EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170903

# Development of food safety risk assessment tools based on molecular typing and WGS of *Campylobacter jejuni* genome

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## Abstract

The 'learning-by-doing' EU-FORA fellowship programme in the development of risk assessment tools based on molecular typing and WGS of *Campylobacter jejuni* genome was structured into two main activities: the primary one focused on training on risk assessment methodology and the secondary one in starting and enhancing the cooperation between the hosting and home organisations, or other joint activities. The primary activities had three subsequent work packages (WPs): WP1 data organisation, WP2 cluster and association analyses, and WP3 development of risk assessment models. The secondary activities have branched into one workshop and the initiation of a cooperation programme between the hosting and home organisations. In the last quarter, the fellow had contributed to the characterisation of some pathogens in possible response to a changing climate, part of the CLEFSA project. The fellow attended various forms of training: online and on-site courses, and also participated at several conferences and meetings for improving his knowledge and skills, contributing to performing the *Campylobacter* risk assessment and source attribution.



**Keywords:** *Campylobacter*, source attribution, risk assessment

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**Acknowledgements:** This report is funded by EFSA as part of the EU-FORA programme.

**Suggested citation:** Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise "G. Caporale", Teramo, Italy, Ardelean AI, Calistri P, Giovannini A, Garofolo G, Di Pasquale A, Conte A and Morelli D, 2019. Development of food safety risk assessment tools based on molecular typing and WGS of *Campylobacter jejuni* genome. EFSA Journal 2019;17(S2):e170903, 18 pp. https://doi.org/10.2903/j.efsa.2019.e170903

**ISSN:** 1831-4732



The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

# Table of contents

# 1. Introduction

## 1.1. About EU-FORA

For building the European Union risk assessment capacity and knowledge community, the European Food Safety Authority (EFSA) initiated the European Food Risk Assessment Fellowship Programme (EU-FORA), as part of the EFSA Strategic Objective. The programme is focused on training in risk assessment and in intensifying exchange and cooperation between different organisations and EFSA. The training is based on 'learning by doing' risk assessment methodologies and practices by involving the fellow in one project linked with food safety from the hosting site, where risk assessment is a significant part. The Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise 'G. Caporale' (IZSAM) initiated many research projects in this field through the Italian National Reference Centre for Veterinary Epidemiology, Programming, Information and Risk Analysis.

## 1.2. General framework

The *Campylobacter* spp. are worldwide distributed bacteria and represent one important microbiological hazard linked with food-borne zoonosis. The gastroenteritis caused by *C. jejuni* and *C. coli* consists of 6 days watery or bloody diarrhoea, self-limiting disease in the majority of the cases, with or without associated fever, weight loss, cramps and headache (Man, 2011; MSD, 2019). Furthermore, *C. jejuni* is associated with a range of other gastrointestinal and extra-gastrointestinal infectious conditions including bacteremia and sepsis, also it may lead to autoimmune conditions known as Guillain–Barré syndrome (GBS), Miller Fisher syndrome, irritable bowel syndrome and Bell's palsy (unilateral facial paralysis), and colorectal cancer (Kaakoush et al., 2015; MSD, 2019). Because the precise role of *Campylobacter* species in the development of these clinical conditions is unknown and the highest prevalence of the gastrointestinal reported conditions caused by this genus, more accurate methodologies in their surveillance and monitoring are necessary. For these reasons the surveillance of *Campylobacter* spp. is part of the EFSA strategy. The legal framework for its monitoring is Regulation (EC) 2160/2003 (European Union, 2003a) on the control of Salmonella and other specified food-borne zoonotic agents and Directive 2003/99/EC on the monitoring of zoonosis and zoonotic agents, however, reporting *Campylobacter* infection is not mandatory in all countries (European Union, 2003b).

Considering the concern in public health of the *Campylobacter* spp. contaminations along the production chain of some food products, the IZSAM is currently involved in several epidemiology studies. The following IZSAM's units are involved: the Italian National Reference Centre for Veterinary Epidemiology, Programming, Information and Risk Analysis (COVEPI), the Italian National Reference Centre for Whole Genome Sequencing of microbial pathogens: database and bioinformatics analysis (GENPAT), and the Italian National Reference Laboratory for *Campylobacter* (NRL).

# 2. Description of work programme

## 2.1. Aims

The first objective of the work programme was 'learning-by-doing' of the fellow in the food safety risk assessment methodology, including collecting, normalisation, and analysis of the data and involving in developing and validation of a set of risk assessment epidemiological tools based on molecular typing and WGS of *Campylobacter jejuni* genomes.

The work programme had three subsequent work packages (WP), focussed on *C. jejuni*:

- WP1. Data organisation: focuses on reviewing the literature, collecting the raw data available, analysing, normalising and organising the data for subsequent epidemiological analyses.
- WP2. Cluster and association analyses: with the purpose to understanding and gaining practical skills in bioinformatics, becoming familiar with tools used in bioinformatics, being trained by doing various statistical methods and approaches for data analysis.
- WP3. Development of risk assessment models: through involvement in the development and validation of a set of risk assessment model considering the main *C. jejuni* genotypes and estimating their contribution in the whole exposure of consumers from Italy.

The second objective was to enhance relationships among home and hosting organisation. The IZSAM is FAO Reference Centre for Veterinary Epidemiology and OIE Collaborating Centre for

Veterinary Training, Epidemiology, Food Safety and Animal Welfare, and finding a solution for agreement and cooperation between organisations was considered.

The fellow has been part of the COVEPI's team involved in the activities of this working programme. In particular, two senior epidemiologists and a statistician have been part of the team, which worked closely with the bioinformaticians of the GENPAT, and the personnel of the NRL for *Campylobacter*. The two senior epidemiologists of the COVEPI's team have acted as mentors for the fellow, assisting him on a daily basis during all the activities carried out. A coordination meeting among the fellow, mentor and supervisor has been held regularly, on a weekly basis, to verify the work done and discuss the implications of the results obtained and plan subsequent activities. The fellow specifically worked on the analysis of the data generated by the molecular typing of *C. jejuni*, and in developing, testing and validation of different tools for the identification of spatiotemporal clusters of epidemiological relevance. The fellow had followed the whole data production and processing process, from the sequencing activities, carried out at laboratory to the bioinformatics analyses and statistical analyses performed on sequence data. In addition, the fellow had benefited greatly by the participation of IZSAM to the activities of the COHESIVE project, with his involvement in selected project meetings.

## 2.2. Activities/methods

### 2.2.1. WP1. Data organisation

In the beginning, the advanced literature search has been conducted regarding the EFSA and international guidance in risk assessment and source attribution based on whole genome sequencing and antimicrobial resistance (AMR), to learn and understand better what is the actual scientific level in the field and what are the best ways to approach the study (and not limited to that, including also the scientific literature in the field) (EFSA BIOHAZ Panel, 2013).

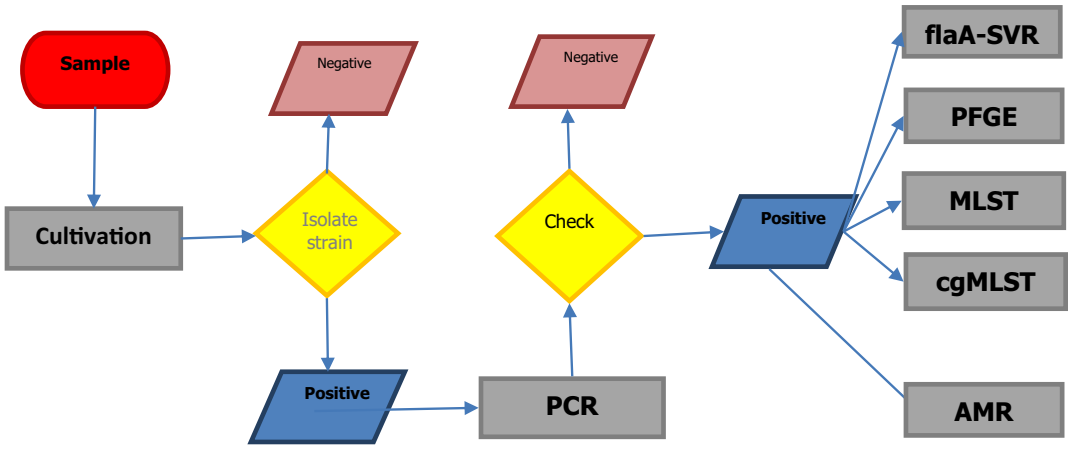


**Figure 1:** Laboratory flow chart for *Campylobacter* spp. analysis

The purpose of WP1 was to obtain the *Campylobacter* database containing the molecular typing results and related epidemiologically relevant metadata, ready to be analysed. The large part of this WP it was made in the first step at the Italian National Reference Laboratory (NRL) for *Campylobacter*. The protocol to *Campylobacter* spp. surveillance is routinely performed by Italian NRL's and is made by cultivation and identification according to EN ISO 10272 part 1 and 2 method (ISO, 2006: Parts 1 and 2). The genotyping characterisation of the isolates are made through species typing by molecularly confirmation by multiplex polymerase chain reaction (PCR) (Wang et al., 2002), flaA-SVR sequencing (Nachamkin et al., 1993), pulsed-field gel electrophoresis (PFGE) (Institue of Environmental Science and Research, 2013) and BioNumerics software version 7.6 (Applied Maths, 2019), in silico multilocus sequence typing (MLST) (Dingle et al., 2008; Jolley et al., 2018; Seemann, 2019), and since 2017, *C. jejuni* isolates are progressively submitted to Core Genome MLST (cgMLST) for a better discriminatory analysis (O'Mahony et al., 2011; Llarena et al., 2017). Part of these sequencing activities is carried out under various national and international projects. The AMR phenotype characterisation is made by testing of susceptibility to seven antimicrobials with a micro-broth dilution method using the 'Sensititre' automated system (TREK Diagnostic Systems, Biomedical Service, Italy) (Kittl et al., 2013; EFSA, 2019).

To date about 3,000 isolates of *Campylobacter* spp., respectively, collected in entire Italy, are available in the IZSAM's strains collection. In this step, the fellow was actively involved in some specific analytical technique linked with cultivation, genotypic and phenotypic characterisation of *Campylobacter*, having the opportunity to develop and improve his laboratory skills and get acquainted with the National Veterinary Information System (https://www.vetinfo.sanita.it/), managed by IZSAM. During this WP, the fellow had learned and understood the entire laboratory workflow (Figure 1) until obtaining the raw data and their recording into the Italian information system. At the same time, the fellow became familiar with the international *Campylobacter* MLST database (https://pubmlst.org/) (Jolley et al., 2018), and used it for comparison and normalisation of the data. IZSAM collects and registers a well-defined set of standardised data for each sample tested in its laboratories. In addition, several samples are collected in the framework of national control plans and all related data are registered into the National Veterinary Information System. All these factors allow IZSAM to retrieve relevant epidemiological data for all tested samples. These epidemiological metadata are fundamental for a correct interpretation of the microbiological results, including the outcomes of molecular typing and phenotyping (ex: antimicrobial resistance). A second step has been done in COVEPI, where a detailed data analysis plan has been developed, including the description of the dataset to be retrieved, the type of data quality checks to be performed and the format of the resulting validated databases. After extracting the raw data, the fellow retrieved, verified, normalised and organised the data for subsequent epidemiological analyses. These data were used in source attribution for human illness through microbial subtyping. (EFSA, 2008, 2019)

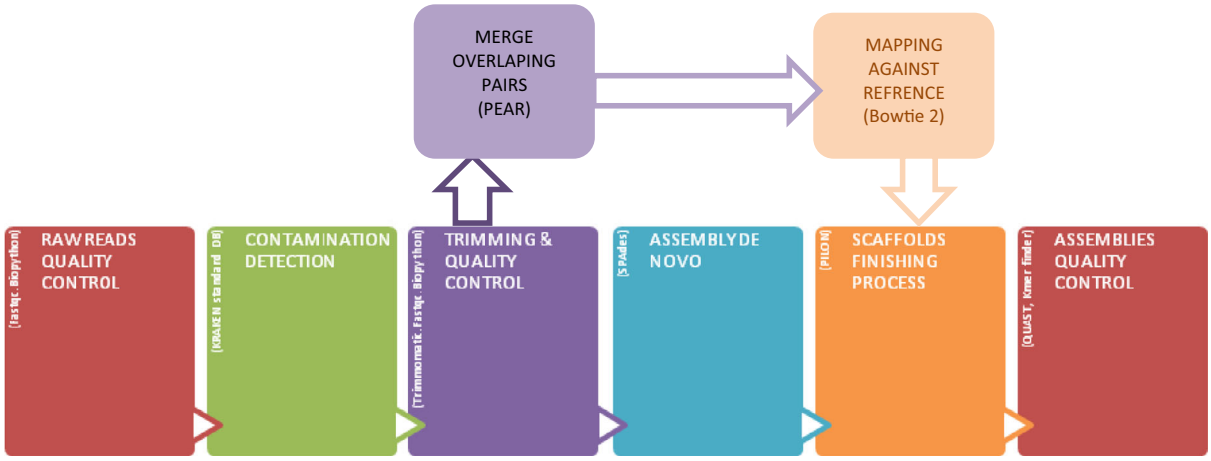


**Figure 2:** Bioinformatics flow chart for *Campylobacter* spp. analysis

### 2.2.2. WP2. Cluster and association analyses

WP2 was focused on obtaining the results from the analyses of molecular typing data and identification of main 'epi-clusters' of *C. jejuni* by analysing the genotypes and phenotypes data as well as epidemiological metadata. In the first step, the fellow had the opportunity to work in the Italian National Reference Centre for Whole Genome Sequencing of microbial pathogens (GENPAT). The bioinformaticians of the GENPAT supported the manipulation and analysis of sequencing data and introduced the fellow in bioinformatics and familiarised him to the bioinformatics software (Figure 3). For comparative analysis based on cgMLST data, the fellow learned and used the minimum spanning tree with GrapeTree software (Figure 2) (Zhou et al., 2018). The second step had been in COVEPI's Statistics and GIS Unit where the fellow was trained in using QGIS geographic information system software. During the third step, in the COVEPI, the fellow analysed the data for the purpose of verifying statistically significant genetic clusters of *Campylobacter* spp. (*C. jejuni)* associated with: the particular species, farm types, food production products; specific phenotypic characteristics, like AMR patterns; or specific spatiotemporal patterns and persistence in specific groups of farms. Working with a big amount of data was challenging, prompting to consider to use some business intelligence software like MicroStrategy.

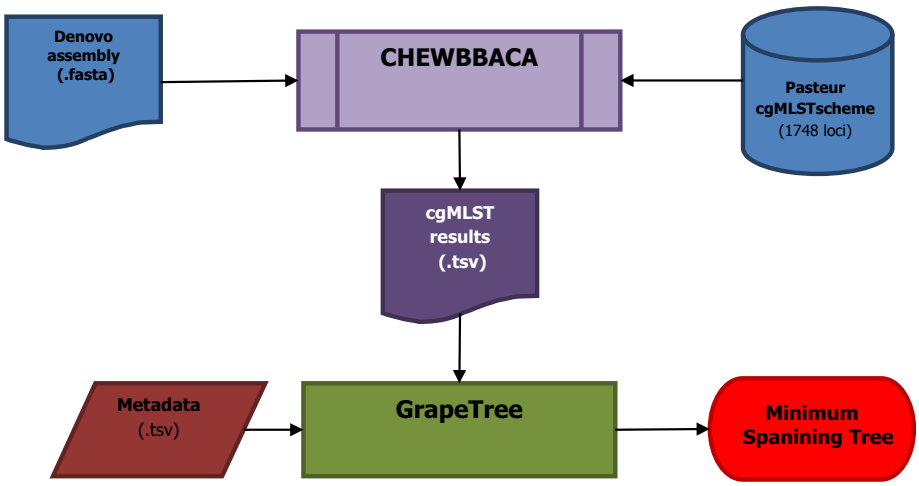


**Figure 3:** The cgMLST data flow chart for *Campylobacter* spp.

### 2.2.3. WP3. Development of risk assessment models

During WP3, in COVEPI unit, different source attribution methods had been used for finding and characterisation of the main *C. jejuni* genotypes and estimating their contribution the whole exposure of Italian consumers. The development and validation of a risk assessment model considering the genotypes and phenotypes were considered. In this step, the fellow analysed the microbial genotypic and phenotypic characteristics of the isolates, to find the common fingerprint and to define the 'epi-cluster'. Within the team, different biostatistical methodologies were tested.

Another approach has been attempted in order to define a particular methodology designated to assess the sources of uncertainties in source attribution models used in *Campylobacter* microbial risk assessment (MRA). The considered methodologies were: failure mode effect analysis (FMEA), fault tree analysis (FTA) and key process indicators (KPI)/quality indicators (QIs). Before starting the assessment, the actors and the stages needed to be defined (pre-preanalytical, preanalytical, analytical, postanalytical and post-postanalytical).

### 2.2.4. Secondary activities

Secondary activities are referred about agreements and cooperation between hosting sites and organisations of origin, cooperation between different organisations and also to other activities not mentioned before, in which the fellow played an active role.

#### 2.2.4.1. Agreements and cooperation

In view of recent epidemics in Europe, taking advantage of this fellowship programme during this period, the foundations have been laid for establishing and strengthening the collaboration between the national reference centres in Italy and Romania, from the IZSAM, Teramo, and the Institute for Diagnosis and Animal Health (IDAH), Bucharest. The first step was to have 2 days 'Animal health risk assessment and vector-borne diseases' workshop during the 2–3 April 2019 for the specialists from IDAH in Bucharest, under scientific coordination of the fellow and participation of the tutors from IZSAM, Paolo Calistri, and Federica Monaco, and from Agricultural Research Council – Onderstepoort Veterinary Research (ARC-OVR), Gert Venter. The second step was the assessment of the needs for professional training in epidemiology for Romanian specialists, for the purpose to find the optimum solution to improve and increase the animal health risk assessment capacity. Depending on the size of the training needs, consideration has been given to the temporary training of a limited number of specialists at the IZSAM, or the initiation of a twinning project, if the needs are more complex. This process is in progress.

In the same context, the European BioSafety Association (EBSA), during their 22nd annual conference at Bucharest, from the total of 42, offered sponsored participation for 24 specialists from Romania including from IDAH and ANSVSA, for participation at the preconference courses (8) in the field of biosafety and biosecurity, and also for participation at the conference (6). Considering the importance of the topics in this region, EBSA increased more than fourth times the numbers of sponsored participation for the persons from Balkan region (Romania, Bulgaria, Republic of Moldova,

Albania, Croatia, Ukraine, Georgia, and Greece) for this year. After this successful experience, different types of collaboration between organisations and their members have started to be considered.

In the last quarter of the EU-FORA programme, the fellow had contributed to the characterisation of some pathogens (like *Campylobacter jejuni*) in possible response to a changing climate, in terms of possible increase in exposure or pathogenicity under a specific climate change scenario, part of the CLEFSA project, coordinated by Angelo Maggiore (EFSA).

#### 2.2.4.2. Additional activities

During 13–15 November 2018, the fellow participated at the Romanian National Sanitary Veterinary and Food Safety Authority (ANSVSA) meeting in Baia-Mare, Romania, for the presentation of the EU-FORA fellowship programme and introduction in risk management, and basic concepts in risk assessment, to the specialists from the national laboratory network.

The fellow participated in the workshop 'Accounting for uncertainty in data-poor scenarios: Case studies on risk analysis in food safety' and at the International Conference on Uncertainty in Risk Analysis, 20–22 February 2019, Berlin at the German Federal Institute for Risk Assessment (BfR), with 'Uncertainty assessment in *Campylobacter* spp. source attribution models: some qualitative approaches' poster presentation at 'Methods for uncertainty analysis' thematic area.

The fellow participated at the 22nd annual international conference 'Burning topics in Biosafety' of the EBSA at Bucharest, Romania, in 2–5 April 2019, where together with the experts Paolo Calistri (IZSAM) and Uwe Mueller-Doblies (MSD), moderated the break-out 'Biosafety and biosecurity in the field in case of an emergency'. Also, the fellow presented two posters: 'The exposure to *Campylobacter* spp. of the food industry workers: a short overview' and 'EFSA EU-FORA – The European Food Risk Assessment Fellowship Programme'.

Like invited guest and collaborator, the fellow participated at the 'Transylvanian Experimental Neuroscience Summer School – TENSS 2019' during 15–16 June 2019, Pike Lake, Romania, organised by the Transylvanian Institute of Neuroscience (TINS).

## 3. Conclusions

### 3.1. Risk assessment in *C. jejuni*

The EFSA EU-FORA 'learning by doing' programme is among the few from Europe which is also addressed to mid-career scientists and open to those who do not necessarily come from the academic field, being a real fortune for people from East European countries. This was a great opportunity for the fellow to consolidate his specialised knowledge and skills in food safety and veterinary epidemiology and public health, by working in a prestigious international and national reference centre. He gained experience by participating in the dedicated program and other activities within the host organisation, better understanding the complex workflow in the specific risk assessment and, at the same time, learned how to investigate an outbreak epidemic, its strengths and weaknesses.

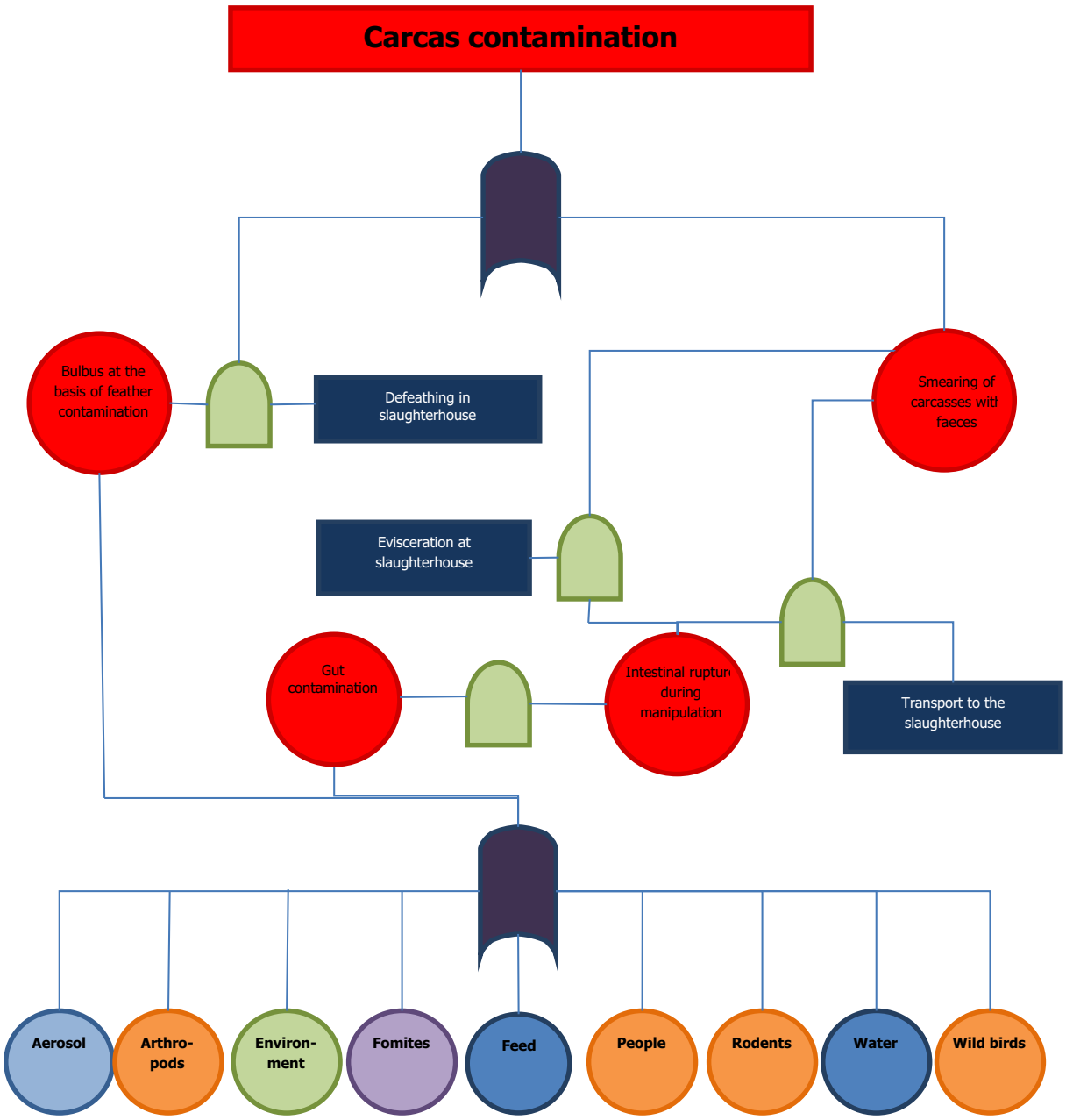


**Figure 4:** Fault tree analysis diagram in poultry carcass contamination, considering the source of *Campylobacter* spp. from poultry

This training offered the opportunity to work both independently and in a team, to integrate the multidisciplinary and evidence-based veterinary medicine approach into assessing the risk of *Campylobacter* and assigning the source in particular.

Applying microbial subtyping methodology in *Campylobacter* source attribution, some characteristics of its population in Italy has been identified. The complexity and the high volume of data provided by cgMLST, at this time, in the absence of a common database, rendered somehow difficult the source attribution based just on this information, anyway, the evidence indicates the existence within *C. jejuni* population of one 'relatively stable' cluster and one 'very dynamic'. Analysing the AMR fingerprint data of the 'relative stabile' *C. jejuni* population, considering the characteristics of the antimicrobial tested, even in the absence of the direct evidence, has supposed that the population had been selected from strains located from another site than the intestinal tract of the animals. The data gathered were not obtained on the basis of a well-defined sampling program, however, the complexity of the information obtained and analysed allows the initiation of a description of the *C. jejuni* population from Italy, that

will represent the foundation for building the common database and harmonised methodology for sub-typing, analysis and storage the data and, in the future, the development of linkage mechanisms (EFSA BIOHAZ Panel, 2013, 2014).

After applying in microbial risk assessment in poultry of some basic cause-effect and effect-cause models (like FMEA respectively FTA) and key process indicators methodologies, a potential scoring system and KPI have been designed and proposed (Table 1) (Wikipedia, the free encyclopedia, 2019a, b) The results were used to draw the flow chart in source attribution in poultry (Figure 4).

During this programme, the fellow had gained skills in using different software like GrapeTree, QGIS, MicroStrategy and had been initiated in using R.

## 3.2. Building cooperation

The future agreement and collaboration between the IZSAM, Teramo, and the IDAH, Bucharest, represents a priority especially for the Romanian part. In this context, several fields for collaboration have been designed, like laboratory activity and epidemiology, public health, and risk analysis.

Many fellows from the 2nd series of the EU- FORA programme had worked together at the poster for the EBSA conference, named 'The exposure to Campylobacter spp. of the food industry workers: a short overview'. The greatest gain of this project is represented by the established human relationships and networking. Starting with relationships with the EFSA coordinators experts, continuing with program coordinators, tutors, experts, and personnel from the hosting sites, and last but not least with the fellow colleagues, this series adds a brick at the building of the future of the EU.

**Table 1:** The list of some key performance indicators (KPI)/quality indicators (QIs) tailored for risk assessment

| Stage | Weighting field | KPI/QIs |
|---|---|---|
| **The KPI with priority 1** | | |
| **Pre-preanalytical** | Weighting the average of the event | a) Number of events, incident or accident/1,000 units of goods<br>b) Number of events, incident or accident/1,000 units of time<br>c) Number of positive *Campylobacter* spp. samples/1,000 units of goods<br>d) Number of positive *Campylobacter* spp. samples/units of time<br>e) Number of positive *Campylobacter* spp. CFU/g per 1,000 units of goods number of positive *Campylobacter* spp. CFU/g per units of time |
| **Pre-preanalytical** | Weighting the average of the manufacturing process | a) Number of goods/units of time<br>b) Number of servings/unit of goods<br>c) Number of personnel/organisation<br>d) Number of personnel necessary/1,000 units of goods<br>e) Number of personnel necessary/units of time |
| **Pre-preanalytical** | Weighting the average of the transport process | a) Number of goods/units of transport<br>b) Number of units of transport/unit of time<br>c) Number of goods transported/units of time |

CFU: colony forming unit.
The KPI was defined with priority 1, compulsory; 2, important; 3, proposed; 4, valuable.

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## Abbreviations

| | |
|---|---|
| AMR | antimicrobial resistance |
| ANSVSA | Romanian National Sanitary Veterinary and Food Safety Authority |
| ARC-OVR | Agricultural Research Council – Onderstepoort Veterinary Research, South Africa |
| BfR | German Federal Institute for Risk Assessment |
| CFU | colony forming unit |

| | |
|---|---|
| cgMLST | Core Genome MultiLocus Sequence Typing |
| CLEFSA | Climate change as a driver of emerging risks for food and feed safety, plant, animal health and nutritional quality (CLEFSA)' project conducted by the EFSA Scientific Committee - Emerging Risks Unit (SCER) |
| COHESIVE | Cohesive –One Health Structure In Europe- is a 3-year project, which aims to develop sustainable One Health approaches with respect to signalling, assessing and controlling zoonoses at the national level within EU countries and across borders |
| COVEPI | Italian National Reference Centre for Veterinary Epidemiology, Programming, Information and Risk Analysis (IZSAM) |
| EBSA | European BioSafety Association |
| EU-FORA | The European Food Risk Assessment Fellowship Programme |
| FAO | The Food and Agriculture Organization of the United Nations |
| flaA-SVR | The DNA sequence of the flagellin A short variable region |
| FMEA | failure mode effect analysis |
| FTA | fault tree analysis |
| GENPAT | Italian National Reference Centre for Whole Genome Sequencing of microbial pathogens: database and bioinformatics analysis (IZSAM) |
| IDAH | Institute for Diagnosis and Animal Health, Bucharest, Romania |
| IZSAM | Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise "G. Caporale", Teramo, Italy |
| KPI | key process indicators |
| MLST | multilocus sequence typing |
| MRA | microbial risk assessment |
| MSD | Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. Kenilworth, NJ, USA |
| NRL | National Reference Laboratory |
| OIE | The World Organisation for Animal Health |
| PCR | polymerase chain reaction |
| PFGE | pulsed-field gel electrophoresis |
| QIs | quality indicators |
| TEENS-2019 | Transylvanian Experimental Neuroscience Summer School –2019 |
| TINS | The Transylvanian Institute of Neuroscience, Cluj-Napoca, Romania |
| VBD | vector-borne diseases |
| WGS | whole genome sequencing |
| WP | work package |

## Appendix A – The on-site courses and training where fellow attended

| Subject | Organisation | Location | Period | Time | Tutor's |
|---|---|---|---|---|---|
| **Internal training on risk assessment methods** | IZSAM | Teramo, Italy | 29.10.2018 | 6 h | Paolo Calistri |
| **Introduction in bioinformatics** | IZSAM | Teramo, Italy | 8.11.2018 | 2 h | Adriano Di Pasquale<br>Antonio Rinaldi |
| **The use of GIS and QGIS software** | IZSAM | Teramo, Italy | 13.3–13.4.2019 | 16 h | Susanna Tora |
| **Microstrategy Dashboarding Data with Dossiers** | Microstrategy | Milano, Italy | 11.3.2017 | 8 h | Stefano Sartorio |
| **Microstrategy Dashboarding Data with Dossiers** | Microstrategy | Roma, Italy | 6.5.2017 | 8 h | Stefano Sartorio |
| **Microstrategy Advanced Reporting** | Microstrategy | Roma, Italy | 7.5.2017 | 8 h | Stefano Sartorio |
| **Microstrategy Enterprise Mobility** | Microstrategy | Roma, Italy | 8.5.2017 | 8 h | Stefano Sartorio |
| **Microstrategy Enterprise Applications** | Microstrategy | Roma, Italy | 9.5.2017 | 8 h | Stefano Sartorio |
| **Transylvanian Experimental Neuroscience Summer School – TENSS 2019 (advanced modelling of biological systems, basic and advanced concepts in signal processing, statistical methods for the evaluation of dynamical systems, principal component analysis, machine learning)** | TINS | Pike Lake, Romania | 15–16 June 2019 | 6 h | Raul C. Muresan<br>Vasile V.Moca<br>Christian Machens<br>Fede Carnevale |

## Appendix B – The online courses and training where fellow attended

| Subject | Organisation | Period | Time | Tutor's |
|---|---|---|---|---|
| **The FoodEx2 classification system and guidance on its harmonised use** | EFSA | 26 September 2018 | 1 h | Sofia Ioannidou<br>Laura Kirwan<br>Alban Shahaj |
| **The FoodEx2 classification system and guidance on its harmonised use** | EFSA | 3 October 2018 | 1 h | Sofia Ioannidou<br>Laura Kirwan<br>Alban Shahaj |
| **How to report surveillance data on Transmissible Spongiform Encephalopathies using the EFSA tool** | EFSA | 21 January 2019 | 1 h | |
| **Learn more about the risk assessment of phthalates used in plastic food contact materials** | EFSA | 15 March 2019 | 1 h | |
| **EFSA's new dedicated support to SMEs** | EFSA | 24 May 2019 | 1/2 h | Remigio Marano<br>Patricia Romero |
| **EPI-interactive webinar: Introduction to R Shiny** | EPI-Interactive | 30 May 2019 | 1 h | Uli Muellner |
| **IHU BioSecurity Free Webinar** | International Hellenic University | 15 May 2019 | 2 h | Gijsbert van Willigen<br>Patrick Rüdelsheim |
| **Medical School Pathology Courses** | Dr. Minarcik's Online Medical School Pathology Course | 1.9.2018–31.5.2019 | 24 h | John R. Minarcik |
| **Illness Outbreaks linked to Enteric Zoonoses and the Interconnectedness of Human and Animal Health** | Pet Poison Helpline | 24 April 2019 | 1 h | Megin Nichols |

## Appendix C – The summary agenda of 'Animal health risk assessment and vector-borne diseases' workshop at Bucharest

| Subject | Tutors |
|---|---|
| **The *Culicoides* diseases in an endemic area** | G. Venter |
| **The vector collection methods** | G. Venter |
| **Workgroup schedule** | G. Venter/A.I. Ardelean |
| **Epidemiological surveillance and risk factors** | P. Calistri |
| **Introduction to risk assessment in animal health** | P. Calistri |
| **Lumpy skin disease: epidemiology and control aspects** | F. Monaco/P. Calistri |
| **Surveillance of West Nile disease in Italy: example of an integrated One Health approach** | F. Monaco/P. Calistri |
| **Geographical information systems: tools for VBD surveillance and control** | P. Calistri |

VBD: vector-borne disease.

# Annex A – Poster Uncertainty assessment in *Campylobacter* spp. source attribution models: some qualitative approaches

"Challenges and Advances in Assessing, Managing and Communicating Uncertainty"
EFSA/BfR International Conference on Uncertainty in Risk Analysis
21–22 February 2019, Berlin, Germany

EU-FORA

## Uncertainty assessment in *Campylobacter* spp. source attribution models: some qualitative approaches

Adrian I. Ardelean [1, 2, 3], Paolo Calistri[2], Armando Giovannini[2], Daniela Morelli [2], Guido Di Donato [2], Francesca Dall'Acqua [2], Antonio Maitino[2], Francesca Cito[2], Maria Luisa Danzetta[2], Daria Di Sabatino [2], Simona Iannetti[2], Valentina Zenobio[2]

[1] European Food Safety Authority EU-FORA Fellow
[2] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Italy
[3] Sanitary Veterinary and Food Safety Directorate Cluj, Romania

### Introduction:

In the majority of the EU countries, with the exception of Nordic countries, about 30-50% of broiler flocks are contaminated when tested at the end of the rearing period, before slaughtering. In the contaminated flocks about 80-100% of animals have *Campylobacter* spp. in the caeca and intestines, without any clinical sign. Not all isolates have virulence characteristics and let to be consider the fact the *Campylobacter* spp. can be part from normal gut microbiota. The prevalence of contaminated carcasses varies among countries. In Italy, and similarly to other EU countries, the proportion of contaminated broiler carcasses is around 40%, but with the great majority of these with low levels of contamination. Many hypotheses have been done to explain this high level of contamination in flocks. A different approach considering the organization activities was taken.
Various approaches have been used to assess the contribution of broiler meat to the burder of campylobacteriosis in humans. The continuous improvement of microbiological risk assessment (MRA) and the development of sophisticated source attribution models, incorporating genomic sub-typing data, are more often used as investigating and food safety control prioritization approaches with a certain success. However, these methods are introducing new sources of uncertainties, often difficult to properly evaluate. It has been already postulated by Donald H. Rumsfeld in 2002 "The silent diagnosis" which underlined that there are two types of unknowns: known unknowns, and unknown unknowns. This study has the purpose to define some methodologies designated to assess the sources of uncertainties in source attribution models used in *Campylobacter* MRA. The present assessment is linked to EFSA EU FORA fellowship program cohort 2018-2019


Fig 1. The food cycle

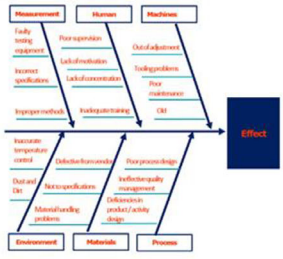


Fig. 2. Cause-effect diagram

### Method:

For the purpose of identifying the uncertainty sources were considered some methodologies as: FMEA, FTA, and KPI/ QIs. Before starting the assessment, the entire workflow has been designed, and the key performance indicators/ quality indicators have been defined. The following stages were defined: pre-preanalytical, preanalytical, analytical, postanalytical and post-postanalytical. Also, the actors were defined for each stage.

Table 1. The health effect factor ($f_h$)

| Nr. crt | The category by effect on health | The value of $f_h$ |
|---|---|---|
| 1 | without harm on individual and collective health | 1 |
| 2 | with minimal harm (without morbidity) | 0.75 |
| 3 | with minor harm (minor morbidity) | 0.50 |
| 4 | with moderate harm (moderate morbidity) | 0.25 |
| 5 | with major harm (major morbidity) | 0 |

### Results:

During the fault tree analysis (FTA) by using cause-effect diagram, some failure mode events were identified. The standard FMEA does not contain any direct index linked with MRA, therefore, for this purpose the health effect factor ($f_h$) was considered (Table1). Another useful scoring to use can be CDC Pandemic Severity Index. After the assessment, was tailored the using of the key process indicators (KPI), some of them are listed in Table 2. The KPI linked with the preanalytical, analytical and postanalytical were also considered. The major risk categories were adapted and proposed to be linked with: air, fomites, effluents, people, biologicals and security.

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Table 2. The list of some key performance indicators (KPI)/ quality indicators (QIs) tailored for RA

| | | | | |
|---|---|---|---|---|
| The KPI with priority 1 | | | | |
| 1 | Pre-preanalytical | 1 | Weighting the average of the event | a) number of events, incident or accident/ 1000 units of goods<br>b) number of events, incident or accident/ 1000 units of time<br>c) number of positive Campylobacter spp. samples/ 1000 units of goods<br>d) number of positive Campylobacter spp. samples/ units of time<br>e) number of positive Campylobacter spp. UFC/ g / 1000 units of goods<br>f) number of positive Campylobacter spp. UFC/ g / units of time |
| 2 | Pre-preanalytical | 1 | Weighting the average of the manufacturing process | a) number of goods/ units of time<br>b) number of servings/ unit of goods<br>c) number of personnel/ organization<br>d) number of personnel necessary/ 1000 units of goods<br>e) number of personnel necessary/ units of time |
| 3 | Pre-preanalytical | 1 | Weighting the average of the transport process | a) number of goods/ units of transport<br>b) number of units of transport / unit of time<br>c) number of goods transported/ units of time |
| The KPI were defined with priority 1, compulsory; 2, important; 3, proposed; 4, valuable | | | | |

### Discussion:

For a powerful MRA a harmonized mutual agreed methodology (referring at FMEA, FTA and KPI) is more than necessary to use, given that it can offer the opportunity to improve it continuously. Considering *Campylobacter* spp. MRA, in particularly for poultry, where there is evidence that contamination occurs in the farm, and it is not very clear how it has happened, the weighting of the entire processes can lead in identifying the source of contamination, also the breaks in the biosecurity measures. New theories presume that the source is linked to surviving in the environment outside or inside the buildings, on the transport vehicles, or/and introducing by personnel during thinning process (the repeated entering of persons into the flock for the selection and removal of animals), so the NGS studies are ongoing trying to explore the accuracy of this hypothesis

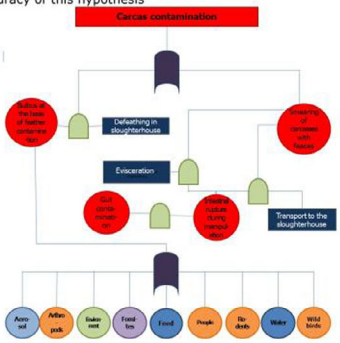


Fig. 3. Fault tree analysis diagram in poultry carcass contamination, considering the source of *Campylobacter* spp. from poultry

This kind of approach has the advantages of defining and monitoring the entire process from an organization, in a uniform way, offering the opportunity to find the gap that can be linked with foodborne diseases. Combining different qualitative methodologies in the MRA, such FMEA, and FTA, and using KPI/ QIs applied to all processes, can lead to enhancing the consistency of the evaluation and can be a useful approach for uncertainty mitigation, proving that these can be useful tools. However, there are some limitations because a deep and exhaustive approach is time and resource consuming. Alternatively, by involving more participants, a standardized approach might enhance in time the MRA through using FMEA and FTA. The KPI/QIs are very useful for total process monitoring and a mutual standardization of them will lead to obtaining benefits.
Through standard definitions and weighting of all processes, a real-time methodology can be developed for risk assessment and for applying more advanced methodologies in the future, such as the one based on the artificial neural network system.

# Annex B – Poster EFSA EU-FORA – The European Food Risk Assessment Fellowship Programme

EBSA22- Annual Meeting of the European Biosafety Association

4-5 April 2019

Caro Hotel, Bucharest, Romania

EU-FORA

## EFSA EU-FORA – The European Food Risk Assessment Fellowship Programme

Stylianos KOULOURIS[1], Cristina Alonso ANDICOBERRY [1], Christoph UNGER [2], Adrian I. ARDELEAN [3]

EU-FORA Fellowship Programme


[1] European Food Safety Authority
[2] Austrian Agency for Health and Food Safety
[3] European Food Safety Authority EU-FORA Fellow at Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Italy

### EU-FORA – The European Food Risk Assessment Fellowship Programme

Is part of the EFSA Strategic Objective to build the European Union risk assessment capacity and knowledge community by attracting early and mid-career scientists to become risk assessors through. Learning by doing, harmonizing risk assessment methodologies and practices across Europe, and intensifying exchange and cooperation among national food agencies and EFSA are some of the activities planned to achieve that goal.

EU-FORA's target audience is focused on the area of biological and chemical food safety, in order to attract scientists with the following backgrounds:

- Molecular Biology,
- Biology,
- Microbiology,
- Biochemistry,
- Chemistry,
- Veterinary/Human Medicine
- Agronomy/Agricultural Science,
- Environmental Science,
- Food Science and Technology,
- Toxicology.

### The EU-FORA concept

Is based on learning by doing and practicing through a 1 year fellowship placement in a competent authority of a Member State, with high capacity in performing Food Risk Assessment, accompanied by a specific and uniform risk assessment training programme for 15 fellows (and 15 additional participants in Parma) during the programme:

- three-week induction training in Parma at EFSA premises in September,
- one-week training module in Vienna at Austrian Agency for Health and Food Safety (AGES),
- one-week training module in Berlin at the German Federal Institute for Risk Assessment (BfR), and
- one-week training module in Athens at Hellenic Food Authority (EFET).

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### The EU-FORA Cohort 2018-2019

is composed of scientists with professional backgrounds in: veterinary medicine, animal science, agriculture, chemistry, biology, ecotrophology, pharmacy, biotechnology, food science and safety, nutrition, marine biology, and toxicology/ecotoxicology.

The participants come from Cyprus, Germany, Greece, Italy, Portugal, Romania, Spain, and UK.

### The EU-FORA Cohort 2019/2020 and 2020/2021

Cohort 3 and 4: EFSA has started a new open call for applications from potential fellows and hosting sites for the cycles 2019/2020 and 2020/2021 of its EU-FORA Fellowship Programme.

**Acknowledgment:** To the staff of EFSA, Austrian Agency for Health and Food Safety, German Federal Institute for Risk Assessment, The Hellenic Food Authority together with all the tutors and technical personnel involved in the trainings. To the EU-FORA fellows cohort 2018-2019: Elena Anastasi, Ricardo Manuel Abreu de Assunção, Maria Cabral, Giorgiana Mihaela. Cătunescu, Eleni Chatzidimitriou, Laura Escrivá Llorens, Carolin Fechner, Juliana Rodrigues Gadelha, Cristiano Garino, Chrystalleni Hadjicharalambous, Márcia de Jesus Monteiro; Dimitrios Pavlidis, Irina Smeu, Christina Vlachou.

# Annex C – Poster The exposure to *Campylobacter* spp. of the food industry workers: a short overview

EBSA22- Annual Meeting of the European Biosafety Association
4-5 April 2019
Caro Hotel, Bucharest, Romania

EU-FORA

## The exposure to *Campylobacter* spp. of the food industry workers: a short overview

EU-FORA Fellowship Programme

**Adrian I. Ardelean[(1)], Paolo Calistri[(2)], Carolin Fechner[(1)], Dimitrios Pavlidis[(1)], Chrystalleni Hadjicharalambous[(1)], Márcia Monteiro[(1)], Christina Vlachou[(1)], Laura Escrivá[(1)], Ricardo Assunção[(1)], Giorgiana Catunescu[(1)]**

*[(1)] European Food Safety Authority, EU-FORA Fellow*
*[(2)] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Italy*

**INTRODUCTION**
Infection by Campylobacter spp. is the main foodborne zoonotic disease in Europe, despite a still high number of cases remaining non-identified. Therefore the risk of exposure for meat food industry workers (0.84 million employees in the EU) is currently underestimated. This infection presents high relevance due to issues related with:
- human safety and security at work
- risk to remain residual source of contamination

**MATERIAL AND METHODS**
A literature-based research and a qualitative risk assessment of worker's exposure to this agent were performed based on the established risk assessment steps (Fig. 1).

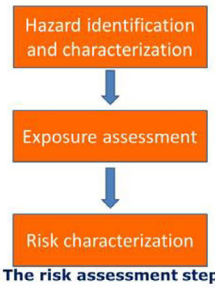


**Fig 1. The risk assessment steps**

**Target population category**
Poultry slaughterhouses employees
**Qualitative methods**
- supporting method (brainstorming)
- scenario analysis
- function analysis

**Control measures evaluated to mitigate the risk**
- engineering control
- administrative control
- personal control

**RESULTS AND DISCUSSION**
**Hazard identification/characterization**
*Campylobacter* spp. is a fastidious bacteria, sensitive to desiccation at high/low temperatures with specific growth requirements. Transmission occurs mainly through oral route by ingestion of undercooked poultry meat and poultry products, or aerosol inhalation (infectious dose: 500-800 CFU).

**Workflow evaluation** (Fig. 2)
→ **Main critical points identified:**
a) place for handling the live bird
b) evisceration
c) digestive tract manipulation
d) waste material manipulation

→ **Possible transmission source:** defeathering process with contaminated aerosols. Further investigations are required.

→ **Control measures for risk mitigation**
a) interruption of the contamination chain
b) engineering control of waste material
c) development of good sewage system
d) good hand hygiene (determinant role in personal exposure)

→ **Early detection measures**
Introduction of an optimized control program for asymptomatic/ symptomatic employees detection according with the level of activity.

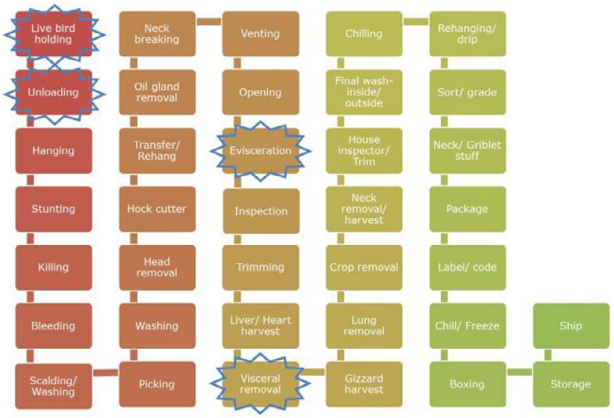


**Fig. 2. The workflow diagramme**
**(highlighted the main critical points identified)**

**CONCLUSION**:
The food industry workers, in our case the personnel from the poultry slaughterhouses, can be exposed to different health hazards. From the category of biological agents, *Campylobacter* spp. is one of high relevance.
According to the performed qualitative risk assessment, some main critical points during the poultry slaughter were identified and could present risk for the workers involved in the process, due to *Campylobacter* spp. exposure. Further research, through a quantitative approach, should be implemented to characterize the risk and contribute to the establishment of control measures.

**Acknowledgment:** EU-FORA fellowship programme (EFSA), cohort 2018-2019.

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