front side, back side and bottom side) were investigated separately as contamination source. Immediately after cutting, the two cutting surfaces were put in contact on Tryptone Bile X-Glucuronide (TBX) Medium agar (agar stamp method), followed by incubation at 37°C for 18 h and viable plate counting on the next day. The process of spiking and cutting was performed always by the same operator, to eliminate this variability source.

In order to investigate the transfer to the knife, the blade of the knife was rinsed on both sides with physiological salt solution and plated on TBX agar, being the preferred method. The trials have been repeated at least four times for each sides.

The analysis of the data was aimed to obtain the values of two parameters: t_1, which describes the transfer from the spiked area of the meat to the knife, and t_2, which estimates the transfer fraction from knife to the cutting surface of the meat. For the enumeration of the fraction of bacteria transferred from the contaminated source to the recipient aspects such as the relative contact surface and the recovery performance of the methods have been taken into account.

Figure 2: Process of cutting (*from left to right*) resulting in exposure of the cutting surfaces

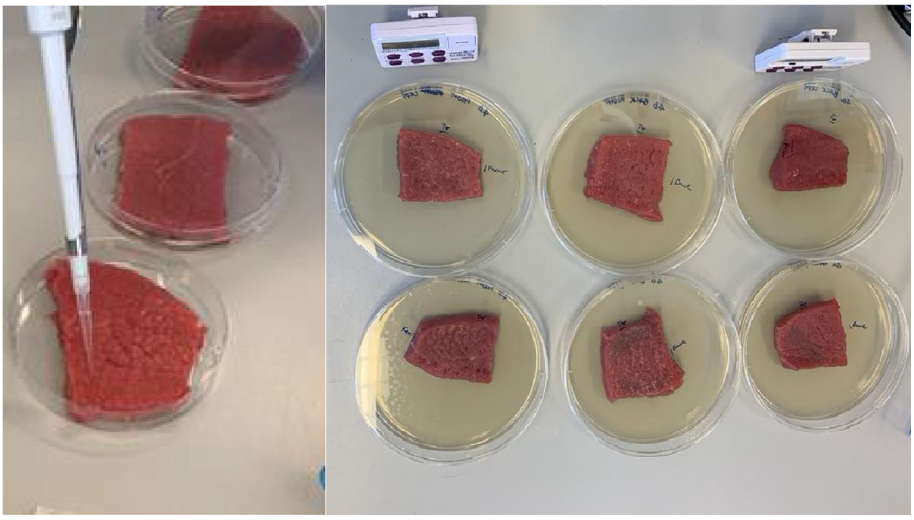

Figure 3: Recovery performance experiment: spiking of the meat *(left)* and meat agar stamp method application *(right)*

3. Conclusions

Transfer of bacteria from a source to a recipient is considered as a cause of food-borne disease (Pérez-Rodríguez et al., 2008). Cross-contamination refers to the direct or indirect transfer of bacteria/ viruses from a contaminated food product to a non-contaminated product (Pérez-Rodríguez et al., 2008, Evers, 2015). This phenomenon is usually associated with contaminated equipment and poor hygiene practices and its occurrence in the consumers' kitchen can be related to disease cases.

The project executed within the EU-FORA fellowship programme provides new valuable modelling and data on cross contamination for QMRA. The cross-contamination model is able to estimate the fraction of bacteria that reaches the consumer for the different scenarios and allows to estimate the importance of the different cross-contamination routes in the transfer of bacteria from contaminated meat to the final dish. The cutting board route presents a higher impact compared to other routes;

moreover, the transfer route 'from hand to fomites' should not be neglected, given the fact that bacteria from the kitchen environment can be reversely transferred to food. Furthermore, the model allows also to estimate the effect of the interventions applied and can help risk managers in defining the best advices to reduce the impact of cross-contamination.

The laboratory recovery trials conducted gave more insight in the microbiological detection methods which could be applied to bacterial transfer investigation. Concerning the results of the trials, it is possible to conclude that the agar stamp method could be an alternative to sampling by a destructive method, giving the possibility to define exactly the area of investigation of the product, represented by the relative contact surface and taking into account only the bacteria available for the transfer. Concerning the knife sampling, the rinsing methods showed higher recovery values compared to swabbing and the combination of rinsing and swabbing. The laboratory experiments on meat cutting provide insight in the complexity of the action of cutting. The transfer from the relative contact surface of the meat (spiked area in contact with the knife) to the knife, named t_1, is high and similar among the sides with the exception of the bottom part which appears lower. The transfer from knife to cutting surfaces, named t_2, is very high, probably due to the characteristic of the blade which enables the detachment of bacteria during the slicing.

The EU-FORA programme allowed the fellow to familiarise with QMRA with a 'learn by doing' approach: from the collection, analysis and harmonisation of data from the scientific literature to the setup of a mathematical model on cross-contamination. Furthermore, combining laboratory work with mathematical modelling can boost scientific understanding and appreciation of the underlying processes and uncertainty of the estimates.

The fellow was exposed also to the best-suited statistical methods to describe the uncertainty associated with microbiological data. In addition, the fellow had the opportunity to become familiar to @Risk, software for risk assessment, which allows for Monte Carlo simulations. The fellow was actively involved in the activities carried out at Z&O (more details in the Appendix section), and attended meetings and seminars organised during the year. The EU-FORA fellowship programme provided an opportunity for a fruitful exchange and collaboration between fellow and supervisor.

3.1. Future goals

The EU-FORA fellowship programme set the basis for future collaboration between the fellow and the hosting site. Further steps foreseen are the publication of the cross-contamination model and the results of the laboratory experiments in scientific journals.

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Abbreviations

CFU	colony forming unit
ECDC	European Centre for Disease Control
EU-FORA	European Food Risk Assessment
QMRA	Quantitative microbiological risk assessment
RIVM	Rijksinstituut voor Volksgezondheid en Milieu – National Institute for Public Health and the Environment, The Netherlands
Z&O	Centre for Zoonoses and Environmental Microbiology

Appendix A

	Description	Date
Training sessions	Workshop Next Generation Sequencing: One tool fits all!	23.1.2020
	Git basic course (RIVM)	7.4.2020
Meetings with scientific presentations	Weekly meeting with Z&O Modelers, with scientific presentations, active interactions and exchange of advices; including discussions and updates on the course of the fellowship project	On Mondays
	Weekly research meeting with the members of the Voedselgroup (Food group), with scientific presentations, exchange of knowledge, including updates on the course of the fellowship programme	On Thursdays until March and on Mondays from April
	Monthly meeting of the centrum Z&O. Meeting with two scientific presentations from the members of the centrum on the activities conducted at RIVM	1.10.2019 5.11.2019 10.12.2019 7.1.2020 4.2.2020 9.4.2020 7.5.2020 11.6.2020 9.7.2020
	'Interactions among infectious agents: Why they're important and how to detect them' (University of Georgia)	10.10.2019
	'Microbiome-mediated defence against enteric infections' (LUMC)	15.10.2019
	'Metagenome analysis for parasites' (Z&O, RIVM)	19.11.2019
	'Climate & Health' (Santé Publique France & RIVM)	16.1.2020
	ZOMAR meeting with presentations of the results from research conducted on antimicrobial resistance	15.10.2019 11.2.2020 17.12.2019
	SIM (Statistiek, Informatica en Modellering) colloquium	14.2.2020
	EPI (Epidemiologie en Surveillance van Infectieziekten) referee presentations	14.11.2019 14.5.2020 25.6.2020
Conferences	One Health EJP – Annual Scientific Meeting	27–29.5.2020
Scientific presentations by the fellow at RIVM	Presentation on previous scientific achievements at the research meeting of the 'Food group' of Z&O centrum	10.10.2019
	Presentation to the 'Modelers group' on the EU-FORA project with focus on the mathematical model	11.5.2020
	Presentation at the research meeting of the 'Food group' of Z&O centrum on the EU-FORA project with focus on the laboratory trials results	8.6.2020
	Presentation 'Cross contamination in the kitchen: modelling and measuring' at the Z&O centrum meeting	9.7.2020
Invited presentation	'EFSA: esperienze a confronto' – Invited contribution on the experiences related to EFSA, online event organized by the University of Perugia (IT)	22.5.2020

	Description	Date
Webinars	Introduction to Risk Analysis using @RISK	22.10.2019
	Webinar global webinar: COVID-19 and companion animals – what we know today (WSAVA)	17.4.2020
	Emerging respiratory viruses, including COVID-19: methods for detection, prevention, response and control (WHO)	17.4.2020
	EFSA Webinar Rapid Assessment of Contaminant Exposure (RACE)	27.4.2020
	Practical use of NGS (One Health EJP)	30.4.2020
	Webinar on Coronavirus detection methods (Istituto Superiore di Sanità, IT)	18.5.2020
	Webinar on Novel Foods and new plant breeding techniques (Foodhub)	3.6.2020
	EFSA webinar – High-risk plants – how does the EU carry out risk assessment of plant commodities?	26.6.2020
	Pandemic! A one health view of emerging infectious diseases	30.6.2020