

**BIOLOGICAL HAZARDS AND CONTAMINANTS UNIT**

**Network on Microbiological Risk Assessment  
Minutes of the 10<sup>th</sup> meeting  
Held on 12-13 May 2014, Parma  
(Agreed on 18 06 2014)**

**Participants**

- **Network Representatives of Member States:**

Country	Name	Country	Name
Austria	Monika Matt	Hungary	László Mészáros
Belgium	Isabel De Boosere	Ireland	Wayne Anderson
Bulgaria	Hristo Miladinov Naydenski	Italy	Monica Gianfranceschi
Croatia	Brigita Hengl	Lithuania	Indre Stoskuviene
Cyprus	Georgios Papageorgiou	Netherlands	Benno ter Kuile
Czech Republic	Vladimír Špelina	Poland	Halina Ścieżyńska
Denmark	Maarten Nauta	Portugal	Elisa Maria Carrilho
Finland	Pirkko Tuominen	Slovakia	Lubomir Valík
France	Pauline KooH	Spain	Fernando Pérez Rodríguez
Germany	Juliane Bräunig	Sweden	Mia Egervärn
Greece	Vassilis Xanthopoulos	United Kingdom	Geraldine Hoad

- **Hearing Experts**

- Lieven De Zutter (for item 5.2)

- **Network Representatives of other countries:**

- Norway: Danica Grahek-Ogden
- Switzerland: Richard Felleisen

- **EFSA:**

- BIOCONTAM Unit: Winy Messens (chair), Michaela Hempen (secretariat), Maria Teresa Da Silva Felicio, Valentina Rizzi,
- AFSCO Unit: Jeff Moon

## 1. Welcome and apologies for absence

The Chair welcomed the participants.

Apologies were received from Juliane Bräunig/Germany and Elisa Maria Carrilho/Portugal

## 2. Adoption of agenda

The agenda was adopted without changes.

## 3. Declarations of interest

In accordance with EFSA's Policy on Independence and Scientific Decision-Making Processes regarding Declarations of Interests (Dols)<sup>1</sup> and the Decision of the Executive Director implementing this Policy<sup>2</sup>, members of networks, peer review meetings, networking meetings and their alternates shall be invited to complete and submit an Annual Declaration of interest (ADoI).

EFSA screened the ADoI filled in by the experts invited for the present meeting. No conflicts of interests related to the issues discussed in this meeting have been identified during the screening process or at the Oral Declaration of interest (ODOI) at the beginning of this meeting.

The Chair thanked the representatives that have submitted an ADoI.

## 4. Agreement of the minutes of the 9th meeting of the Network on Microbiological Risk Assessment held on 19-20 November 2013, Parma.

The minutes were agreed by written procedure on 29 11 2013 and published on the EFSA website on 05 12 2013.

## 5. Topics for discussion

### 5.1 Food-borne infections caused by enteropathogenic *Yersinia*

Hristo Naydenski (Bulgaria) presented a study on enteropathogenic *Yersinia*. The aims of the study were to 1) compare the pulsotypes of 20 strains of *Yersinia enterocolitica*, 2) to study the relationship between *Y. enterocolitica* and *Y. pseudotuberculosis* strains and 3) to evaluate the epidemiological relevance of PFGE. Bulgaria also tested a real time PCR protocol for the detection and quantification of *Yersinia* in raw milk.

### 5.2 Isolation and spread of enteropathogenic *Yersinia* spp. throughout the pork production chain

Lieven De Zutter from Ghent University presented on behalf of Inge Van Damme a research on *Yersinia* spp. throughout the pork production chain. 70-80% of human yersiniosis is estimated to be related to the pork reservoir. The samples included pig tonsils, pig carcasses and minced meat. This study shows that the occurrence of enteropathogenic *yersiniae* in pigs at slaughter in Belgium is high as the organisms were isolated from 56% of tonsils and 26% of faecal samples, with 65% of pigs being positive in at least one of both samples. The prevalence of *Y. pseudotuberculosis* in pig tonsils and faeces at slaughter in the present study was lower compared to *Y. enterocolitica*. *Yersinia* contamination of pig carcasses originates mainly from positive pigs delivered to the abattoir and cross-contamination from previously slaughtered pigs.

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<sup>1</sup> <http://www.efsa.europa.eu/en/keydocs/docs/independencepolicy.pdf>

<sup>2</sup> <http://www.efsa.europa.eu/en/keydocs/docs/independencerules.pdf>

### **5.3 Investigation of toxin production by *Bacillus cereus*, characterization and detection of the strains responsible for food poisoning (BACEREUS project)**

Isabel De Boosere (Belgium) presented results from a project on *Bacillus cereus*. One part of the study elaborated on the conditions that are necessary for enterotoxin production. The project used a dynamic gastrointestinal simulation model and found that vegetative *B. cereus* cells do not seem to survive the gastro-intestinal passage and do not compete well with intestinal microbiota. *B. cereus* spores survive and germinate to a certain extent, but no enterotoxins seem to be produced or may be rapidly degraded. Possibly, ingested *B. cereus* spores germinate and multiply locally in the mucus layer of the intestine with enterotoxin production close to the gut epithelium.

### **5.4 TRiMiCri: a free tool for risk based microbiological criteria, developed in a Nordic project**

Maarten Nauta (Denmark) gave an introduction to a software tool, TRiMiCri, which assists in the development of risk based microbiological criteria. The definition of a microbiological criterion (MC) is based on the current health risk and the risk reducing effect. There are two types of risk based MC: a microbiological limit and a relative risk limit. TRiMiCri was developed for *Campylobacter* in chicken meat with the aim to explain how such an approach could be applied. The tool is a downloadable stand-alone program with tutorial, available at [tools.food.dtu.dk/trimicri](http://tools.food.dtu.dk/trimicri).

The MRA Network members suggested considering a more in-depth discussion of the tool during the November Network meeting.

### **5.5 Risk map Austria concerning microbiological hazards in food**

AGES (Austrian Agency for Health and Food Safety) conducted a general risk map for hazards in Austria which was presented to the MRA Network by Monika Matt (Austria). Each stage from farm to fork is defined as one possible hazard carrier for the risk carrier of interest: human being and economy. This risk map consists of several subsets, e.g. animals as hazard carriers and economy as risk carrier. In a first step an expert elicitation was conducted, which resulted in a list of twenty hazards. Pathogens in food and malnutrition were ranked as the most important threat for the population concerning food.

Within the detailed risk map of microbiological hazards in food a logarithmic weighted risk estimate is calculated for fourteen microorganisms. In short, the multiplication of probability of illness with the sum of logarithmic weighted damage measures and the proportion of illness due to food gave a risk estimate. The top three microbiological hazards in Austria are *Campylobacter*, *Salmonella* and *Toxoplasma gondii*.

The risk map Austria is used by risk managers and within the AGES to plan working programs, scientific programs and human resources.

### **5.6 EFSA Activities in the field of Molecular Typing of Food-borne Pathogens**

In December 2013, the EFSA BIOHAZ Panel has adopted Part I of a self-tasking mandate on the evaluation of methods and applications of molecular typing<sup>3</sup>. Adoption of Part II on surveillance and data management activities is foreseen for July 2014.

Teresa Da Silva Felicio (EFSA) summarised the approach and main conclusions of Part I of this mandate. The BIOHAZ Panel concluded that molecular typing methods should ideally provide appropriate discriminatory power, reproducibility, capability for international harmonisation and reduced handling of and exposure to pathogens in the laboratories. No current typing method, whether phenotypic or molecular, complies with all these expectations. Several methods are often used in combination in order to obtain the resolution needed. The detection of outbreaks and their investigation in real-time would be

<sup>3</sup> <http://www.efsa.europa.eu/en/efsajournal/pub/3502.htm>

enhanced by the generation of fully comparable molecular typing data from human, veterinary and food laboratories prior to submission to a central or connected databases. A major challenge of using data generated from molecular typing methods in source attribution models, in particular WGS data, will be to define meaningful subtypes providing an appropriate level of discrimination for source attribution.

EFSA is also involved in other activities on molecular typing, such as PulseNet International, Global Microbial Identifier, 100k Genome Project, staff training on molecular epidemiology, a procurement call including activities on molecular characterisation using Whole Genome Sequencing (WGS) of *Listeria monocytogenes* strains and the EFSA Scientific Colloquium on WGS of food-borne pathogens and its application for public health protection.

Valentina Rizzi (EFSA) presented the EFSA activities on molecular typing data collection at EU level. ECDC collects molecular typing data from food-borne pathogens isolated from human cases and EFSA collects similar data from food, feed and animal isolates, in close collaboration with relevant EURLs. The data collection covers initially *Salmonella*, VTEC and *Listeria* with PFGE and MLVA (*S. Typhimurium*) methods. Other methods may be added later.

A list of questions related to molecular typing was discussed during the break out session.

### **5.7 Challenges and feasibility of analysis of microbial risks throughout the entire food chain**

Benno ter Kuile (the Netherlands) introduced the concept of Food Chain Analysis In order to estimate the relationship between the microbiological risks at the different stages of the chain and the final product, a thorough analysis of the entire chain is needed. This analysis can be divided in several stages:

- Summing up of the risks and disease load,
- Description of the chain and the points at which risks may be introduced,
- Prioritizing of the public health risks,
- Listing of potential risk factors and interventions,
- Development of an overall risk-matrix and formulating advice to the risk-manager.

Questions on food chain analysis were discussed during the break out session.

### **5.8 Break-out sessions**

#### **5.8a Break out session on molecular typing for source attribution/outbreak investigation**

The following question was discussed: What are the challenges to integrate molecular typing data into routine monitoring, surveillance and outbreak preparedness?

Challenges are:

- Complexity of methods and lack of equipments and experienced technicians
- Cooperation between different actors (epidemiologists/ microbiologists and from human and food/veterinary side)
- Harmonisation of methods and interpretation
- Availability and harmonisation of software and tools
- Establishing link between epidemiological and analytical results
- Access to data from industry and private labs
- Storage of data and legal issue
- Information on sampling strategy (representativeness of the isolates)
- Cost for implementation

#### **5.8b Break out session on food-chain analysis for risk assessment**

The following questions were discussed:

What kind of data are needed to reliably connect disease load and food chain?

- Microbiological growth and inactivation data throughout chain
- Dose-response data
- Human consumption data
- Dilution of pathogen concentration
- Distribution of pathogen in food
- Attribution data based on epidemiology
- Data on reporting system in human cases

How do you attribute a microbial load at the end of the chain to the different stages?

- Food chain risk assessment
- Load at the end is the sum of growth/inactivation at different stages, which consists of different types of organisms
- Molecular data may allow to decide what parts of the chain are involved or more important to focus on for control measures
- Consumer behaviour is part of the chain
- Some questions are easy, others difficult and may need a complex model to answer

Can we predict competing and compensating effects of measures?

- Can be done, but not frequently implemented
- Some measure could actually be counter-productive. Eg *Listeria* increased with introduction of cooling chain
- Expert elicitation to help focus modelling
- Some things you cannot predict, emerging risks
- Time (shelf life or storage time) impacts estimations
- Consequences of interventions on other risks (chemical for example)

Does a chain analysis yield anything that separate analysis of product / bug combinations would not reveal?

- It is an integrated approach to identify at which point in the food chain an intervention would be most effective and includes a cost-benefit analysis
- This approach may be better suited for risk communication
- May predict unintended consequences

### **5.9 Heat inactivation of pathogens in meat (PATHOGENCOOK project)**

Isabel De Boosere (Belgium) presented a research project that assessed heat resistance of various pathogens and their survival in meat from different animal species during cooking. The meat was inoculated, stored over night at 7°C and then fried in pan (steak and hamburger) or stir fried (meat strips). Meat preparations of ground meat (hamburgers) yielded highest number of countable results. Temperatures which were actually reached during cooking are generally overestimated. Because of the obtained bi-phasic survival curves, the acknowledged “safe harbour” of 2 minutes at 70°C does not achieve a 6 log reduction of all vegetative pathogenic cells in broth and it is not sure if obtaining an equivalent heat treatment in the meat matrix will accomplish a complete elimination of high numbers of pathogens present.

### **5.10 What is actually meant by “risk assessment”?**

Maarten Nauta (Denmark) raised a discussion on what the MRA Network actually means by “risk assessment” and how this term is used by EFSA and Codex Alimentarius. The definition of microbiological risk assessment could be interpreted in the broad sense, i.e. any

scientific research to support MS food safety risk managers also including risk management or in a more narrow sense as defined by Codex Alimentarius.

The network members had different views on this, partly supporting a narrow definition but others favouring a broader view. The terms of reference of the MRA Network are defining the tasks of the network in a larger context.

#### 5.11 Public health risk from raw milk – questions from WG of BIOHAZ Panel

Michaela Hempen (EFSA) provided feedback on the replies to the questionnaire on raw milk that was sent to the network members by the BIOHAZ Panel WG on public health risks related to the consumption of raw milk. EFSA acknowledged the feedback received from 19 MSs. It seems that most data available are on cow milk, to a lesser extent on goat milk, far fewer on sheep milk. There is hardly any information on milk from horses and donkeys. There is variability across MS with regard to the legislation on the sale of raw milk and on the use of vending machines.

#### 5.12 Current and recent activities of the BIOHAZ Panel

The following recent and ongoing mandates of the BIOHAZ Panel were presented:

- The evaluation of the safety and efficacy of peroxyacetic acid solutions for reduction of pathogens on poultry carcasses and meat (EFSA-Q-2013-00601<sup>4</sup>, adopted in March 2014)
- *Carbapenem* resistance in food animal ecosystems (EFSA-Q-2013-00010<sup>5</sup>, adopted in December 2013)
- Public health risk related to the maintenance of the cold chain during storage and transport of meat. Part 1 (meat of domestic ungulates) (EFSA-Q-2013-00646<sup>6</sup>, Adopted in March 2014)
- Public health risk related to the maintenance of the cold chain during storage and transport of meat. Part 2 (minced meat of all species) (EFSA-Q-2013-00648, deadline July 2014)
- Risk posed by pathogens in food of non-animal origin:
  - *Salmonella* and *Norovirus* in leafy greens eaten raw as salads (EFSA-Q-2012-00238<sup>7</sup>, adopted March 2014)
  - *Salmonella* and *Norovirus* in berries (EFSA-Q-2013-00179<sup>8</sup>, adopted May 2014)
  - *Salmonella* in melons (2013-00176; deadline September 2014), *Salmonella* and *Norovirus* in tomatoes (EFSA-Q-2013-00177; deadline September 2014), and *Salmonella*, *Yersinia*, *Shigella* and *Norovirus* in bulb and stem vegetables, and carrots (EFSA-Q-2013-178, deadline December 2014).
- Public health risks of table eggs due to deterioration and development of pathogens (EFSA-Q-2013-00400, deadline July 2014)
- Development of a risk ranking toolbox for EFSA BIOHAZ Panel (EFSA-Q-2013-00014, deadline December 2014)

<sup>4</sup> <http://www.efsa.europa.eu/en/efsajournal/doc/3599.pdf>

<sup>5</sup> <http://www.efsa.europa.eu/en/efsajournal/doc/3501.pdf>

<sup>6</sup> <http://www.efsa.europa.eu/en/efsajournal/doc/3601.pdf>

<sup>7</sup> <http://www.efsa.europa.eu/en/efsajournal/doc/3600.pdf>

<sup>8</sup> <http://www.efsa.europa.eu/en/efsajournal/doc/3706.pdf>

## 6. Any Other Business

### 6.1 Guideline for coordination of the information flow between Advisory Forum members, Focal Points and national representatives in EFSA's Scientific Networks

Jeff Moon (EFSA) presented the guidance for scientific network representatives which results from a self-review of EFSA's scientific networks and was discussed in EFSA's Advisory Forum. The document aims at providing guidance on good practice for improving communication at national level at the different EFSA counterparts. It also provides a general description of the main roles of the different actors. The draft guideline is currently presented for consultation by network coordinators at EFSA and the focal points drafting group. The final guidance document is expected by September 2014 and will be shared with the network when it is published.

### 6.2 Renewal of membership of BIOHAZ Panel

The call for renewal of the Scientific Committee and eight Panel (including the BIOHAZ Panel) has been launched in April 2014, deadline 18 June 2014<sup>9</sup>. The new members will be appointed for the following three-year term starting in July 2015.

### 6.3 Scientific colloquium N°20 "Whole Genome Sequencing of food-borne pathogens for public health protection"

EFSA's Scientific colloquium on whole genome sequencing will take place on 16-17 June 2014 in Parma<sup>10</sup>. Discussions will focus on assessing the latest scientific information, on strengthening alliances with the relevant EU and international bodies to initiate discussions on the use of WGS methods for food safety applications and drive EFSA's ongoing efforts in the collection of molecular typing data by proactively anticipating the specific requirements of WGS data.

### 6.4 Call for tenders on *Listeria monocytogenes* in ready-to-eat foods

EFSA launched two calls for tender "Closing gaps for performing a risk assessment on *Listeria monocytogenes* in ready-to-eat (RTE) foods". Activity 3 concerns the comparison of isolates from different compartments along the food chain, and from humans using whole genome sequencing (WGS) analysis (OC/EFSA/BIOCONTAM/2014/01). The deadline was 12 May 2014. Activity 2 concerns a quantitative risk characterisation of *L. monocytogenes* in RTE foods; starting from the retail stage (OC/EFSA/BIOCONTAM/2014/02). The deadline is 27 June 2014.

## 7. Next meeting

The next MRA network meeting will be held in Parma on 25-26 November 2014.

<sup>9</sup> <http://www.efsa.europa.eu/en/press/news/140409.htm>

<sup>10</sup> <http://www.efsa.europa.eu/en/events/event/140616.htm>