BIOLOGICAL HAZARDS AND CONTAMINANTS UNIT

# Network on Microbiological Risk Assessment Minutes of the 17th meeting

**Held on 10/11 October 2017, Parma**

**(Agreed on 06/11/2017)**

**Participants**

- **Network Representatives of Member States (including EFTA Countries):**

| **Country** | **Name** |
|---|---|
| Austria | Monika Matt |
| Belgium | Lieven De Zutter, Inge Van Damme |
| Bulgaria | Hristo Najdenski |
| Cyprus | Christos Kourtis |
| Croatia | Brigita Hengl |
| Czech Republic | Barbora Mackova |
| Denmark | Maarten Nauta |
| Estonia | Mati Roasto |
| Finland | Jukka Ranta |
| France | Pauline Kooh |
| Germany | Anja Buschulte |
| Greece | Panagiota Gousia |
| Hungary | Arienn Berikcs |
| Ireland | Lisa O'Connor |
| Italy | Dario De Medici |
| Lithuania | Indre Stoskuviene |
| Netherlands | Aarieke de Jong, Frits Franssen |
| Poland | Elzbieta Mackiw |
| Portugal | Luisa Peixe |
| Romania | Isabela Madalina Nicorescu |
| Slovakia | Lubomir Valik |
| Slovenia | Pavel Pollak |
| Spain | Fernando Perez Rodriguez |
| Sweden | Jakob Ottoson |
| United Kingdom | Joanne Edge |
| Norway | Danica Grahek-Ogden |
| Switzerland | Francoise Fridez |

- **EFSA:**

BIOCONTAM Unit: Michaela Hempen, Francesca Latronico, Ernesto Liebana (chair), Winy Messens, Pietro Stella.

## 1. Welcome and apologies for absence

The Chair welcomed the participants. No apologies were received.

## 2. Adoption of agenda

The agenda was adopted without changes.

## 3. Agreement of the minutes of the 16th meeting of the Network on Microbiological Risk Assessment held on 4/5 April 2017, Parma[1]

The minutes were agreed by written procedure on 29 May 2017 and published on the EFSA website 30 May 2017.

## 4. Topics for discussion

### 4.1. Overview of activities on foodborne parasites

The representative from France gave an overview of Anses activities on foodborne parasites which include surveillance systems in humans, food and animals in France. She presented past and recent risk assessments and research activities such as datasheets on foodborne parasites, specific work on particular parasites (e.g. *Toxoplasma, Cryptosporidium, Giardia, Anisakis, Echinococcus multilocularis*), risk profiles and surveys.

### 4.2. *Giardia* and *Cryptosporidium* in shellfish

The Croatian Food Agency has conducted a study to assess the presence of *Cryptosporidium* and the *Giardia* in shellfish to determine whether there is a danger to human health. The presentation provided some background on these parasites, human disease and occurrence in shellfish from coastal areas of Croatia. This study was the first molecular confirmation of *C. hominis* and *G. duodenalis* in shellfish in Croatia.

### 4.3. COST Action TD1302 CYSTINET

The COST Action TD1302 CYSTINET is the European Network on taeniosis/cysticercosis[2], which has been running for 4 years, including 35 countries (of which 27 EU). The Action is a strong, extensive, multi-disciplinary scientific network that aims to increase the knowledge and understanding of the *Taenia saginata* and *Taenia solium* zoonotic disease complexes, focusing on the EU. Results include updates on occurrence of *T. solium* and *T. saginata* in the EU, the economic impacts and risk factors of these neglected parasitic foodborne zoonoses, but also evaluations in the diagnostic preparedness of the EU and recommendations towards prevention.

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[1] https://www.efsa.europa.eu/sites/default/files/event/170404-2-m.pdf
[2] www.cystinet.org; http://www.cost.eu/COST_Actions/fa/TD1302?

### 4.4. Risk assessment on the dairy food supply chain

The Netherlands Food and Consumer Product Safety Authority (NVWA) is conducting a series of food supply chain risk analyses. Each analysis consists of three parts: a risk assessment carried out by the Office for Risk Assessment and Research of the NVWA, an assessment of the supervision activities by the supervision department and an assessment on fraud by the intelligence and investigation services (NVWA-IOD). These three "pictures" taken of the same landscape will show what the most important hazards/risks are, what already is covered by supervision, where the "gaps in the system" are and what should be the ideal situation with respect to knowledge-based and risk-oriented supervision. This presentation focussed on the Dutch dairy chain. Main hazards addressed were *Bacillus* spp., *Campylobacter* spp., *Cronobacter* spp., *E.coli* (incl. STEC), *L. monocytogenes*, *Salmonella* spp. and *Staphylococcus* spp.

### 4.5. *Yersinia* spp. in minced pork

The study aims to evaluate the effect of different processing scenarios along the farm-to-fork chain on the contamination of minced pork with human pathogenic *Y. enterocolitica*. A modular process risk model (MPRM) is used to perform the assessment of the concentrations of pathogenic *Y. enterocolitica* in minced meat produced in industrial meat processing plants. The model describes the production of minced pork starting from the contamination of pig carcasses with pathogenic *Y. enterocolitica* just before chilling. The endpoints of the assessment are (i) the proportion of 0.5 kg minced meat packages that contain pathogenic *Y. enterocolitica* and (ii) the proportion of 0.5 kg minced meat packages that contain more than 1000 pathogenic *Y. enterocolitica* at the end of storage, just before consumption of raw pork or preparation. Comparing alternative scenarios to the baseline model shows that the initial contamination and different decontamination procedures of carcasses have an important effect on the proportion of highly contaminated minced meat packages at the end of storage. The addition of pork cheeks and minimal quantities of tonsillar tissue into minced meat also has a large effect on the endpoint estimate. Finally, storage time and temperature at consumer level strongly influence the number of highly contaminated packages. The study is published (Van Damme et al., 2017).[3]

### 4.6. Qualitative and quantitative data on ESBL *E. coli* in faeces, tonsils and pig carcasses

During a first study, the abundance of cefotaxime resistant *Escherichia coli* (CREC) in the faeces and tonsils of 96 pigs during slaughter was tested. CREC (based on enumeration results on Tryptone Bile X-glucuronide agar supplemented with 1 mg/L cefotaxime) were detected in 72 faecal samples (75%; 95% CI: 64 to 83%) and 45 tonsil samples (47%; 95% CI: 35 to 59%), in numbers up to 5.5 and 5.6 $\log_{10}$ CFU/g, respectively. On average, around 1/10,000 E. coli in both faeces and tonsils were cefotaxime-resistant, though large variations were observed between pigs (Van Damme et al., 2017).[4]

During a second study, the occurrence of cefotaxime-resistant *E. coli* (CREC) on nine different carcass areas of 104 pig carcasses was studied. In 49% (95% CI: 29-69%) of the carcasses, CREC could be isolated and enumerated with

[3] http://www.sciencedirect.com/science/article/pii/S0740002016307729
[4] http://www.sciencedirect.com/science/article/pii/S0378113517307605

proportions of positive samples varying between carcass areas from 1% (loin region) to 23% (head) and maximum numbers ranging from -0.6 $\log_{10}$ CFU/cm$^2$ (loin and elbow before evisceration) to 1.7 $\log_{10}$ CFU/cm$^2$.

### 4.7. Review on *Listeria monocytogenes* outbreak in Germany

The investigation of a L. monocytogenes outbreak with 78 cases in Southern Germany from 2012-2016 was presented as an example for the use of Whole Genome Sequencing to identify the source of the outbreak. Epidemiological investigations pointed to pork meat products distributed in supermarkets at supra-regional level in Southern Germany. Food authorities intensified official control sampling of pork meat products and sent isolates to BfR for molecular typing. A match was found in a pork belly product manufactured in Southern Germany. Follow-up sampling at retail and at production level in affected company found four other products and two other *L. monocytogenes* strains (Kleta et al., 2017).[5]

### 4.8. Health advice given to pregnant women and vulnerable groups concerning *L. monocytogenes*

The Norwegian Scientific Committee for Food Safety (VKM) issues a health advice for pregnant women and vulnerable groups on *L. monocytogenes*. The Norwegian Food Safety Authority asks VKM to calculate the likelihood of developing listeriosis linked to consumption of different categories of fish and seafood products, raw and heat treated meat, dairy products and fresh produce. The results of the assessment can be expected in June 2018.

### 4.9. New and recent activities of the BIOHAZ Panel

The BIOHAZ Panel adopted an opinion on Public health risks associated with hepatitis E virus as a foodborne pathogen which is published on the EFSA website.[6]

New mandates received are a self-tasking mandate on public health risks associated with foodborne parasites[7] and a Second scientific opinion on hazard analysis approaches for certain small retail establishments and food donations.[8]

### 4.10. Draft opinion on *Listeria monocytogenes* contamination of ready-to-eat foods and related risks for human health in the European Union

Around 2,200 confirmed human listeriosis cases were reported in the European Union and European Economic Area (EU/EEA) in 2015. Time series analysis (TSA) for the EU/EEA indicated a significant increasing trend of the monthly notified incidence rate of confirmed human listeriosis in the female age group 25-44 years old (yo), and in the female and male groups ≥ 75 yo during 2008–2015. In 2015, the incidence rates were higher for the male than for female age groups over 25 years old (except for the 25–44 age groups; mainly pregnancy-related) and were highest for the ≥ 75 group. A conceptual model was the basis for identifying factors in the food chain as potential drivers for *Listeria*

[5] https://wwwnc.cdc.gov/eid/article/23/10/16-1623_article
[6] https://www.efsa.europa.eu/en/efsajournal/pub/4886
[7] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2017-00460
[8] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2017-00565

*monocytogenes* contamination of ready-to-eat (RTE) foods and listeriosis illness. Their potential contribution of the factors related to the host, the food, the national surveillance systems or the bacterium to the trend of human listeriosis cases/incidence rates were evaluated by answering eight assessment questions. These were evaluated through importance analysis using a developed *L. monocytogenes* generic quantitative microbiological risk assessment (gQMRA) model. Factors that may have had an impact on the trend were classified based on the quality of the available evidence. Among the evaluated factors, those considered likely to be responsible for the increasing trend in cases are the increased population size of the elderly and susceptible population except for the 25–44 female age group. For the increased incidence rates and cases, the likely factor is the increased proportion of susceptible persons in the age groups over 45 yo of both genders.

The draft opinion was open for public consultation until the end of September 2017[9]. Before its adoption by the BIOHAZ Panel (foreseen in December 2017), the draft opinion will be revised, taking into account the comments received during the public consultation. EFSA organised a meeting with stakeholders on 19-20 September 2017 to present the draft opinion and gather feedback[10].

### 4.11. Closing gaps for performing a risk assessment on *L. monocytogenes* in RTE foods: Activity 2, a quantitative risk characterization on *L. monocytogenes* in RTE foods; starting from the retail stage

A quantitative risk characterization of *Listeria monocytogenes* in various ready-to-eat (RTE) food categories in the European Union (EU) was performed starting from the retail stage. Growth of *L. monocytogenes* considering interaction with lactic acid bacteria was modelled from retail to consumption using temperature-time profiles during transport and storage. This information was combined with the Pouillot dose-response models to estimate the number of listeriosis cases per servings as well as the annual number of listeriosis cases in the EU associated with the consumption of the RTE foods. Cooked meat and sausage presented most cases, sliced pâté packaged in normal atmosphere presented the highest listeriosis risk per million servings. With respect to the estimation of the total number of cases per population group, considering each food subcategory separately, the higher risk population group corresponded to elderly, followed, in most cases, by pregnant and healthy, with the exceptions of cooked meat and hot smoked fish in which pregnant presented higher risk than elderly. In the light of results, it seems necessary that educative programs and specific recommendations are specially oriented to the most susceptible population groups so as to mitigate the risk. The full report is published on the EFSA website.[11]

### 4.12. *L. monocytogenes* in RTE in Belgium

At Ghent University, several challenge tests have been conducted in 2016-2017 in a variety of raw milk soft, semi-soft and semi-hard cheeses. Artificial *L. monocytogenes* contamination was used to study the growth potential of the

[9] https://www.efsa.europa.eu/en/consultations/call/170724-0
[10] https://www.efsa.europa.eu/en/events/event/170919-2
[11] http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2017.EN-1252/pdf

pathogen in a semi-hard cow milk cheese and durability studies were used to assess the growth potential in a naturally contaminated semi-hard raw goat milk cheese. It was noted that in both cases, no growth of the pathogen was noted, and thus the results of the challenge testing coincided with the results of the durability testing. However, survival of the pathogen throughout the shelf life was noted in red-smear semi-hard cheese made of raw (goat or cow) milk. Thus, it should be kept in mind that in particular for consumption by pregnant women or other specific vulnerable groups being susceptible to listeriosis, also low numbers of *L. monocytogenes* are not tolerated in these ready-to-eat foods, and thus absence per 25 g (preferably confirmed by using a multiple sample subunit approach (n= 5) for *L. monocytogenes* testing) needs to be the preferred food safety objective to aim for.

## 4.13. Prevalence and counts of *Listeria monocytogenes* in different categories of RTE food products in 2012-2016 in Estonia

The Estonian university of life sciences has conducted a study on prevalence and counts of *L. monocytogenes* in RTE foods. Highest prevalence was found in salted fish and fish products. In a study from 2008-2010, mostly RTE foods were analysed, but also raw meat and fish (Kramarenko et al., 2013).[12] In a study from 2012-2013, RTE fish and meat products with long shelf life were analysed. The highest prevalence was found in lightly salted fish fillet products (Kramarenko et al., 2016).[13]

Within five years in total of 30,016 RTE food samples were analysed and 3.6% (CI95 3.4% - 3.8%) were found to be positive for *L. monocytogenes*. Highest prevalence among food categories was found for RTE fish and fish products (11.6%; CI95 10.9% – 12.3%) Enumeration of *L. monocytogenes* was performed for 14,342 RTE food samples and official food safety criterion 100 CFU/g was exceeded by 46 samples (0.3%).

## 4.14. Risk assessment on *Listeria monocytogenes* in RTE fish products

The public health risk of *L. monocytogenes* in cold smoked and salt cured salmon was assessed for two population groups differing in dose-response and consumption. This was based on occurrence data at retail level, predictive modelling, and food consumption 48h interviews.

Quantitative modelling was implemented with R & OpenBUGS, consisting of sub-models designed to be modifiable for both microbiological and chemical risk assessment. At retail, prevalence was estimated 22.5% (CI95: 20-25%). 81% of the positive concentrations were below the limit of enumeration (but assuming at least one bacteria in the sample of 25 grams, those have CFU>1/25). Accounting for both exact concentrations (above limit) and those below/between limits, parameter uncertainty was described as posterior distribution, and this was used in predictions, resulting to $P(CFU > 100) \approx 10\%$. Exponential dose-response model was used for predicting the number of cases (exploiting also data on consumption frequencies and consumption amounts). Parameter of the dose-response model was based on values reported in WHO report. Assuming no growth, no cases were predicted. With different scenarios for growth (time &

[12] http://www.sciencedirect.com/science/article/pii/S0956713512003799
[13] http://www.sciencedirect.com/science/article/pii/S0956713516300809

temperature) a distribution of cases was predicted, most notably for the elderly population.

### 4.15. Discussion on *Listeria monocytogenes*

The representative from the Netherlands presented discussion topics on *L. monocytogenes*: 1) Do we select for less infectious isolates?, 2) What is the role of fresh meat? Is RTE food really the most important risk product?, 3) Label vs customer use: RTE or not?, 4) Challenge testing temperature profile, and 5) Absence in 25g? The discussion time was not enough to clarify the questions raised and it was suggested to follow it up using a questionnaire to the MRA network members.

### 4.16. QMRA model to evaluate *Trichinella* infection risk via controlled housing, backyard farming and wild boar consumption

The Dutch National Institute for Public Health & the Environment developed a quantitative risk assessment model for trichinellosis. Different scenarios were run to a) compare risk from different housing systems, b) estimate actual risk and c) explore effect of descreased test sensitivity.

The QMRA for *Trichinella* showed that controlled housing of pigs is effective to prevent human trichinellosis incidence as compared to non-controlled housing. Not testing 120M EU pigs from controlled housing could lead to at most 5 - 6 cases of human trichinellosis per year for the whole EU, additional to hundreds of cases due to pigs from non-controlled housing and consumption of game meat. Testing of pigs from non-controlled housing is paramount, preferably using the EU-RM / ISO 18743 or at least equally sensitive test methods. A slight decrease of Trichinella test sensitivity has a large impact on estimated trichinellosis incidence, affecting mainly endemic countries with high proportions of non-controlled housing. Part of the study presenting the QMRA for *Trichinella* is published (Franssen et al., 2017)[14] and an additional publication concerning the questions raised above is underway.

## 5. Any Other Business

The presentations of the meeting are made available on DMS.

## 6. Date for next meeting

The next meeting will be held on 24/25 April 2018 in Parma.

## 7. Closure of the meeting

The chair thanked the participants and closed the meeting.

[14] http://www.sciencedirect.com/science/article/pii/S0168160516305669