Network on Microbiological Risk Assessment Minutes of the 19th meeting

Held on 21-22 May 2019, Parma

(Agreed on 05 July 2019)

Participants

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

Country	Name
Austria	Monika Matt
Belgium	Lieven De Zutter
Bulgaria	Hristo Najdenski
Croatia	Brigita Hengl
Cyprus	Georgios Papageorgiou
Czech Republic	Barbora Macková
Denmark	Johanne Ellis-Iversen
Estonia	Mati Roasto
Finland	Jukka Ranta
France	Moez Sanaa
Germany	Anja Buschulte, Marion Gottschald
Greece	Panagiota Gousia
Hungary	Adrienn Berkics
Ireland	Lisa O'Connor
Italy	Dario De Medici
Lithuania	Indre Stoskuviene
Netherlands	Aarieke De Jong
Poland	Elzbieta Mackiw
Portugal	Luisa Peixe
Romania	Isabela Nicorescu
Slovakia	Lubomir Valík
Slovenia	Pavel Pollak
Spain	Elena Carrasco Jimenez
Sweden	Jakob Ottoson
United Kingdom	Paul Cook
Norway	Danica Grahek-Ogden
Switzerland	Françoise Fridez

- **Hearing Experts**

  Karen Remm (agenda item 4.1)

- **EFSA:**

BIOCONTAM Unit: Pietro STELLA, Michaela HEMPEN, Maria Francesca IULIETTO, Winy MESSENS.

21 May 2019

1. Welcome and apologies for absence

The Chair welcomed the participants.

Apologies were received from Hungary and Portugal.

2. Adoption of agenda

The agenda was adopted without changes

3. Agreement of the minutes of the 18th meeting of the Network on Microbiological Risk Assessment held on 24/25 April 2018, Parma[1].

The minutes were agreed by written procedure on 25 May 2018 and published on the EFSA website on the 31 May 2018.

4. Topics for discussion

4.1 Raw milk in vending machines: results from Switzerland, Germany and Austria

The guest speaker from Lower Saxony State Office for Consumer Protection and Food safety (LAVES), Karen Remm, and the representative from Austria presented results on similar studies on raw milk quality from vending machines in Switzerland, Germany and Austria.

In the German study, 159 samples of raw cow milk from farm-gate vending machines, collected between 2016 and 2018, were investigated for their microbiological quality and the occurrence of bacterial foodborne pathogens. Total viable counts (TVC) were > 10^5 cfu/ml for 23%, while *Escherichia coli* was >10^3 cfu/ml in 8% of samples and coagulase-positive staphylococci were >10^3 cfu/ml for 6% of samples. *Salmonella* spp. was not detected but 2 samples out of 139 were positive for *Campylobacter*, 7 out of 154 were positive for Verotoxin-producting *E.coli* (VTEC), and 6 out of 166 samples where *Listeria monocytogenes* positive.

The study conducted in Austria included 74 samples of raw cow milk, collected in July/August 2017. 22 samples exceeded limits for TVC (taking into account that according to the Austrian national regulation for raw milk, the limit for TVC is 50,000 cfu/ml). *S.* Dublin and *C. jejuni* were detected once, VTEC was isolated in two samples while *L. monocytogenes* was not isolated.
A Swiss study (Zulauf et al. 2018[2]) investigated the microbiological quality of raw milk sold directly from farms to consumers. 73 samples of raw cow milk marketed at farm level were investigated for their microbiological quality and the occurrence of bacterial foodborne pathogens. TVC were mainly (67.1 %) in the range from

[1] https://www.efsa.europa.eu/sites/default/files/event/180424-m.pdf

[2]Zulauf M., Zweifel C., Stephan R. (2018) Microbiological quality of raw milk sold directly from farms to consumers in Switzerland, Journal of Food Safety and Food Quality.

10^3 to 10^5 CFU/ml, while *Escherichia coli* and coagulase-positive *Staphylococci* were each detected in 30.1 % of the samples. TVC results for raw milk from vending machines (34.4 % above 10^5 CFU/ml) were clearly higher than those from pre-filled bottles, emphasizing the importance of ensuring correct cleaning and disinfection procedures of vending machines.

4.2 Shigatoxin-producing *Escherichia coli* and *Campylobacter* in milk tank filters

The representative of Sweden presented the results of a study on the occurrence of *Campylobacter* spp., *Salmonella* spp. and shigatoxin-producing *Escherichia coli* (STEC) in milk filters from Swedish dairy farms. Altogether 302 milk tank filters were collected from three regions in Southern Sweden. Each one from a different farm that voluntarily participated providing the filter after morning milking (spring-autumn). Filters were incubated in enrichment broth which was screened by PCR. Isolation was performed for PCR-positive broths; 214 STEC, 91 Campylobacter and 2 Salmonella respectively. Forty-five isolates from 42 STEC-positive samples were investigated by WGS for presence of virulence factors (stx1,stx2, eae). Serotyping was conducted for 29 isolates. The most common serotype was O145:H28. Two isolates belonged to O26:H11 and one to O157:H7. Only the latter was of high pathogenicity. Thirty-eight (13 %) samples were positive for *Campylobacter* (34 samples for *C. jejuni* and 4 samples for *C. lari). Salmonella* spp. was not isolated from any of the filters. Pathogen occurrence was higher in farms with more than 50 cows and farms with an untethered milking system.

4.3 Quantitative microbiological risk assessment of *Salmonella* Dublin in raw milk cheese

The representative from France presented a qualitative evaluation of a sampling plan for raw milk cheese and a quantitative assessment of the effect of such sampling on the risk of salmonellosis conducted by Anses.[3]
In the context of the outbreak of salmonellosis associated with two types of cheeses made from unpasteurized milk in late 2015 and early 2016, the French Ministry of Agriculture requested a qualitative and quantitative assessment of a sampling plan. According to the sampling plan, 0.4% of cheese samples were positives for *Salmonella* spp.
The pathogen concentration changes during cheese manufacturing, aging distribution, until consumption. Risk reduction was assessed comparing different scenarios. The results of the stimulation indicated that the effectiveness of the sampling plans is sensitive to the level of contamination at the time of sampling. Sampling at the end of acidification resulted as the more effective.

4.4 Dehairing process as a source for *Salmonella* contamination of pig carcasses

The representative from Belgium presented some results from the project Safemeat. The aim of the study was to investigate the source of contamination of *Salmonella* spp. in pig carcasses at the slaughterhouse. 105 pig carcasses were sampled and 64% were found positive for *Salmonella* spp. The investigation

[3] https://www.anses.fr/fr/system/files/BIORISK2016SA0168.pdf

included samples from rectum content, carcass swabs of breast and elbow before/after evisceration and mouth swabs.
The microbiological analysis showed that the number of positive samples from rectum and mouth swabs increased after dehairing. Samples from the dehairing machine were positive and the temperature detected was in between 28.4°C and 38.2°C, confirming that the contamination mainly occurred at the dehairing process.

4.5 *Listeria monocytogenes* on beef carcasses before chilling in slaughterhouses and sources for this contamination

The Belgian representative presented the results of the research that investigated the contamination rate and contamination sources of *Listeria monocytogenes* in carcasses in Flemish slaughterhouses. The first step of the research consisted in swab sampling of the carcasses in three slaughterhouses. *L. monocytogenes* was isolated in all the three slaughterhouses and 42 out of 90 carcasses resulted as positives, from different area of the carcass. To map the contamination routes, samples from hides and carcasses from four slaughterhouses were collected showing 97% and 47% positive results respectively.
The pulsotypes of the strains evidenced two scenarios: the direct transfer from hides to carcases and a possible persistent strain in the environment.
For the persistence research step, samples were collected in the clean area from carcasses and carcass splitter. The results showed the presence of *L. monocytogenes* in carcases after splitting and from the carcass splitter even if in different time of the year.
In conclusion, hides, but also persistent *L. monocytogenes,* are important source of contamination at the abattoir.

4.6 Evaluation of listeriosis risk related with the consumption of non-prepacked RTE cooked meat products handled at retail stores in Greece

The Greek representative presented the main output of the joint research between EFET and Aristotle University of Thessaloniki, granted by EFSA on non-prepacked RTE cooked meat products sliced at retail food service environments. The main goal was to assess the exposure of consumers to *L. monocytogenes* related to the consumption of non-prepackaged RTE cooked meat products handled at retail food service environments in Greece. An exposure assessment model was developed based on the prevalence and concentration of *L. monocytogenes* at the retail level, its growth during domestic storage, and consumption data. The model will be further used to evaluate mitigation strategies at both retail and domestic level such as better hygiene conditions at retail level, regulation of a use-by date for non-prepackaged RTE cooked meat products and improvement of domestic storage temperature.

4.7 Foods considered unable to support growth of *Listeria monocytogenes*

The representative from Ireland presented the discussion on foods considered unable to support the growth of *L. monocytogenes*. Considering the criteria for *L. monocytogenes* in ready to eat foods in Reg. (EC) 2073/2005, the attention was focused on multicomponent food with a shelf-life of <5 days.

The update of the Regulation (Reg. (EC) 2019/229) consider sprouted seed as able to support the growth of *L. monocytogenes* therefore covered by the criterion for ready-to-eat foods; while the *fresh, uncut and unprocessed vegetables and fruits* are legally considered unable to support the growth of *L. monocytogenes* (cat. 1.3). Nevertheless, the evidences of survival and growth of *L. monocytogenes* on whole fresh produce according to some published studies raised some concerns.
In addition, it was emphasized the importance of a clear labeling in case of products that have to been thoroughly cooked or reheated before consumption to avoid the consumer to be exposed to food safety risks.
It was agreed to follow-up the discussion with a questionnaire from Ireland to ask network members for their views and experience on this discussion.

4.8 Evaluation of freshness and microbiological safety of portioned raw meat on special offer sales at the end of expiration date

The representative from Croatia presented a research on quality and microbiological safety of portioned raw meat at the end of expiration date. Up to 10% of total food waste is related to the date of labelling of the shelf life, in fact after the "use by" date, the food must not be placed in the market. 150 pre-portioned raw meat samples and 150 minced meat samples from different species were collected. Microbiological analyses were conducted on the last day of the expiry date of the product according to the provision of the EU regulation and national microbial criteria guide.

The freshness was assessed including sensory evaluation, pH and ammonia. *Salmonella* spp. was detected in 10 samples; 22 samples of poultry meat showed *Enterobacteriaceae* concentration of more than 10^5 cfu/gr. The increased number of aerobic mesophilic bacteria and *Enterobacteriae* were associated with poor sensory characteristics thus indicating the impossibility to extend the shelf life of these products.

22 May 2019

5. Welcome and apologies for absence

Apologies were received from Ireland.

6. Topics for discussion

6.1 Tracing the source of foodborne disease outbreaks using FoodChain-Lab

The German representative introduced FoodChain-Lab[4], a free open source software for traceability in foodborne disease outbreak investigations with the aim of tracing back and forward suspicious food items along the supply chain.
The tool allows integrating available tracing information into one database, including the processing, visualization and analysis of the data. An example of a successful application of a preliminary version was the EHEC outbreak in 2011.

[4] https://foodrisklabs.bfr.bund.de/foodchain-lab/

The presentation was followed by a practical session introducing the tool and its functions.
The network members were invited to access and use the tool for their own investigations and to ask BfR for any assistance needed, including the organisation of practical workshops at their own organisations.

6.2 Contamination and antimicrobial resistance in prawns and pangasius from Asia

The representative from Denmark presented the results of the research conducted to address the following question: what is the risk of introducing antimicrobial resistance (AMR) via shellfish and pangasius from Asia? Pangasius is imported as fillet for further cooking, prawns are imported as raw and pre-cooked and the pre-cooked ones are often ready to eat.

300 frozen samples were collected and analyzed: 100% of the pangasius fillets samples were contaminated with *Enterococci*, 52% with *E.coli*; 89.7% of prawns samples were contaminated with *Enterococci* while 25% with *E.coli*. 10 multidrug resistant *E.coli* were isolated.

A high proportion of contaminated samples may pose a high risk of AMR gene import. Most of the resistance genes evidenced with this study are already present in Danish products but mobile quinolones resistance genes have also been identified. In addition, one isolate showed resistance to ESBL, macrolide, colistin and mobile fluoroquinolone resistance.

In conclusion, it is not possible to exclude that these products may pose a risk to consumers by introducing AMR genes that are still rare in domestic food sources.

6.3 *Campylobacter* on chicken carcasses - including assessment of risk change between 2013-2017

The representative from Denmark further presented the surveillance system of *Campylobacter* in slaughter carcasses in Denmark (including 1,000-1,500 leg skin samples from chicken, annually analyzed). The data collected are used in the exposure model, that allows a continuous risk assessment against baseline.

6.4 Quantitative microbiological risk assessment on *Campylobacter* in the broiler meat chain

The representative from France presented a report on the update of the knowledge on *Campylobacter* contamination of broilers and the assessment of the impact of interventions at different stages of the food chain in France.[5]
The Working group conducted an extensive literature review of control measure of *Campylobacter* in the poultry production and their improvement since the publication of the EFSA opinion on *Campylobacter in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain* (2011).[6]
Campylobacter contamination and interventions were considered before slaughter, during the slaughter process and at the consumer phase; modelling of the French poultry food chain from rearing to consumption was used for assessing

[5] https://www.anses.fr/en/system/files/BIORISK2016SA0183RaEN.pdf
[6] https://doi.org/10.2903/j.efsa.2011.2105

the risk of consumer disease and selecting/optimizing the control measures; cost/benefit analysis of risk reduction was also conducted. The model predicts the number of campylobacteriosis cases in France and risk reduction was calculated for each intervention.
In conclusion, on the farm, the model showed that most effective interventions are those targeting at a reduction of carcass contamination; at the slaughterhouse air-cooling appears to be an effective intervention. Finally, at the consumer phase, cleaning of hands and utensils can reduce the risk of campylobacteriosis.

6.5 Game meat: *Toxoplasma*, *Trichinella* and HEV

The Swedish representative presented three risk assessment reports related to wild boar, focusing on *Trichinella*, *Toxoplasma gondii* and Hepatitis E.
Franssen et al. (2017)[7] published a farm-to-fork model for the risk of human trichinellosis from pork and wild boar meat that can be considered as a support for risk-based monitoring of *Trichinella*. Roth et al. (2016)[8] reported the wild boar prevalence in Sweden as 15%; furthermore, genetically similar hepatitis E virus strains infect both humans and wild boars in the Barcelona area, Spain, and Sweden. [9]
In conclusion, confirming the traditional advices in case of game meat consumption, wild boar meat should pass a certified slaughterhouse and in case of uncertainty if the game meat has been tested, wild boar meat should be cooked thoroughly.

6.6 Game meat: health assessment of human pathogenic parasites

The representative from Germany presented the results of the risk assessment of parasites in game with reference to *echinococcosis*, *cysticercosis* and *Alaria alata*, extended to trichinellosis, sarcosporidiosis, toxoplasmosis.
According to the EU legislation and to the German animal food hygiene regulation (Tier-LMHV), wild animal carcasses are generally subject to official *post-mortem* inspection.
A non-systematic literature research was carried out and the exposure was estimated using the prevalence in wild animals combined with the game meat supply in Germany and the consumption (average <1g of game/day). Even if data are mostly insufficient, the risk of parasitosis from eating game meat is considered very low. For the so-called, 'extreme consumers' there is a possibly higher risk as well as for vulnerable population groups. In conclusion, game and game meat products need sufficient heat treatment and, in particular for sensitive population groups, thorough cooking is required.

6.7 Screening of Ready-to-Eat Meat Products for Hepatitis E Virus in Switzerland

The representative from Switzerland presented the results of a study on Hepatitis E virus (HEV). HEV has considerable genetic diversity, with four major genotypes:

[7] https://doi.org/10.1016/j.ijfoodmicro.2016.10.029
[8] https://doi.org/10.3390/v8090259
[9] https://doi.org/10.1111/tbed.13115

genotypes 1 and 2 are found only in humans, whereas genotypes 3 and 4 are found in both humans and several animal species (domestic swine, wild boar and deer) and they can be transmitted through the food chain.
Products containing raw pork liver at highest risk investigated. An enhanced HEV surveillance was put in place, including the obligation to report positive findings of HEV by PCR since 1.1.2018.
Data from Moor et al. (2018)[10] on *Screening of Ready-to-Eat Meat Products for Hepatitis E Virus in Switzerland* were presented. Pork liver sausages and raw meat sausages from the Swiss retail market were tested for the presence of HEV. The RNA of the virus has been detected in 18 samples: 11 samples of total 42 for liver sausages, 7 samples of the total 190 for raw meat sausages. The significance of the presented work was a current overview of the HEV prevalence in ready-to-eat meat products on the Swiss retail marked and an improvement of the extraction efficiency of the HEV detection method.
In conclusion, food business operators should apply strategies to minimize risks (i.e. heat treatment of products, control of raw meat) while consumers (in particular vulnerable ones) who want to minimize the risk of an HEV infection should avoid eating raw meat products, in particular those containing raw liver.

6.8 Recent and ongoing mandates of BIOHAZ Panel

The BIOHAZ secretariat presented the recently adopted and ongoing mandates of the BIOHAZ Panel.

Recently adopted opinions by the BIOHAZ are on:

- *Salmonella control in poultry flock and its public health impact* (EFSA-Q-2017-00692)[11]
- *Hazard analysis approaches for certain small retail establishments and food donations (EFSA-Q-2017-00565)*[12]
- *Public health risks associated with food-borne parasites (EFSA-Q-2017-00460)*[13]

New mandates received are:

- Scientific opinion as regards specific maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed (EFSA-Q-2019-00221)[14]
- Scientific opinion on the evaluation of public and animal health risks in case of a delayed *post-mortem* inspection in ungulates (EFSA-Q-2019-00124)[15]

[10] https://doi.org/10.1007/s12560-018-9340-x
[11] https://doi.org/10.2903/j.efsa.2019.5596
[12] https://doi.org/10.2903/j.efsa.2018.5432
[13] https://doi.org/10.2903/j.efsa.2018.5495
[14] http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?3
[15] http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?4

- Use of the so called "tubs" for transporting and storing fresh fishery products (EFSA-Q-2019-00053)[16]
- Public health risk posed by *Listeria monocytogenes* in frozen fruit and vegetables including herbs, blanched during processing (EFSA-Q-2018-01006).[17]

In relation to EFSA-Q-2018-01006 on Listeria in frozen fruits and vegetables, EFSA is requesting the network members to send any data they may have on *L. monocytogenes* in these food products. A data request will be sent to the network after the meeting specifying the required information.

7. Any other business

The network members expressed their concern about the reduction of the number of network meetings from two annual meetings to only one. This reduction results in fewer possibilities for networking and exchange of information. The network members encourage EFSA and network member organizations to find solutions that would allow two meetings per year. A solution envisaged is the possibility to schedule only one meeting per year but extended to one day and a half. The chair promised to inform EFSA managers about this concern and the suggestions made and to look into possible solutions.
The chair then summarised some follow-up activities identified during the meeting: Netherlands to circulate questions on criteria for raw milk, Ireland to send questionnaire on criteria for *Listeria* in RTE foods, network to reply to Croatia's data request, if not done year and Croatia to provide a summary of results and to reply to the data request from EFSA on Listeria in frozen fruit and vegetables.

8. Date for the next meeting

The next meeting will be held on 5 and 6 May 2020 in Parma.

9. Closure of the meeting

The chair thanked the participants and closed the meeting.

[16] http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?5
[17] http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?7