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Application of data science in risk assessment and early warning

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Abstract

The food supply chain has been recognised by the EU as a critical infrastructure, and its complexity is the main cause of vulnerability. Depending on the food matrix, natural and/or deliberate contamination, foodborne diseases or even food fraud incidents may occur worldwide. Consequently, robust predictive models and/or software tools are needed to support decision-making and mitigating risks in an efficient and timely manner. In this frame, the fellow participated in data collection and analysis tasks, so as to provide additional predictive models. The working programme, covered a wide range of aspects related to risk assessment including identification of emerging risks (quantitative), microbiological risk assessment, authenticity assessment, spatio-temporal epidemiological modelling and database formation for hosting predictive microbial models. The training and close integration, in the open-source, in-house (German Federal Institute for Risk Assessment (BfR)) developed software tools under the framework of FoodRisk-Labs (https://foodrisklabs.bfr.bund.de.) for data analysis, predictive microbiology, quantitative microbiological risk assessment and automatic data retrieval purposes allowed for the independent use. Moreover, the fellow actively contributed to the update of the upcoming Yersinia enterocolitica risk assessment, and also in authenticity assessment of edible oils. Over the course of the year, the fellow was closely involved in international and national research projects with experts in the above-mentioned disciplines. Lastly, he consolidated his acquired knowledge by presenting his scientific work to conferences, and BfR-internal meetings.

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1. Introduction

1.1. General Description of the followed programme

The project took place in the department Biological Safety (Dep. 4) of the German Federal Institute for Risk Assessment (BfR) and was supervised by the Unit Food Hygiene and Technology, Supply Chains, Food Defense (Unit 41) of this department. The department Biological Safety deals with health risks for humans, which may arise more particularly from microorganisms, the toxins formed by them and other microbial metabolites. This includes bacteria but also viruses, parasites and TSE pathogens. The department is involved in establishing the cause of outbreaks of food-borne diseases and zoonoses. It has a number of national reference laboratories for the diagnosis and fine typing of pathogens, antibiotic resistance and the microbiological contamination of foods (a task anchored in food legislation). In this scope, Unit 41 deals with the identification and evaluation of hazards that may be present in food. Performing vulnerability assessments concerning these hazards and developing risk mitigation strategies are related unit's tasks. Unit 41 is furthermore involved in microbiological risk assessments (MRAs) and provides the national expert for the European Food Safety Authority (EFSA) Scientific Network on MRA. Other key foci are national and international research projects that aim at the development of new data and knowledge-driven models supporting the efficient generation of risk assessments. In this context, several open-source software tools have been developed under the umbrella of FoodRisk-Labs (https://foodrisklabs.bfr.bund.de) which aim to facilitate the generation of quantitative microbial risk assessments (QMRAs). Examples are Predictive Microbial Modelling Lab (PMM-Lab; aims to ease and standardise the statistical analysis of experimental microbial data and the development of predictive microbial models), FoodProcess-Lab (for the application of predictive microbial models on food process chains), Food Safety Knowledge Lab (FSK-Lab: an open-source software supporting exchange of risk assessment models) and the open Food Safety Model Repository (openFSMR; a community-driven search engine for predictive microbial models). Parallel activities are the creation of data analysis pipelines supporting food safety research questions and national and international research projects, like: FoodAuthent, RAKIP, AGINFRA+, DEMETER. Under the internal BfR structure, the Unit 41 is highly connected with other units of BfR, such as Unit 83, performing among others assessments of food integrity by means of untargeted analytical methods.

The fellow being part of the Unit's risk assessment team was supervised by two senior research scientists of Unit 41: Dr. Anja Buschulte, Veterinarian, Senior Research Scientist and Matthias Filter, Biochemist, Senior Research Scientist. Further support was given by other research scientists of the Unit with many years of professional background in the field of either performing MRA and/or data analysis or software development. Cross-unit activities gave the opportunity to get a closer insight into other risk assessment-related issues.

1.2. *Y. enterocolitica* in various food matrices

Codex Alimentarius recommends following and applying risk-based approaches and metrics that can more directly and transparently establish the stringency of control measures (Codex Alimentarius, 1999). This supports the statement by Roberts and Jarvis (1983), 'a mathematical model that quantitatively describes the combined effect of the environmental parameters can be used to predict growth, survival or inactivation of a microorganism and thereby contribute important information about product safety and shelf-life'. In the end, such models could be applied in the whole food-supply chain. The employment of models may be useful for decision-making purposes to prevent risks for human and animal health (Prandini et al., 2008). Indeed, numerous publications, models and data sets describing the behaviour of microbial hazards in food already exist. However, this information is widely spread in the scientific literature and usually not available in a harmonised format. Therefore, structured and annotated databases that bring this information together could efficiently close this gap.

According to annual reports of the European Food Safety Authority (EFSA and ECDC, 2018), *Campylobacter* spp., *Salmonella* spp. and *Yersinia enterocolitica* are the three most common enteropathogenic bacteria responsible for food-borne disease. *Y. enterocolitica* belongs to the family Yersiniacea and the genus Yersinia. Despite the numerous species of this genus, only virulent strains of the species *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica* have been found to be human pathogens (Cornelis et al., 1998; von Altrock et al., 2015). *Y. enterocolitica* and, to a much lesser extent, *Y. pseudotuberculosis* are the causative agents of the so-called Yersiniosis, a gastrointestinal disease of humans. Despite this fact, the identification of virulent strains is still a challenge (Bancerz-Kisiel et al., 2018).



Epidemiological studies have shown that Y. enterocolitica occurs in various niches, such as humans, animals, food and the environment (Rahman et al., 2011). The majority of isolates recovered from asymptomatic carriers, infected animals, contaminated food, untreated water and contaminated environmental samples are non-pathogenic having no clinical importance (Fredriksson-Ahoma and Korkeala, 2003). Other non-food-related sources of Y. enterocolitica could be domestic animals, such as dogs and cats (von Altrock et al., 2015; Duan et al., 2017). The main reservoir of Y. enterocolitica is the pig. Even though Yersiniosis can be caused by different foods and water, there is evidence of the link between pigs, pork carcasses and wild boars and associated products (Fredriksson-Ahomaa et al., 2007; Virtanen et al., 2012; EFSA BIOHAZ Panel, 2014; von Altrock et al., 2015). Prevalence data in pork and minced meat and a study by the Robert Koch Institute (RKI; Rosner et al., 2012) indicate that the consumption of raw minced pork, probably as so-called 'Mett' or 'Hackepeter', can be considered the most important risk factor for Yersiniosis in Germany (DE). These traditional German pork products are 'ready to eat' and considered as eaten raw, as they consist of raw pork meat, which has only be prepared, e.g. with the addition of spices. Besides, Yersinia spp. is also found in raw milk, which can serve as an excellent medium for reproduction due to its pH value and chemical composition. Furthermore, outbreaks linked to fresh produce (ready to eat salads, spinach) have also been documented (Sakai et al., 2005; MacDonald et al., 2012; Espenhain et al., 2019). For instance, vegetables can be contaminated with this pathogen through direct contact with wildlife faeces or through contaminated water, e.g. during irrigation, harvesting or transport (EFSA, 2007).

Y. enterocolitica is able to grow in a wide temperature range $(0-42^{\circ}C)$ and the ability of this species to multiply at low temperatures is of considerable concern. In this context, it is particularly important that Yersinia spp. can even grow at -2° C in fresh pork (EFSA BIOHAZ Panel, 2014) and can survive and remain infectious for several weeks in frozen food (BfR, 2013). Common heating methods, such as cooking and pasteurisation, eliminate the pathogen. Heating to a core temperature of at least +70°C for 2 min is deemed to be sufficient to eliminate Yersinia (BfR, 2013). However, insufficient cooking and pasteurisation (BfR, 2013; Longenberger et al., 2014), and/or post-process cross-contamination still remain a threat. The species Y. enterocolitica consists of six biotypes (BT) and more than 50 different serotypes which are very heterogeneous in terms of their pathogenic potential. At European level, the strains of BT 4 (serotype O:3) and BT 2 (serotype O:9) are often associated with clinical cases in humans (EFSA, 2007). In DE and in the European Union (EU), Y. enterocolitica strains of bioserovar 4/O:3 are the most common cause of Yersiniosis (RKI, 2019). In terms of pathogenesis, Y. enterocolitica is responsible for a wide variety of clinical manifestations, ranging from mild gastroenteritis (abdominal pain and diarrhoea) to invasive syndromes like terminal ileitis and mesenteric lymphadenitis to death (CDC, 2016). At present, only limited data on real prevalence are available. This may be explained by the low recovery rates of isolates from food samples, which in turn may be due to the limited sensitivity of cultivation methods Fredriksson-Ahoma and Korkeala (2003). The ail gene, which is associated with the pathogenicity of Y. enterocolitica, has in general a low prevalence among the Y. enterocolitica isolates (Bancerz-Kisiel et al., 2018). To our knowledge, little information related to the minimum infective dose is published. Robins-Browne (2013) reported that a median infective dose for Yersiniosis in humans is not known but is likely to exceed 4 log colony-forming units (cfu). The Public Health Agency of Canada states an infective dose of 10⁶ cells (Public Health Agency of Canada). Lastly, Bhunia (2008) reported that generally a high dose of seven to nine log cells of Y. enterocolitica is necessary to cause the disease. Furthermore, it can be assumed that the infective dose depends on the food matrix and the immune status of exposed consumer groups (BfR, 2013).

In summary, the consumption of raw minced pork has been shown to be the main risk factor for Yersiniosis infections in DE (Rosner et al., 2012; BfR, 2013). The EFSA estimates that the largest proportion of food-borne outbreaks occurring in the EU originate in the household, with meat and meat products being the most frequently implicated food (EFSA, 2016). Typical raw pork products such as Mett, fresh raw sausages and fermented products, which are very popular in German-speaking countries, therefore pose food safety challenges (Lücke and Zangerl, 2014). Against this background, the question arises to what extent these typical products must be considered as potential sources of infection and what influence household practices have in this context.

1.3. Food integrity in the food supply chain/Vulnerability assessment in various food matrices

Globalisation and the growing complexity of the food chain, as well as the recent food scandals (e.g. melamine in milk, horsemeat in lasagne, fipronil in eggs and adulterated edible oils) have become



a challenge for food safety authorities. Food fraud/adulteration is according to Elliott (2014) considered as food crime, thus, violation of the general food law (European Commission, 2002). In parallel, EFSAs emerging risks infrastructure has categorised food authenticity and fraud as 'medium-term issues' (medium levels of uncertainty) based on the knowledgeability (EFSA, 2018). One of the most reported Rapid Alert System of Food and Feed (RASSF) cases, especially at border inspections, is related to mislabelling and/or food fraud topics. Among the several outcomes of the emerging risks framework include issues related to edible oils (EMA, 2017). Taking into account all the above, one of the proposed actions is a predictive analysis that allows to derive trends or patterns from the data which help to determine what the driving activities and behaviour are in order to predict which future problems/threats are likely to emerge (EFSA, 2018). In this concept, BfR is involved in the development of a so-called Emerging Risk Knowledge Exchange Platform (ERKEP) within the DEMETER project and explores rapid, simple and in-field applicable fingerprinting methodologies to identify food fraud, e.g. by performing preliminary screening of edible oils.

2. Description of work programme

2.1. Aims

The overall aims of the fellowship project were:

- Learning best practice on data analysis principles (including transparency, harmonisation, validation, documentation and good data analysis practices).
- Getting insights about tasks of the department concerning MRA with emphasis on the use of scientific data for risk assessment.
- Getting familiar with open-source software tools for data analysis and data mining KNIME, R and emerging risk identification frameworks as e.g. SiLeBAT-NewsRadar.
- Getting expertise in tools for predictive microbial modelling (PMM-Lab, Combase and FSK-Lab).
- Getting expertise in relevant tools for QMRA (FSK-Lab, FDA-iRISK) and spatio-temporal epidemiological modelling (STEM).
- Going deeper in the databases concepts and participate in the update of the openFSMR.
- Participation in experimental research together with data analysis tasks in a microbiological and a chemometrical lab.

The Unit 41 aimed to integrate the fellow in upcoming MRA tasks of BfR, such as an update of the BfR 'Y. *enterocolitica* risk assessment'. In this way, the fellow extends his knowledge in the field of MRA, acquires additional theoretical background and additional lab experience. Finally, it was agreed to offer to the fellow the opportunity to participate in the exploration of the potential of a new handheld NIR sensor for the rapid and reliable authentication and identification of possible adulterations of edible oils.

2.2. Activities/methods

The fellow got close insight into the area of (quantitative) MRA. He participated in BfR internal activities in this area and explored the currently ongoing software tools for the related community. Fellow-specific trainings were organised from the Unit 41, such as on KNIME, FSK-Lab, PMM-Lab, STEM and KNIME-based FoodAuthent workflows. In addition, the fellow was closely involved in related international and national research projects (AGINFRA+, DEMETER, RAKIP and ORION) carried out in Unit 41 and participated actively in workshops and trainings organised in these projects. Apart from that the fellow joined actively the weekly team meetings that monitor the status of the group members` work. Other related training opportunities provided by Unit 41, Department 4 and BfR were various seminars, symposia and meetings, such as Junior Research Group Meetings, PreDoc Symposium, Colloquium presentations, Modellers Meeting and *Yersinia* risk assessment meetings. Additionally, the fellow participated in externally activities proposed by BfR researchers. For instance, he attended the winter semester 2018/2019 course on `Infectious disease epidemiology' and Colloquium for Statistic Methods at the RKI, Berlin, DE. Last but not least, the fellow participated in two International Conferences:

- i) the conference co-organised by EFSA and BfR on 'Uncertainty in Risk Analysis', with a workshop on 'Accounting for uncertainty in decision making' and the title: 'Accounting for uncertainty in data-poor scenarios: cases studies on risk analysis in food safety' in Berlin, DE
- ii) the 'European Symposium on Food Safety' of the International Association of Food Protection (IAFP) in Nantes, France.



In detail, the fellow pursued the following research during the 1-year internship:

- A) Update of the openFSMR. openFSMR is a user-friendly tabular web portal, containing detailed meta-information on each predictive microbial model that is available in a third party software tool or as a publicly accessible files in the Predictive Modelling in Food Markup Language (PMF-ML) format. PMF-ML is a software-independent information exchange format for models specifically designed for predictive microbial models. Following the proposal of Plaza-Rodriguez et al. (2015) to establish food safety model repositories, a screening of the literature for integrated microbial models in software took place. Extracted information from Tenenhaus-Aziza and Ellouze (2015), and other known and unknown sources lead to 17 publicly available or commercial software tools. Each was screened individually for each separate model. The different input and outputs model parameters were recorded and grouped. A data matrix was prepared in the openFSMR Google sheet, with rows representing the integrated tertiary model describing the metadata following MIRIAM guidelines (Le Novere et al., 2005) and the input parameters of each model. The results of this study as well as the final database (https://sites.google.com/site/openfsmr/) were presented as a poster at the European Symposium of Food Safety of the International Association for Food Protection (IAFP) 2019 in Nantes, France. The aim of this database is to provide risk assessment authorities and food business operators a mapping of ready to use available predictive microbial models.
- B) Research on data sources to support modelling and potential risk assessments on Hepatitis E and A virus (HAV and HEV) in foods. As only limited data could be identified to estimate the exposure to HEV from food, this research was used to get deeper understanding of the FDAiRisk risk assessment tool. In parallel, published data related to inactivation of the viruses were collected and analysed with PMM-Lab.
- C) Re-implementation of published microbial models. Published validated microbial models were re-implemented using the in-house software PMM-Lab, FoodProcess-Lab and R – specifically, the models on growth and survival of *Listeria monocytogenes* and *Y. enterocolitica* under dynamic growth/death-inducing conditions, in Italian style fresh sausage (Iannetti et al., 2017), and *Hepatitis A* inactivation in spinach (Bozkurt et al., 2015).
- D) Collection and data analysis of publicly available data sets related to growth/inactivation/survival of Y. enterocolitica, in order to develop additional ready to use predictive microbial models. It is well known that Y. enterocolitica is a ubiquitous agent (EFSA and ECDC, 2018) and can survive and/or multiply in various food matrices. Thus, models that can be used to predict its behaviour are of interest. Data on microbial growth/survival of Y. enterocolitica were collected from the scientific literature and COMBASE (https://www.combase.cc/index.php/en) and processed with the Predictive Microbial Modelling Lab software v1.06 (https://foodrisklabs.bfr.bund.de/pmmlab de/). PMM-LAB is an extension of the open-source platform KNIME (http://www.knime.org), providing tailored functionality of microbial data analysis in a transparent, modular way. The collected datasets cover specific food matrices: raw and cooked meat, seafood, milk and 'uncategorized food' for a wide range of environmental conditions. Following a two-step modelling approach, the data points were initially fitted through different primary equations, and then, the dependency of relevant environmental parameters (mainly temperature) was modelled through a secondary equation. Alternatively, a one-step modelling approach was followed. Part of the results will be presented orally and as poster at the Junior Researcher's Zoonoses Meeting 2019 in Berlin, DE.
- E) Participation in the process of updating a QMRA of *Y. enterocolitica* in typical German ready to eat products. Experimental data from various challenge tests related to the behaviour of the hazard in fermented sausages and salami were utilised for predictive modelling purposes. In parallel, the fellow participated in a lab experiment related to 'Mett', by spiking different levels of *Y. enterocolitica* in spiced raw minced pork. The final step of this project foresees an indepth study of the current available scientific articles on the bacterial survival in similar products. The ultimate aim is to develop a precise mathematical model which can explain and describe the behaviour of the pathogen in certain matrices or assess the observed values with already existing models from the openFSMR repository.
- F) Authenticity assessment of edible oils. The need to establish on-site procedures to mitigate the risk of fraudulent practices have triggered the application for in-field methodologies. The fellow participated in a feasibility study to explore the potential of a handheld NIR sensor



measuring from 740 to 1070 nm for the robust and reliable authentication of edible oils, which are widely consumed in DE. The preliminary results were presented orally at the Junior Research Group Meeting 'Authenticity along the supply chain' and will also be part of a presentation during the National Food Chemistry Conference 2019 in Dresden, DE.

3. Conclusions

Globalisation has led to a wide distribution of raw materials and goods. The higher the complexity of the food supply chain, the higher its vulnerability and the more difficult risk assessments become. Consequently, robust predictive models and/or software tools are needed to support decision-making in an efficient and time-effective manner.

3.1. *Y. enterocolitica* response in various food matrices

The occurence of *Y. enterocolitica* in traditional German products still remains a threat for the public. The experiments performed at BfR, revealed that these typical products can pose a risk to the public health. Preliminary results are in line with those of other authors for similar products (Lindqvist and Lindblad, 2009; Ivanovic et al., 2015; Mitrovic, 2016) reporting that the use of starter cultures to produce sausages significantly affects the survival of microbial pathogens such as Y. enterocolitica. In other products, such as Mett, the presence of respective amounts of salt could not lead to a significant reduction and/or elimination of Y. enterocolitica. Taking into consideration that the reduction of pathogen levels may be insufficient if fermented sausages undergo inadequate maturation prior to refrigerated storage (Lindqvist and Lindblad, 2009), production procedures should be re-evaluated to ensure safe food. Therefore, such products should preferably not be consumed by particularly risk groups (such as pregnant women, small children, elderly and immunocompromised persons). Address-oriented risk communication is particularly important in this context, as these products are consumed especially in rural areas also by small children and senior citizens who are often unaware of the risk of a food-related illness. Last but not least, own experimental work confirmed that the detection methods for Y. enterocolitica need to be improved. With regard to other aspects, it was shown that the growth of Y. enterocolitica differs according to the food matrix. New consumer trends, such as the consumption of raw milk (a matrix which can support the growth of this and other pathogens), should be critically evaluated by the authorities, as they could pose a risk and thus lead to an increase of food-borne diseases.

3.2. Assuring authenticity in the food supply chain

In the past laborious, time-consuming and sophisticated lab methodologies were a bottleneck to ensure authenticity of food products. Nowadays convenient, portable, easy to use untargeted analysis approaches become available that might provide solutions in that area and can increase the number of controls with limited budget in a time-efficient period at all stages of the supply chain. In experiments performed by the fellow, the inter sensor analytical variability and repeatability was investigated by measuring the same samples with a new sensor, yielding similar results. The experimental research carried out revealed that there is a high potential for reliable screening of edible oils by applying miniaturised NIR sensors.

Summarising, this was a very fruitful year. Overall the project was very inspiring, allowing a deep insight in the activities of a Federal authority dealing with food safety. Thanks to the EU-FORA programme and the expertise of the hosting site the fellow gain a lot of experience in various areas. For instance, the activities carried out allowed him to expand his scientific knowledge both in practice and on a theoretical level. During the fellowship, the fellow was introduced to and gained experience in the use of modern open-source software tools for MRA. He became more familiar with cloud computing in a data-driven community. He adopted concepts such as transparency, harmonisation and documentation and exposed himself in a highly academic environment, having interaction with scientists from different disciplines. By joining and actively participating in various meetings and seminars organised internally or externally throughout the year, new ideas and questions were raised. The fellow is convinced that he will be able to use the knowledge in the near future to support food safety at national and European level. For sure the fellow will disseminate his acquired knowledge in the different software tools. Both the fellow and the supervisors agree that the EU-FORA programme provides an excellent opportunity to exchange opinions, experiences and methodologies on relevant public health issues and to build a professional and personal network that can serve as a basis for future cooperation.



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Abbreviations

AGINFRA+ Accelerating user-driven e-infrastructure innovation in Food and Agriculture

- BfR German Federal Institute for Risk Assessment, Berlin, Germany
- CDC Centres for Disease Control and prevention
- cfu colony-forming units
- DE Deutschland
- DEMETER DEtermination and METrics of Emerging Risks
- EMA Economically Motivated Adulteration
- FSK Food Safety Knowledge
- FSMR Food Safety Model Repository
- HAV Hepatitis A virus
- HEV Hepatitis E virus
- IAFP International Association of Food Protection
- MRA Microbiological Risk Assessment
- PMM Predictive Microbial Modelling
- QMRA Quantitative Microbiological Risk Assessment
- RAKIP Risk Assessment Knowledge Integration Platform
- RASSF Rapid Alert System of Food and Feed
- RKI Robert-Koch Institute
- STEM spatio-temporal epidemiological modelling