



PROTECTION OF CONSUMERS

by microbial risk mitigation through combating segregation of expertise



ABOUT PROMISE

PROMISE is a EU funded research project with the overall objective to improve and strengthen the integration, collaboration and knowledge transfer between the new and old member states of the European Union and its candidates countries. The goal is to tackle common food safety threats and hence to protect the European consumers.

PROMISE integrates stakeholders like public health authorities and national food safety authorities from the old and new member countries in order to ensure the exploitation of research results into standardization and harmonization efforts and hence to contribute to sustainability of project outcomes.

The PROMISE consortium will work on both exogenous and indigenous neglected routes of pathogen transmission. Exogenous routes of transmission are those where the source is spatially segregated from the consumer sphere in the EU-27, whereas indigenous routes of transmission are those where a close link between the pathogen eco-niche and a vulnerable food supply chain exists.



This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 265877.

www.promise-net.eu

PROMISE STRATEGY

Across Europe food borne illness is a major component in considerations of public health. Food safety issues include community wide concerns such as disease associated with common pathogens and more localized, potentially transferable, problems such as transmissible spongiform diseases or animal viruses.

PROMISE will deliver sound scientific results and risk assessment to form the basis of policy formation to manage food safety and protect consumers. PROMISE will assess the risks for the European consumer of two selected neglected routes of transmission being illegal imports and environmental contamination. Through the integration of National Food Authorities the results will have an impact directly onto the policy-making process in Europe.

PROMISE will provide tools for improved data interpretation, exploitation and modeling expertise on food supply chains and will thus strengthen risk assessment capability and modeling capacity in European Union and Candidate Countries. This will lead to better understanding of epidemiological prevalence data and reasons for their heterogeneity. Improved understanding of uncertainty and variability in the information supply will be used to inform and target dissemination and communication processes that clearly embrace new member states.

PROMISE will develop lecture guidelines, on-line and print training material to be used initially by all project partners. In a further step an open access to this documentation will be provided to other interested institutions. PROMISE will improve existing and develop new methods for improved stakeholder communication. The communication of food related risks is very sensitive, and must be adapted to suit the target audience.

PARTNERS

PROMISE COORDINATION & PROJECT MANAGEMENT

Scientific Coordination
Martin Wagner: martin.wagner@vetmeduni.ac.at

Project Management & Dissemination
Andreas Moser: moser@rtd-services.com

The PROMISE Consortium comprises twenty partners from fourteen different countries in Europe. The following overview shows their specific know-how and expertises:

University of Veterinary Medicine Vienna – Austria

Coordinator: Prof. Martin Wagner, Institute for Milk Hygiene, Milk Technology and Food Science (www.vetmeduni.ac.at)

Federal Institute for Risk Assessment - Germany

Prof. Bernd Appel, Department of Biological Safety (www.bfr.bund.de)

Institute of Food Research – United Kingdom

Dr. Gary Barker, Application of computational techniques in food safety science (www.ifr.ac.uk)

Agricultural University of Athens - Greece

Panos Skandamis Ph.D., Food Hygiene (www.aua.gr)

Teagasc Moorepark Food Research Centre - Ireland

Dr. Kieran Jordan, Food Safety (www.teagasc.ie)

University of Burgos - Spain

Dr. David Rodriguez-Lazaro, Food Science and Molecular Microbiology (www.ubu.es)

Veterinary Research Institute Brno – Czech Republic

Dr. Ivan Rychlik, Salmonella research (www.vri.cz)

University of Ljubljana - Slovenia

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Centre for Agricultural Research of the Hungarian Academy of Sciences – Hungary

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Food Safety Authority – Ireland

Dr. Lisa O'Connor, Food Science (www.fsai.ie)

Hellenic Food Safety Authority – Greece

Angelos Vakalopoulos, Food of Animal Origin Enterprises Control (www.efet.gr)

Turkish Food Safety Authority – Turkey

Serap Nazir, TFSA Board (www.tfsa.tr)

National Sanitary Veterinary and Food Safety Authority – Romania

Liviu Rusu, General Direction of Food Safety (www.ansvsa.ro)

Public Health Authority of the Slovak Republic

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RTD Services – Austria

Andreas Moser, Management & Dissemination (www.rtdservices.com)



PROTECTION OF CONSUMERS

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Food borne illnesses are a major concern in public health.

Strengthening the knowledge transfer on food safety issues in Europe.

Integrating stakeholders from old- and new-member countries to ensure harmonization and standardization in Europe.

Assessment of risks for European consumers on food-borne pathogens from illegal imports and environmental contamination.

Providing tools for improved data interpretation and modeling expertise on food supply chains.

Strengthening risk assessment and modeling capacity in the EU and candidate countries.



Visit the
PROMISE ACADEMY
www.promise-academy.eu

DEAR PROMISE
COLLEAGUES AND FRIENDS,

It's time again for our PROMISE newsletter. We wish you a happy and successful year 2014!



It was great seeing all (or most) of you in Dublin during the TTWS and the Consortium meeting. 2/3 of the project lifetime are over and obviously we are very good on track. Nevertheless, the last year will be the hardest. We thank you in advance for your full commitment and cooperation. Surly, our personal meeting in Dublin (thanks Kieran!) gave us again a motivation kick to all of us.

All the best for the remaining winter time... **days are getting longer again!**

The management team:

*Martin, Markus
and Andreas.*



SUCCESSFUL REVIEW MEETING.

On 24th October 2013, the PROMISE consortium had a review meeting together with the scientific officer from the EC and the external expert in Vienna, Austria. The meeting went quite well and the workpackage leaders presented the work done so far after 18 months of project duration. The work so far is quite impressive and fully in time with the workplan

2nd Technical Training workshop held in Dublin on 18th / 19th of November 2013.

WP 04: The 2nd Technical Training Workshop (TTWS) was held successfully prior to the Consortium meeting in Dublin.



Following the promising start Burgos last year, we have now organized the second training workshop. 23 "Juniors" and "Seniors" came together in the 2-half-day event. Again, the didactic mix was hands-on, praxis oriented and interactive.

While more of the exchange measures have finalized already, the PROMISE ESR's are getting more and more integrated into the Team and building up their own network. A short video documentation of this Training Workshop is available on the project home page.



PARTNER ACTIVITIES.

Faculty of Veterinary Medicine,
University of Zagreb
Good example for Dissemination Activities.

Here are some of the activities in disseminating information about PROMISE, realized by Estella and her team. This should inspire and motivate all of us to bring PROMISE to the public.

On 19th of July 2013 in Osijek, in the premises of Croatian Chamber of Economy, Veterinary Faculty University of Zagreb in cooperation with Croatian Food Agency (HAH) organized the seminar: "New trends of sampling food in EU: milk and dairy products".

This seminar was also the opportunity for cooperation with the FP7 BASELINE project and to spread the information on FP7 project PROMISE. In that light, VET informed all participants with the scope and purpose of PROMISE. The participants also received the information on the current state of the play of the PROMISE project, especially in the light of Croatian role in this project. As a reflection to this, two big articles, one in the daily newspaper and second in the Cro.Vet.J. were published.

These facts motivate Croatian PROMISE team to continue the spreading of the information on PROMISE project to the wider scientific community. Due to that, PROMISE project was also presented within the poster session on the 5th International Congress "VETERINARY SCIENCE AND PROFESSION" ZAGREB, October 3rd and 4th, 2013, under the organization of Faculty of Veterinary Medicine, University of Zagreb. The poster was presented by the PhD student Sandra Gutic and published within in the Book of Abstracts (Gutić, Sandra; Horvatek Tomić, Danijela; Kozačinski, Lidija; Lukač, Maja; Prukner-Radovčić, Estella).

The Croatian team will continue with the activity of informing the public on the PROMISE project news!

Best regards,
Estella

Message from the Coordinator

Dear All,
Pre-Christmas present from Austria,



We have successfully submitted our paper to Food Microbiology. Many, many Thanks to all of you, for collaboration, for contributions, for answers to enquiries. Many Thanks for Bea for helping me with finalizing the ms. Kieran, Thanks for helping us with the English!

I am proud of the PROMISE spirit.
Let see what the reviewers suggest!

Best regards

Martin

Management issues

A revised report of our 18-months interim report has been submitted. Our project officer had required some (minor) changes and further explanations. But now this part is done. We just can wait and hopefully the next payment will come in the first months of 2014.

Next meetings 2014

As agreed in Dublin, this is just to remind you the next PROMISE events:

General Annual Meeting:
Greece, 18-20 June



Final Meeting and Symposium:
Vienna, 18-19 December



WHO IS WHO IN PROMISE.

My name is Nihan ERYILMAZ and I am a member of Kalite Sistem Laboratories Group (KSL). I received a bachelor in biology from Middle East Technical University. I worked in National Funding agency of Turkey (TUBITAK) which is responsible from coordination of National and European Programmes and I have over three years experience in management of Research, Technology Development and Innovation projects, particularly EU Framework projects. 3 months ago, I have changed my job and attended to Kalite Sistem Laboratories Group as Business Development and EU Projects expert.

My role in PROMISE: Overall responsibility of Project management and coordination within KSL group. Dissemination activities for the Project, communication with Project partners, following up technical and financial project issues, preparation of interim and final project reports and scientific research coordination and communication within the internal group are under my responsibility for PROMISE Project.

My research interest: I am not directly involved in scientific research in KSL group but I am coordinating the works done for the projects and I am preparing the scientific reports for the projects. For PROMISE Project my interest focuses on pathogen analysis on different food samples from Istanbul market and preparation and organization of technical trainings and integration of PROMISE academy.

Personal interest: I really love travelling and getting to know about world culture. I love and compassion for animals. I like to spend time reading, watching old movies, being with my family and my cats.



Again, a good start into 2014...
may all your wishes and dreams come true!



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FOOD SAFETY

for the protection of consumers





The overall objective of the project PROMISE was to improve and strengthen the integration, collaboration and knowledge transfer between the new and old member states of the European Union and its candidate countries. A common research, training and dissemination network was implemented in order to tackle food safety threats to protect the European consumer. PROMISE was and is a highly active network of twelve academic partners of multiple disciplines including six national food safety authorities. PROMISE has been funded by the 7th Framework Programme for Research, Technology and Demonstration of the EU.

Research focused on investigating neglected routes of transmission of food-borne pathogens, either on a macro dimension (global transmission and illegal imports) and a micro dimension (cross contamination scenarios at food processing companies) and modeling of prevalence found. A series of training workshops completed by a mobility program for early stage and senior researchers was an anchor of the PROMISE activities. PROMISE furthermore integrated stakeholders like public health authorities and national food safety authorities from the old and new member states through the PROMISE academy in order to ensure the exploitation and dissemination of research results. The project lasted from 2011- 2014 but research efforts will go on at the different partner institutions.



THE METHODOLOGY & STRATEGY

The PROMISE consortium worked on exogenous and indigenous neglected routes of pathogen transmission. Exogenous routes of transmission are those where the source is spatially segregated from the consumer sphere into the EU-27: PROMISE assessed whether and to what extent illegal food imports by individuals through ports, airports and at borders are a risk for the European Union.

2,621 animal food samples were taken at airports, ports, black and food markets selling predominantly home-grown food. The pathogens investigated were *Campylobacter* spp., *Listeria monocytogenes*, *Salmonella* spp. and potentially hazardous *E. coli*. Some partners were looking for Staphylococci and multi-drug resistant *E. coli*. Food originating from more than 50 countries worldwide that has been carried along by travellers in their luggage was assessed with standardized methods.

On the other hand indigenous routes of transmission are those where a close link between the pathogen eco-niche and a vulnerable food supply chain exists. Till the PROMISE project less was known about the impact of environmental contamination on the safety of food supply chains. *Listeria monocytogenes* was the chosen pathogen to improve the exchange of methods and concepts for unravel pathogen transmission in processing facilities, thus increasing the control efficiency of food supply chains.

Three technical training workshops took place in Burgos (Spain), Dublin (Ireland) and Hydra (Greece) and were organized to enhance the know-how of young researchers and to prepare them for future scientific challenges in the fields. The consortium created a platform, the PROMISE academy, for the collection, organization and preparation of the workshop materials and for a web based delivery. The PROMISE academy (www.promise-academy.eu) was identified as the most suitable portal for giving access to the workshop materials.





Main air- and sea-ports, as well as ground border stations, serve as the new borders of many countries that have been opting for the Schengen treaty and could be therefore hot spots for illegal food import. Sampling points were located in Southern Europe and Central Europe and comprised airports (Vienna, Austria; Frankfurt, Germany; Berlin, Germany; Ljubljana, Slovenia; Budapest, Hungary; Bilbao, Spain), seaports (Hamburg, Germany) and ground border stations with food and veterinary inspection service, open to non-EU and non-Schengen countries. In addition, PROMISE analysed samples from specific local markets in Romania and Turkey.

The consortium agreed on certain ISO methods for the detection of foodborne pathogens and worked closely together with the national food safety authorities. 2,621 samples have been collected and have been analysed. 500 isolates have been identified that were of interest for further characterization by molecular research.

Due to data from the airport Vienna, 2,4% of the travellers carry food in their luggage. The average amount of food carried by travellers was between 2,7- 4,2 kg and only few nationalities contribute to the majority of food commodities confiscated at airport Vienna: Turkey, Egypt and China – 77% of all passengers were checked. Hygiene of this products was ominous in many cases (11% of food samples exceeded the *E. coli* limits as given in EU DIR 2073/2005). Food confiscated from travellers could be contaminated at rates higher than described in samples taken from domestic production (regarding Austria: *L. monocytogenes* prevalence was double). Neither at airport Bilbao, Frankfurt or Vienna *Campylobacter* has been found.

Based on the experiences made during PROMISE it turned out that in many cases travellers carrying food in their luggage are not the end users and may be used for purchase in ethnic food selling premises. Due to the limited distribution of only a couple of kg of food, the public health impact is expected to be rather low. But travellers are often not aware of legal restrictions about importing food into the European Union.

One main recommendation by PROMISE is that more detailed information should be distributed to passengers about the legal restrictions on importing food into the EU.

ENVIRONMENTAL CONTAMINATION

PROMISE assessed in-house cross-contamination aspects in food processing and chose *Listeria monocytogenes* as a model organism. Due to the ability of survival in food processing environments (FPE), *Listeria monocytogenes* is recognized as hardly eradicable from FPEs. Twelve European FPEs tested for *L. monocytogenes* by a harmonized approach at small-scale direct marketers and small- and medium-sized enterprises (SMEs) were all contaminated, where as the contamination was smaller in dairies than it was in meat FPEs. Food contact surfaces were contaminated a three-fold lower rate than non-contact food surfaces. The study showed that FPE contamination is a major driver of cross-contamination and a cornerstone for improvement of preventive action.

Three different contamination scenarios (sporadic contamination, hotspot contamination, widely distributed contamination) were described and show that disinfection must be implemented on a daily basis. Strict compartmentalization from dirty to clean areas are needed but also additional barriers between these areas are necessary. The results indicate that the major *L. monocytogenes* vehicles were shoes, transport vehicles and boxes. A sanitation plan should be developed and should include the establishment of a critical control area (CCA) concept. A critical control area should encompass all rooms after the last effective decontamination step in the processing chain.

Disinfection plans in a CCA should be most carefully developed including the latest state of knowledge with a special focus in the avoidance of dilution failures occurring on wet surfaces. Furthermore, drying of the surfaces after cleaning is highly recommended. Close monitoring of CCAs could improve processing environment hygiene with respect to *L. monocytogenes*.



KNOWLEDGE TRANSFER AND NETWORKING

Training workshops and PROMISE academy.

Each training workshop included a series of informative presentations mixed with practical sessions in which participants were encouraged to experience some relevant activities – from constructing cell walls to Monte Carlo computer simulation. At the PROMISE academy website (www.promise-academy.eu) each workshop is accessible in a variety of forms including:

- An e-learning version of each workshop component (with notes)
- A live recording of talks and actions and of many workshop components
- Short description of everyone who presented workshop materials

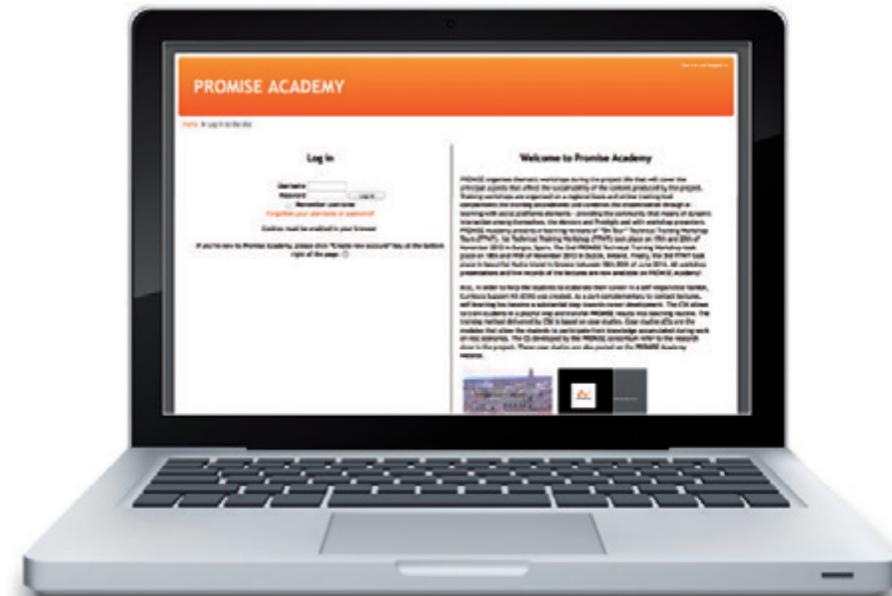
The PROMISE academy website also includes a short video introduction to the technical training workshop series.

A special achievement is a toolbox that helps the students to elaborate their career in a self-responsible fashion. The training method delivered is based on case studies. Case studies (CS) are interactive modules that allow the students to participate from knowledge accumulated during work on real scenarios. As a best practice, CS are built on field experience and are self-explanatory and both ask and give answers. CS are challenging and aim at intertwining multiple sources of information. The CS are introduced by teachers to the trainees and then being run by students. Seven case studies are published on the PROMISE academy website and e-learning platform.

PROMISE mobility program.

The idea of the mobility program has been that Early Stage Researchers (ESR) exchange and link with Senior Researchers (SR) and to achieve an efficient knowledge exchange. Thirteen ESRs took the opportunity for exchange. Five SRs visited PROMISE partner organisations for a short-term visit to enhance the relationship and discuss further cooperations.

Visit the PROMISE website to learn more about the exchanges and the profiles of each Early Stage Researcher.



Models for uncertainty and variability of prevalence estimates.

In Europe the EFSA and the ECDC collect, collate and publish data about food borne illness and zoonoses within the community each year. National data is submitted annually by each EU MS and covers a variety of issues from reported outbreaks of food borne illness to the prevalence of zoonotic agents in flocks of laying hens. The data can be used to indicate changes in time (trends) or to make comparisons between MSs (spatial patterns) but these signals are difficult to visualise and interpret. PROMISE explored the origins of uncertainty and variability in the national reports and whether additional analyses or modelling approaches could assist with harmonization or could improve the interpretation of food safety information.

An initial search for public domain data that supplements the official reports, which involved many PROMISE partners and produced a small database of additional sources, confirmed that the EFSA annual summaries, and the national submissions which support them, represent the dominant EU food safety information supply.

Annual summary statistics are most easily considered as a spatially segmented, coarse grained, time series. In PROMISE traditional time series analyses were explored and an alternative scheme based on discrete changes, more commonly associated with biosurveillance, is recommended. Traditionally the segmentation of safety information by geographical region (MS) is represented by a coloured map (choropleth) but a review reveals many exciting new visualization and infographics techniques that could be used to include additional aspects of the data including uncertainties, effects of borders and agent types.

There is a complex relationship between the actual incidence of food borne illness or zoonoses (which follow true trends) and the number of reports that are submitted to surveillance systems. Underreporting is responsible for a major part of the uncertainty associated with summary information. Following on from previous work performed by the MedVetNet group national reporting of pathogen specific food borne illness has been examined and considerable variability exists. Within the reporting process some key features can be identified, such as the way in which bloody diarrhoea is reported by cases and by practitioners, and these form the basis for guiding improved reporting and increased harmonization.

The assessment of potential cross-contamination in food processing enterprises has been summarized into specific recommendations for food business operators (FBOs) and to help them during implementation of monitoring critical environmental points (CEP).

- Understand the nature of *L. monocytogenes* contamination as an important route of transmission via food processing environments (FPEs).
- Take FPE based contamination serious and develop a FPE monitoring concept as core of the Good Hygiene Practices (GHP).
- Improve your methodological arsenal and build the FPE monitoring on rapid detection of *L. monocytogenes* by Polymerase Chain Reaction (PCR) methods.
- Choose the right sampling sites and methodology. Sampling is the most critical procedural step in any circumstances.
- Choose the right sampling frequency. First time sampling of the FPE should be based on a broad sampling approach. Test a restricted number of sampling sites frequently rather than test a lot of sites only once.
- Establish critical control areas and clearly define these areas where FPE contamination is not acceptable under any circumstances. Critical control areas should be clearly marked.
- Trace the route of transmission of isolates most importantly in CCAs where the risk for contamination of the food is the highest and pay attention to not sanitized niches.
- Mind phases of reconstruction and be aware of the high risk of cross-contamination during these times. Try to avoid access of craftsmen and external people to areas of production as much as possible.
- In cases of disseminated contamination, review critically the floor sanitation procedures applied and use drain water sampling to control the efficiency of sanitation.
- Structure your data and do not just collect data. Use external support to establish an internal decision making process based on the self-control data.
- Document carefully your progress and efforts on good hygiene practices.

Further details on the PROMISE website or directly contact **Martin Wagner** (martin.wagner@vetmeduni.ac.at)

PARTNERS

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- University of Veterinary Medicine Vienna – Austria
- Federal Institute for Risk Assessment - Germany
- Institute of Food Research – United Kingdom
- Agricultural University of Athens - Greece
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- University of Burgos - Spain
- Veterinary Research Institute Brno – Czech Republic
- University of Ljubljana - Slovenia
- Centre for Agricultural Research of the Hungarian Academy of Sciences – Hungary
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Legal Notice

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Ethical Issues

The PROMISE Consortium undertakes to respect all basic ethical principles as outlined in the Charter of European Fundamental rights, including human dignity, cultural, religious and linguistic diversity, equality and anti-discrimination, freedom of expression and of information and respect for the environment.

Additional information

The PROMISE Website can be assessed via: www.promise-net.eu



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Dr. Gary Barker, Application of computational
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Panos Skandamis Ph.D., Food Hygiene (www.aau.gr)

Teagasc Moorepark Food Research Centre - Ireland
Dr. Kieran Jordan, Food Safety (www.teagasc.ie)

University of Burgos - Spain
Dr. David Rodriguez-Lazaro, Food Science and
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Dr. Ivan Rychlik, Salmonella research (www.vri.cz)

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PROGRAM

MONDAY, 17.11.2014

www.promise-net.eu



promise

10:45 Uhr

Official opening of the meeting

SESSION 1

Do routes of scant attention for the transmission of food-borne pathogens exist?

11:00 - 11:30

Luca Cocolin

Behavior of pathogenic bacteria in the food chain:

***Listeria monocytogenes* as a case study**

DISAFA-University of Turin, Italy

11:30 - 11:50

Martin Wagner

***Listeria monocytogenes* in food confiscated at airport Vienna**

University of Veterinary Medicine,

Vienna, Austria

11:50 - 12:10

Anca Nicolau

Neglected routes of macro transmission of food-borne pathogens:

Do black markets exist?

Universitatea Dunarea de Jos,

Galati, Romania

12:10 - 12:30

David Rodríguez Lázaro

Food - borne viruses in food confiscates sampled at Airport Bilbao

Instituto Tecnológico Agrario de Castilla y León,

Spain



12:30 - 12:50

Kathrin Rychli

Novel virulence features in *L. monocytogenes* isolated from food from a Romanian black market

University of Veterinary Medicine Vienna,

Vienna, Austria

12:50 - 13:10

Bela Nagy

Food travelling globally: VTEC types and antibiotic resistance markers

Hungarian Academy of Science,

Budapest, Hungary

13:10 - 13:30

Dagmar Schoder

Is Internet cheese a matter of concern?

University of Veterinary Medicine,

Vienna, Austria

13:30 -14:30

Lunch Break

SESSION 2

L. monocytogenes as a model of a saprophyte in food processing environments

14:30 - 15:00

Marios Georgiadis

EFSA Baseline study for *L. monocytogenes*: Prevalence and risk factor analysis

European Food Safety Authority,
Parma, Italy

15:00 - 15:20

Jordi Rovira

Prevalence of *L. monocytogenes* in European food processing environments

University of Burgos,
Spain

15:20 - 15:40

Kieran Jordan

Dynamics of *L. monocytogenes* contamination in farmhouse cheese making

TEAGASC Research Center,
Cork, Ireland

15:40 - 16:00

Beatriz Melero

Monitoring pathogens colonizing a Spanish meat processing plant

University of Burgos,
Spain

16:00 - 16:20

Stephan Schmitz-Esser

Composition of the microbiome in water and biofilms of *L. monocytogenes* positive drains

University of Veterinary Medicine, Vienna, Austria

16:20 -16:50

Coffee Break

SESSION 3

The competition of pathogens in their natural or artificial habitat

16:50 - 17:20

Hilde Kruse

Emerging foodborne diseases – a WHO perspective

World Health Organization,
Copenhagen, Denmark

17:20 - 17:40

Meryem Muhterem

Evolution of *L. monocytogenes* persistence in a food processing environment

University of Veterinary Medicine,
Vienna, Austria

17:40- 18:00

Evangelia Zilelidou

Growth and virulence of *Listeria monocytogenes* in co-cultivation models

Agricultural University of Athens,
Athen, Greece

18:00 - 18:30

Andreas Fahrnleitner

Opening the „black box“ of microbial fecal pollution of ground water resources

Vienna University of Technology,
Vienna, Austria

18:30 *Departure by bus to the vineyards of Vienna*
Reception for BacFoodNet and PROMISE participants at the
“Heurigen Zawotzky”, Reinischgasse 3, 1190 Vienna

22:00 *Departure from the Heurigen and transfer to the Hotels*



LUCA COCOLIN

Institution: DISAFA-University of Turin | Turin, Italy

Position: Associate professor at the University of Turin, Italy. Executive Board Member of the International Committee on Food Microbiology and Hygiene, part of the International Union of Microbiological Societies and Editor-in-Chief of the International Journal of Food Microbiology.

In addition: He is co-author of more of 200 papers on national and international journals. In Scopus (www.scopus.com visited October 18, 2014), he has 163 papers reviewed, which were cited 3326 times, resulting in an h index of 33.

Expert in the field of food fermentations and more specifically in the application of culture independent molecular methods for the study of the microbiota of fermented foods. Moreover, since the end of the 90's he is interested in the molecular behavior of foodborne pathogens (*Listeria monocytogenes*, *Campylobacter* spp. and others) in the food chain.

COAUTHOR: Valentina Alessandria, Marios Mataragas, Paola Dolci, Kalliopi Rantsiou

Behavior of pathogenic bacteria in the food chain: *Listeria monocytogenes* as a case study.

Despite the efforts dedicated by food producing companies, official authorities and research institutions to reduce the prevalence and incidence of foodborne pathogens, they still represent relevant health risks for the consumers. Examples of such evidence are the frequent outbreaks provoked by the consumption of foods contaminated with pathogenic bacteria. One of the last episodes is represented by the 2011 outbreak of the O104:H4 *Escherichia coli* in Germany from the consumption of sprouts. Several thousands were affected and about 50 deaths were recorded.

Due to the important repercussions which food safety has on the society, not only by the health point of view, but also economically, deeper investigations are necessary to develop tools to combat and reduce the incidence of foodborne pathogens.

In the last 10 years the possibility to study the behavior of bacteria, through the use of microarrays, reverse transcription quantitative PCR (RT-qPCR) and, more recently, RNA seq has opened up new possibility to comprehend how they express specific genes in response to environmental parameters and throughout the food chain. Moreover prediction models can be developed, which are able to anticipate the behavior of pathogenic bacteria.

In this study we will present the results obtained by applying molecular methods (microarrays and RT-qPCR) to investigate the transcriptomic response of *Listeria monocytogenes* when subjected to several stresses in vitro (mainly pH and salt) and in situ (during fermentation of sausages). The results obtained underline the heterogeneity of the strains used in the study and they highlight how this intra-species diversity has to be taken into consideration for risk assessment. Moreover, it was underlined the important difference of the outcomes when performed in vitro and in situ.

The study demonstrate how transcriptomics can be efficiently used to better understand the behavior of *L. monocytogenes* in the food chain.



MARTIN WAGNER

Institution:	University of Veterinary Medicine Vienna, Austria
Position:	Head of the Institute for Milk Hygiene, Milk Technology and Food Science, Department of Farm Animal and Public Health in Veterinary Medicine
In addition:	<p>1981 Enrollment at the University for Veterinary Medicine</p> <p>1986 First interest in painting, several group exhibitions</p> <p>1991 Diploma at the VMU</p> <p>1991 Research fellow at the Ludwig Boltzmann - Institute for Immune- and Cytogenetics, Vienna (Prof. W. Schlegler) Project „Detection and Definition of genetic variants in hens and cattle“</p> <p>1991-1993 Post graduate studies at the Institute for Animal Breeding and Genetics (Prof. Dr. B. Mayr) Project „Genome analysis of raptor species“</p> <p>1993 Graduation Dr. medicinae veterinariae</p> <p>1993-2008 Assistant at the Institute for Milk Hygiene, Milk Technology and Food Science, Research focus: Molecular detection and differentiation of foodborne pathogens, Molecular epidemiology and public health issues</p> <p>1993-1996 Establishment of the molecular working group at the IMMF</p> <p>1998-1999 Research fellow at the Biocenter in Würzburg (Prof. Dr. W. Goebel) Project “Comparative molecular virulence of clinical <i>Listeria monocytogenes</i>-isolates</p> <p>1999-2000 Post doctoral research at the Complutense University Madrid, Grupo Patogénesis Molecular Bacteriana, Veterinary Faculty, 28040 Madrid, Spain (Prof. Dr. J. A. Vazquez-Boland) on “Virulence and molecular epidemiology in <i>Listeria</i> spp.</p> <p>7-10/ 2000 Research stay in Würzburg and Madrid</p> <p>22. 9. 2000 Docentship, Subject: Food Hygiene</p>

In addition:	2000-05 Vice Chair of the Institute for Milk Hygiene, Milk Technology and Food Science
	2000-08 Coordinator working group Molecular Food Microbiology
	2003- Coordinator of the research focus IV „Food Safety and Risk Analysis“
	1.11. 2006 Head of the Christian Doppler-Laboratory for Molecular Food Analytics
	1.8.2008 Appointment as Full Professor for Molecular Food Microbiology, Head of the Institute for Milk Hygiene
Awards:	1995 Hermann Zittmayr-Award of the Austrian Milk Science Board
	2000 Armin Tschermak von Seysenegg-Award of the UVM
	2000 Hygiene-Prize of the Austrian Society for Hygiene, Microbiology and Preventive Medicine
	2005 Hygiene-Prize of the Austrian Society for Hygiene, Microbiology and Preventive Medicine (Co-author)
	2007 Hygiene-Prize of the Austrian Society for Hygiene, Microbiology and Preventive Medicine (Co-author)
	2005 Most successful researcher in acquisition of funds

Microbial pathogens in food confiscated at airport Vienna.

THE 7th FP EU funded project „Protection of Consumers through Mitigation of Segregation of Expertise (PROMISE)” studies food-borne pathogens transmitted into EU-28 through less researched gates. One part focused on travelers carrying food in their hand luggage.

At Austria`s main airport Wien Schwechat, more than 11 tons of food are annually confiscated by border authorities from travelers hand luggage. We accompanied the authority for 8 month and took 600 food samples of animal origin carried along by passengers



ANCA NICOLAU

Institution: Universitatea Dunarea de Jos | Galati, Romania

Position: Professor of Food Microbiology, Rapid methods and Automation in Microbiology and Hygiene for Food Business Operators at the Faculty of Food Science and Engineering from the „Dunarea de Jos“. Head of the faculty ISO 17025 accredited Microbiology Laboratory

In addition: Anca Ioana NICOLAU graduated as food technologist (1985) and obtained her PhD title in Biotechnology (1999). She is habilitated to coordinate PhD thesis in the domain of Industrial Engineering, specialization Food Industry.

Her current research is related to the detection of pathogens in food and food processing environments and to the destruction of microorganisms using emerging technologies. She is member of the technological platform Food for Life Romania, where she acts as member of the operational committee and vice-president of the working group Quality, processing and food safety. She represents Romania in COST Action FA 1202: A European Network for Mitigating Bacterial Colonisation and Persistence On Foods and Food Processing.

COAUTHOR: Andrei Sorin Bolocan, Luminita Ciolacu, Elena Alexandra Oniciuc, Kathrin Rychli, David Rodriguez Lazaro, Martin Wagner

Neglected routes of macro transmission of food-borne pathogens: Do black markets exist?

Food black markets are existing all over the world as result of economic strife, unemployment and the high cost of living. Surprisingly, the black market for food is not small. The Europe is not excepted from black markets presence, some of them existing right at the EU borders, as it happens in Romania where the so called Moldavian markets represent illegal points for food trade due to small border traffic.

The occurrence of *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter* and *Escherichia coli* O157:H7 was investigated in 200 food products purchased from such a black market in Galati, Romania. An overall prevalence of 7.5% for *L. monocytogenes* and 8% for *S. aureus* was revealed, whereas neither *Campylobacter*, nor *E. coli* O157:H7 and *Salmonella* could be detected. *L. monocytogenes* was frequently present in fish (17%) and meat products (9.5%), while *S. aureus* attained similar levels in dairy (11%), fish (11%), and meat products (8%).

One isolate of *S. aureus* detected in a pork lard sample was MRSA. This isolate belonged to sequence type 398, harbored SCCmec type V, tested negative for the presence of the PVL genes and was resistant to ciprofloxacin, tetracycline and ceftazolin, besides all β -lactams. Among the other 31 *S. aureus* isolates, which were methicillin-sensitive, 29% were resistant to penicillin, 9.7% to tetracycline and 3.2% to ciprofloxacin.

L. monocytogenes isolates showed distinct virulence profiles, due to a high diversity in the amino acid sequence of main virulence factors: PrfA, internalin A (InIA) and listeriolysin O (LLO). The amino acid sequences of PrfA, InIA and LLO were identical for strains belonging to the same serotype. In total, 30 different amino acid substitutions, resulting in seven different InIA variants, two of which have not yet been described, were detected.

Although the risk associated with the foods sold in Moldavian markets is not high, black marketed foods are sources of new pathogenic strains.



DAVID RODRÍGUEZ-LÁZARO

Institution: Instituto Tecnológico Agrario de Castilla y León | León, Spain

Position: Senior scientist at ITACyL and assistant Professor of Microbiology at the University of Burgos

In addition: Doctor in Veterinary Medicine (DVM), specialised in Food Science (BSc and MSc) and Molecular Microbiology (PhD). He has performed research stays in the Danish Institute for Food and Veterinary Research (Denmark), University of Prague (Czech Republic), the Food and Environmental Research Agency (UK), and the University of Bristol (UK). He was a Leverhulme visiting Professor in the Institute of Advanced Sciences in the University of Bristol during the years 2004 and 2005 and Marie Curie Research Fellow in the Faculty of Medical and Veterinary Sciences in the University of Bristol (UK) until 2007.

His research interest is focused on the establishment of reliable, quantitative molecular strategies for detection of important food-borne pathogens from environmental sources and various types of food-stuffs, the characterisation of the prevalence of the main foodborne pathogens in food and food related environments, and the development of emergent food preservation processes and their effects in the microbial virulence. He has participated in a number of coordinated EU-funded projects such as PROMISE, BASELINE, VITAL, FOOD-PCR, SACROHN, and MONI-QA, having established active links with the leading European research groups working in Food Safety.

He has published more than 90 international scientific papers and book or book chapters regarding to Food Safety. He is currently member of the editorial board of “Applied and Environmental Microbiology”, “International Journal of Food Microbiology”, “Food and Environmental Virology” and “International Journal of

Food Contamination” and the editor-in-chief of the Journal “Food Analytical Methods”. He was awarded with the “XV Jaime Ferrán Award” in 2013 by the Spanish Society for Microbiology for his understanding promising scientific career in Microbiology.

COAUTHOR: Marta Díez Valcarce, Rebeca Montes Briones, Marta Hernández, Jordi Rovira

Food-borne viruses in food confiscated samples at Airport Bilbao

A potential source of transmission of food borne viral infections is (neglected) cross-border routes. The rapid alert systems are valuable monitoring tools for food contamination in legal imports; however these are not the only entrances into the EU. New threats can be carried in the baggage from countries outside the EU. It constitutes a real threat as emergent pathogens such as hepatitis E virus (HEV), and human norovirus could be introduced into the EU.

This study was performed from May 2012 to May 2013. A total of 122 meat samples that has been previously confiscated from travellers’ luggage from 19 countries at the International Bilbao airport (Spain) were analysed for the presence of HEV, hepatitis A viruses (HAV), and human norovirus genogroups 1 and 2 (NoVG1 and NoVG2).

Sixty-seven food samples (54.9%) were positive for least one enteric virus (HEV, NoVG1 and NoVG2), whereas HAV was not found. Interestingly, 65 samples were positive for HEV (53.28%), and 38 pork samples were positive for that virus (65.52%).

These results demonstrate the dissemination of HEV and other enteric viruses through neglected routes as illegally imported foods, and highlight the need for more cross-border containment measures to reduce possible health risks for EU consumers.



KATHRIN RYCHLI

- Institution:** University of Veterinary Medicine | Vienna, Austria
- Position:** Postdoc, Institute of Milk Hygiene, Veterinary University of Vienna.
- In addition:** Main areas of research
- Virulence and stress response of *Listeria monocytogenes*.
 - Discovery of new antimicrobial compounds of epigenetically modified fungi.
- | | |
|-----------|---|
| 10/2009- | Group leader, Institute for Milk Hygiene, Veterinary University of Vienna. |
| 2008-2009 | Postdoc, Department of Internal Medicine II, Medical University of Vienna, Prof. Dr. Wojta. |
| 2005-2008 | PhD studies, Department of Internal Medicine II, Medical University of Vienna, Prof. Dr. Wojta: Regulation of angiogenic biomolecules in the human heart. |
| 2003-2004 | Diploma thesis, Department of Biochemistry, University of Vienna. |
| 1998-2004 | Studies of Chemistry, University of Vienna |

COAUTHOR: Luminita Ciolacu, Anca I Nicolau, Martin Wagner

Novel virulence features in *L. monocytogenes* isolated from food from a Romanian black market.

Listeria monocytogenes is a facultative intracellular foodborne pathogen responsible for listeriosis. Within a recent study, in which we investigated neglected exogenous routes of transmission of foodborne pathogens into the European Union, we have isolated 15 *L. monocytogenes* strains in food products, which were imported from the Republic of Moldavia to Romania and illegally sold at a local market.

The aim of this study was to characterize the subtype and virulence potential of these 15 *L. monocytogenes* strains. Multilocus sequence typing revealed that these *L. monocytogenes* strains belong to six different sequence types (ST2, ST8, ST9, ST20, ST121 and ST155). In addition, *in vitro* virulence assays using human intestinal epithelial Caco2 and macrophage-like THP1 cells showed a high strain variability regarding the invasion efficiency in Caco2 cells (0.04 -6.99%) and the intracellular growth rate in both cell types. Both ST121 strains and the ST9 isolate were unable to invade Caco2 cells; and all ST155 strains showed no proliferation inside macrophages and revealed low cytotoxicity. Furthermore we performed sequence analysis of three main virulence factors: PrfA, internalin A (InIA) and listeriolysin O (LLO). The Romanian food isolates showed a high diversity in the InIA and LLO amino acid sequences, whereas the amino acid sequence of PrfA of all strains was identical. Overall, the amino acid sequences of PrfA, InIA and LLO were identical for strains belonging to the same ST. We detected in total 30 different amino acid substitutions, resulting in seven different InIA variants, two of which have not yet been described. The three strains, which were unable to invade Caco2 cells, harboured a premature stop codon resulting in truncated InIA. Furthermore, we detected four different amino acid substitutions in the LLO sequence, which are present in four variants. The number of LLO mutations correlates negatively with intracellular growth in Caco2 and THP1 cells; and subsequently with cytotoxicity.

In conclusion, we show that *L. monocytogenes* isolated from food samples from a Romanian black market show distinct virulence profiles, due to a high diversity in the amino acid sequence of main virulence factors.



BELA NAGY

Institution: Hungarian Academy of Science | Budapest, Hungary

Position: Professor emeritus and project leader at the Institute of the Hungarian Academy of Sciences

In addition: Received his DVM degree at University of Veterinary Science, Budapest (1965) and his PhD in veterinary microbiology, Budapest (1978), DSc (1993), memb. of Hung. Acad. Sci (1998).

He worked in the Veterinary Diagnostic Service for 20 years, and has been working at the Veterinary Medical Research Institute of the Hungarian Academy of Sciences since 1991 (present short name: Inst.Vet.Med.Res. of HAS-CAR, Budapest (abbreviated:VMRI). He is a professor emeritus and project leader. His main area of research has been enteric microbiology and foodborne zoonoses (E. coli, Salmonella, rota-, and corona viruses, Cryptosporidia). As a visiting scientist at the Iowa State University and NADC (Ames, Iowa USA) he has worked in two terms (1974-76 and 1990-91) with Dr. H.W. Moon and colleagues, and discovered with them new fimbriae (987P and F18) of enterotoxigenic E. coli.

By utilization of the new knowledge he and his colleagues developed new diagnostic tools and patented new vaccines. At the VMRI he established for 20 years he led the research team of "Enteric bacteriology and foodborne zoonoses". Between 1996-2012 he lead 4 PhD works (and co-lead one work) on pathogenic E. coli and Salmonella as well as on antimicrobial resistance, which areas he continues to study also within EU FP7 PROMISE.

COAUTHOR: Martin Wagner, Sonja Smole-Mozina, Jasna Kovac, MarDagmar Schoder, Anja Strauss, Sabine Schlager, Janine Beutlich, Bernd Appel, Marija Lusicky, Mojca Cimerman, Pavel Aprikian, Judit Pászti, István Tóth, Renáta Kugler and Ama Szmolka

Food travelling globally: VTEC types and antibiotic resistance markers.

A major goal of WP1 of PROMISE, was to test foods of animal origin confiscated at airports and ground ports, for the presence of foodborne pathogens by using standardized guidelines [ISO: for O157 verotoxigenic E. coli (VTEC/STEC), and agreed protocols for non-O157 VTEC] and for multidrug resistant (MDR) E.coli.

Results of isolations and of characteristics of the above selected foodborne pathogens from >1800 confiscated food samples were as follows: O157 VTEC was not detected, but one EHEC O26 strain was present. All together 15 strains of non-O157 VTEC, collected by 3 Partners, were identified, mainly from food of ruminant origin. Phenotypic and genotypic (microarray) analysis of VTEC indicated no resistant phenotypes and no genotypic determinants of antimicrobial resistance. However, VTEC strains of various, sometimes unusual O-groups, carried an abundance of E. coli fitness and virulence genes beside stx1 and/or stx2. PFGE and MLST indicated a high genetic diversity of the strains, and MLST typing led to the establishment of three new ST types of E. coli. The 28 strains of MDR E. coli were characterized by a low numbers of virulence genes but by a wider range of antimicrobial resistance genes and a higher frequency of class 1 integrons.

The above results may not raise major or immediate food safety concerns, although they only represent a small fragment of illegal food import to the EU, which may bring a large reservoir of new virulence or antimicrobial resistance genes of E. coli in food smuggled into the EU.



DAGMAR SCHODER

- Institution:** University of Veterinary Medicine | Vienna, Austria
- Position:** Assistant professor at the Institute of Milk Hygiene at the Department of Veterinary Public Health and Food Science, the University of Veterinary Medicine, Vienna, Austria
- In addition:** She began her career studying veterinary medicine in Vienna. She conducted post doctoral research there at the Institute of Milk Hygiene and since 2001 has been lead researcher in the laboratory for Listeria monitoring.
- Since 2008 she has been head of the department's Global Food Safety working group.
- Schoder's research focuses on global food safety. Above all this concerns the control and prevention of Listeria monocytogenes in the food processing environment. Simultaneously, an interest in risk assessment regarding food safety in less and least developed countries has led her to ethnological research. This has involved traditional methods of food processing by indigenous people in East Africa and tracing illegal food trade and intentional food contamination. Dagmar's awards include the Heinrich Stockmeyer Science Award in 2011, the Vienna-Future Award in 2007 for the category of most creative researcher, the AWD Award in 2007 for the most successful junior researcher in the acquisition of funding, the Hermann Zittmayr Award of the Austrian Milk Science Board in 2006 and the Alfred Kleibel Award of the Austrian Society of Veterinary Medicine in 2002.
- Since 2007 Dagmar Schoder has been president of Veterinarians Without Borders Austria. She is also a board member for Veterinarians Without Borders Europe.

COAUTHOR: A. Strauß, K. Szakmary-Brändle, M. Wagner

How safe is European Internet cheese? A purchase and microbiological investigation.

The suitability for consumers of a variety of raw milk cheeses purchased over the Internet was investigated in terms of packaging, labelling, physicochemical parameters and microbiological safety. 108 purchases from seven European countries were examined. The prevalences of Salmonella spp., L. monocytogenes, E. coli and coagulase positive staphylococci (SA) were determined. All 108 samples were described on websites as raw milk cheeses and thereby qualified for this study.

However, after delivery it was noted that 4.6% (5/108) of cheeses were labelled to be manufactured from heat-treated or pasteurized milk. Delivery duration ranged from 24 hours to six days, receipt cheese temperatures ranged between 5 - 23 °C, whereas in 61.5% of all cases the temperature was higher than 15 °C. Cheese labelling was examined in respect of EC Guideline 2000/13 and Regulation No. 853/2004. Only 17.6 % (19/108) of cheeses were properly labelled. In 50.9%, 48.9%, 46.3% and 39.8% of all cases (i) specific storage requirements, (ii) name and address of the manufacturer/packer or seller, (iii) net weight and (iv) minimum shelf life, were missing. Even the labelling information "made from raw milk" was not apparent on 36% of all cheese items delivered. None of the 108 investigated cheeses showed a pH \leq 5.0 and aw value \leq 0.94. For 2 samples (0.9%) and 11 samples (10.2%) the pH and the aw value was \leq 4.4 or \leq 0.92 at least at one of the three time points. E. coli and SA could be detected in a total of 29.6% (32/108) and 8.3% (9/108) of samples, respectively. The foodborne pathogen L. monocytogenes was detected in 1.9% of all samples, one of which had counts of 9.5×10^3 CFU/g, whereas Salmonella spp. was not detected. Results reveal that labelling and hygiene concerns about the safety of Internet purchased cheeses in Europe are justified.



MARIOS GEORGIADIS

- Institution:** European Food Safety Authority | Parma, Italy
- Position:** Scientific Officer at the Assessment and Methodological Support Unit (AMU)
- In addition:** He holds a degree in Veterinary Medicine from the Aristotle University of Thessaloniki, in Greece and a Masters in Preventive Veterinary Medicine and a Ph.D. in Epidemiology from the University of California, Davis in United States of Amerika.

COAUTHOR: A. Strauß, K. Szakmary-Brändle, M. Wagner

Analysis of the baseline survey on the prevalence of *Listeria Monocytogenes* in certain ready-to-eat foods in the EU, 2010-11.

A European Union-wide baseline survey on *Listeria monocytogenes* was carried out in 2010 and 2011 in certain ready-to-eat foods at retail: 3053 batches of packaged (not frozen) hot or cold smoked or gravad fish, 3530 packaged heat-treated meat products and 3452 soft or semi-soft cheeses were sampled from 3632 retail outlets in 26 EU Member States and one country not belonging to the EU. The fish batch samples were analysed on arrival at the laboratory and at the end of shelf-life, whereas the meat products and the cheese samples were analysed only at the end of shelf-life.

The EU-level prevalence in fish samples at the time of sampling was 10.4 % and at the end of shelf-life was 10.3%, while for meat and cheese samples at the end of shelf-life it was 2.07% and 0.47%, respectively. The EU-level proportion of samples with a *Listeria monocytogenes* count exceeding the level of 100 cfu/g at the end of shelf-life was 1.7%, 0.43% and 0.06% for fish, meat and cheese samples, respectively, while for fish at the time of sampling it was 1%.

Furthermore, Generalized Estimating Equations were used to investigate the statistical association between several factors on which information was gathered during the baseline survey, and two outcomes: prevalence of *Listeria monocytogenes* and proportion of samples with counts exceeding 100 cfu/g, in the surveyed fish and meat products.

Finally, a statistical model was developed that allowed the use of estimates of the proportion of samples with an *L. monocytogenes* count >100 cfu/g obtained from a single-unit sample survey of a population of RTE foods, in order to estimate the probability that if a five-unit sample had been taken from the same population, no individual unit, out of $n = 5$ units constituting the sample, would have exceeded the level of 100 cfu/g.



JORDI ROVIRA

- Institution:** University of Burgos | Burgos, Spanien
- Position:** Professor of Food Science and Technology of the University of Burgos
- In addition:** Degree in Biology and PhD in Cellular and molecular biology for the University of Navarre (Spain).
- Head of the Department of Biotechnology from the meat company Campofrío Alimentación, since 1989 till 1993.
- Head of the transfer of knowledge of University of Burgos, since 1994 till 1997.
- Professor of Food Science and Technology of the University of Burgos since 1994 till nowadays.
- Main topics of interest: Food microbiology (spoilage and pathogens), meat and fish products, food fermentations.

COAUTHOR: Meryem Muhterem-Uyar, Martin Wagner and 13 more from PROMISE project (Marion Dalmaso, Andrei Sorin Bolocan, Marta Hernandez, Anastasia E. Kapetanakou, Tomáš Kuchta, Stavros G. Manios, Beatriz Melero, Jana Minarovicová, Anca Ioana Nicolau, Jordi Rovira, Panagiotis N. Skandamis, Kieran Jordan, David Rodríguez-Lázaro, Beatrix Stessl)

Prevalence of *L. monocytogenes* in European food processing environments.

Listeria monocytogenes enters the food processing facility via environment, or contaminated raw materials. To increase the understanding of *L. monocytogenes* environmental contamination in the meat and dairy food sector, six European scientific institutions sampled twelve food processing environments (FPEs) in a harmonized methodological approach. The selection of six previously assumed uncontaminated (UC) FPEs and six contaminated (C) FPEs was based on the *L. monocytogenes* occurrence information originating from the time prior to the current study. An aim of the study was to highlight, that FPEs regarded for years as uncontaminated, may also become *L. monocytogenes* contaminated and repeated environmental sampling could help to identify the potential sources of contamination.

From a total of 2,242 FPE samples, *L. monocytogenes* was present in 32% and 8.8% of meat and dairy processing environments, respectively. In the actual study, each FPE was contaminated with *L. monocytogenes* on at least one sampling occasion. Three contamination scenarios could be observed: (i) sporadic contamination in the interface of raw material reception and hygienic areas, (ii) hotspot contamination in the hygienic processing areas (iii), and widely disseminated contamination in the entirely FPE. These data demonstrate that *L. monocytogenes* are common colonizers of FPEs throughout Europe and that a consistent cross-contamination risk exists. To avoid food contamination, a risk assessment approach should assign risk levels to critical control areas (CCAs) and identify those where cross-contamination should be essentially excluded.



KIERAN JORDAN

Institution: TEAGASC Research Center | Cork, Ireland

Position: Chairperson of the International Dairy Federation Standing Committee on Microbiological Hygiene and a Member of the Biological Safety sub-committee of the Food Safety Authority of Ireland.

In addition: Kieran Jordan works in microbiological food safety and milk quality. His main areas of interest include survival, growth and persistence of *Listeria monocytogenes* in processing environments, using molecular methods to understand epidemiology, routes of transfer and sources of the bacterium.

He also works on other food pathogens, including *Staphylococcus aureus*, *Cronobacter sakazakii*, *E. coli* O157:H7, *Campylobacter*, and on milk quality issues related to the dairy industry. These interests are reflected in a number of publications in international journals in, particularly in relation to epidemiology and persistence of *L. monocytogenes*, residues in milk.

Dynamics of *L. monocytogenes* contamination in farmhouse cheese making.

Listeria monocytogenes, which causes listeriosis, is primarily a foodborne pathogen that is widespread in the environment. Therefore, it will be found in food processing environments from where it can contaminate food. It is important that food business operators are aware of the presence of *L. monocytogenes* in the processing environment so that targeted measures can be taken to control it.

This presentation will focus on process environment sampling of farmhouse cheesemaking facilities that was undertaken as part of the PROMISE project. Extensive sampling was undertaken to determine if contaminated facilities remain contaminated and presumed uncontaminated facilities remain uncontaminated. The results indicated that all facilities can be contaminated with *L. monocytogenes* and that the contamination status can be defined in order to facilitate the type of targeted action necessary to control the contamination and reduce the risk of contamination of food.





BEATRIZ MELERO

Institution: University of Burgos | Burgos, Spain

Position: Researcher in the Area of Food Technology in the University of Burgos since 2008

In addition: PhD in Food Science and Technology from the University of Burgos rated "Apto Cum Laude" (2012), with the mention of European Doctor and PhD Prize (2013). Participate in two research lines: the study of different strategies to increase the shelf life and food preservation and the study of Food Safety Management Systems for food safety, particularly focused on the microbiological hazards in food industries. Within these lines, has participated in seven competitive projects with public funding: 2 European, 1 national, 1 regional and 1 local. Has also participated in two research contracts with local food industries. These investigations have led the publication of 6 papers in scientific journals located in the first quartile, 2 book chapters and authored and co-authored numerous communications in international and national conferences.

Since 2010 she has participated in various subjects of the Bachelor's Degree in Food Science and Technology, and Bachelor's Degree in Agroalimentary Engineering and the Rural Environment. She has also participated in one competitive European teaching innovation project with public funding.

COAUTHOR: Beatriz Manso, Martin Wagner, Beatrix Stessl, Jordi Rovira, David Rodríguez-Lázaro

Study of *Listeria monocytogenes* colonization in a new dairy processing facility and its molecular characterization.

Listeria monocytogenes colonization in a new dairy processing facility was studied during one year. The dairy processing plant is divided into two facilities separated 13 Km from each other. A total of 536 samples were taken, including non-food contact and food contact surfaces and food samples, with a total of 45 positive samples. The prevalence of *L. monocytogenes* was similar in each building (8.72 % in building I and 8.18 % in building II, the new one) but *L. monocytogenes* was detected for the first time in the third visit in building II while it was always present in building I.

Molecular characterization (serotyping, PFGE and MLST) was performed for all the 45 isolates, that were divided into 7 PFGE types and 7 sequence types (ST). The salting area (building I) showed a high diverse contamination (presence of the 7 PFGE types) while building II was colonized with the PFGE type 3 that appeared for the first time in the third visit and was spread across the facility until the last visit. This colonization was mainly caused by the lack of hygienic barriers when operators change their working place because of different production needs. Thus, food quality managers should make an effort to improve operator's training as well as provide appropriate and well distributed hygienic measures.



STEPHAN SCHMITZ-ESSER

- Institution:** University of Veterinary Medicine | Vienna, Austria
- Position:** Group leader, Institute for Milk Hygiene, University of Veterinary Medicine Vienna
- In addition:** Research interests:
- Persistence and adaptation of *Listeria monocytogenes* in food production environments
 - Microbiome characterizations of farm animals
 - Cheese rind microbiology
- 2010 - 2014 Postdoc, "Ecology of food-borne pathogens", Institute for Milk Hygiene, University of Veterinary Medicine Vienna
- 2004 - 2010 Postdoc, Department of Microbial Ecology, University of Vienna
- 2001-2004 PhD thesis: "Molecular interaction between a chlamydia-related endosymbiont and its *Acanthamoeba* host"
- 2003-2004: Department of Microbial Ecology, University of Vienna
- 2001-2003: Department of Microbiology, TU München, Germany
- 1996-2001 Studies of Biology, TU München, Germany, main subject: Microbiology

COAUTHOR: Elisa Schornsteiner, Martin Wagner

Community analysis of *Listeria monocytogenes* -contaminated and uncontaminated dairy plant floor drains by 16S rRNA amplicon pyrosequencing.

Controlling *Listeria* (*L.*) *monocytogenes* is of great concern for food safety. Floor drains are an important source for contamination and recontamination of food production plants with food-borne pathogens. However, the microbial community of floor drains has only rarely been investigated until now. We hypothesize that the survival of *L. monocytogenes* in floor drains is dependent on the co-occurrence of other microbes.

The aim of this study was to characterize the microbial community of drain water- and biofilm in two Austrian dairy plants using Roche/454 pyrosequencing of 16S rRNA gene amplicons. The community composition of three *L. monocytogenes*-contaminated and two -uncontaminated floor drains were analyzed along the time line. In order to compare the community composition of drain water and -biofilm, four and three floor drains from the contaminated and the uncontaminated dairy plant, respectively, were sampled at one time point. All samples were tested for the presence of *L. monocytogenes* using quantitative PCR and cultivation after enrichment in half and full-strength Fraser broth.

In total, 24 drain samples including biofilm and drain water samples from the *L. monocytogenes*-contaminated and the uncontaminated dairy plant were sequenced and analyzed using mothur. After quality control 94,889 reads remained (approx. 4,350 reads per sample). The communities in the floor drains were dominated by three phyla (Proteobacteria, Firmicutes and Bacteroidetes; more than 94.5% of all reads). Already on phylum level, the community composition of most analyzed samples was highly different. The most abundant families were: Streptococcaceae, Lactobacillaceae, Flavobacteriaceae and Pseudomonadaceae. In drains from production areas, product-associated bacteria e.g. *Lactococcus* were highly abundant. The presence of *L. monocytogenes* reads was shown, although at low abundance.

Here we show first deep insights into the community composition of floor drains which might allow the detection of possible co-occurring taxa which might help controlling *L. monocytogenes*.



HILDE KRUSE

Institution: World Health Organization | Copenhagen, Denmark

Position: Programme Manager, Food Safety, WHO Regional Office for Europe

In addition: Dr Hilde Kruse graduated as a DVM from the Norwegian College of Veterinary Sciences in 1990. She holds a PhD in microbiology from the same university and is a diplomat of the European College of Veterinary Public Health.

During her PhD studies, addressing the epidemiology of antimicrobial resistance, Dr Kruse spent half a year at the US Centers for Disease Control and Prevention (CDC). After she received her PhD degree, she worked as an associate professor in food hygiene at the Norwegian College of Veterinary Sciences, before she left for Washington DC for one year as a Fulbright fellow working as a food safety policy analyst at the US Food and Drug Administration (FDA) and at the US Department of Agriculture, Food Safety and Inspection Services.

Thereafter she held different positions at the National Veterinary Institute in Norway (senior scientist and laboratory leader in food microbiology (1996-1999), head of the Norwegian Zoonosis Center (2000-2005), department director for Health Surveillance (2005-2006). Since 2007, Dr Kruse is the Programme Manager Food Safety for the WHO Regional Office for Europe. From 2003 to 2009, Dr Kruse was a member of the European Food Safety Authority's (EFSA) Scientific Panel on Biological Hazards. Dr Kruse is the author of more than 50 scientific articles in peer reviewed journals, is a frequent speaker at international conferences and meetings and is often giving interviews on food safety, zoonoses and antimicrobial resistance to the media.

Emerging foodborne diseases – a WHO perspective.

Food safety is a basic individual right (FAO/WHO 1992). Nevertheless, ingestion and handling of contaminated food causes significant illness and death worldwide. Food- and waterborne diarrheal diseases kill an estimated 2.2 million people annually, most of whom are children. This only represents the tip of the iceberg, as most cases of foodborne disease are not reported due to limitations of the surveillance systems.

While everyone is exposed to foodborne health risks, it is the poor who are most exposed and vulnerable to these risks. There is also a vicious cycle between nutrition and food safety as undernutrition augments vulnerability to foodborne disease and foodborne disease augments micronutrient deficiencies and thereby growth and development impairment.

Factors that play a role in the epidemiology of emerging food-borne problems include changes in the pathogens; demographics, food consumption habits, beliefs and consumer trends; environmental changes and pollution; changes in the health or agriculture systems; new technologies; travel and migration; trade in food, animal feed and animals; poverty; and crises and emergencies. An example of a change in the pathogen complicating the picture is antimicrobial resistance. This immense public health problem also is a food safety issue as use of antimicrobial agents in food production can cause the development of antimicrobial resistance that can spread to humans through the food chain.

In an increasingly interconnected world, officials must apply a global perspective in their contingency planning and response to foodborne hazards and ensure efficient intersectoral cooperation and communication. Food safety is by nature an area that requires „health in all policies“, „whole-of-Government“ and „whole-of-society“ approach. WHO, in collaboration with our partners at regional and international levels, are supporting countries in their efforts to address foodborne disease efficiently, including strengthening capacities for detection, surveillance of and response to foodborne diseases, facilitating information-sharing, and raising awareness, with the overall aim of reducing and preventing foodborne disease in Europe and across the globe.



MERYEM MUHTEREM

- Institution:** University of Veterinary Medicine | Vienna, Austria
- Position:** PhD student at the Institute for Milk Hygiene, Milk Technology and Food Science
- In addition:** She studied Nutritional Sciences at the University of Vienna and obtained her diploma degree in 2012 with the thesis “Hygiene status of ready-to-eat leafy green salads”. Currently, she is working on her PhD thesis “Key features for the adaption and survival of *Listeria monocytogenes* in the food processing environment”.
- Her interest is focused on food microbiology, molecular epidemiology and bioinformatic analysis of bacterial genomes.



COAUTHOR: Martin Wagner, Stephan Schmitz-Esser, Beatrix Stessl

Key features for the adaption and survival of *Listeria monocytogenes* in the food processing environment.

Persistent *L. monocytogenes* and *Listeria* spp. isolates (characterized by PFGE and MLST typing) are hypothesized to be better adapted to the food producing environment (FPE).

Therefore, the whole genome of 15 *L. monocytogenes* and *Listeria* spp. strains established in the same food processing environment was sequenced applying the Illumina sequencing technique.

The *L. monocytogenes* core-genome of the 12 selected strains was highly syntenic. Differences were detected in mobile elements of the accessory genome. Eight *L. monocytogenes* and two *L. innocua* strains indicated the presence of 15-90 kbp plasmids. The long-term *L. monocytogenes* persistent strains, assigned to genetic lineage I [sequence type (ST) 5] and lineage II (ST204), harbored a plasmid comparable to plm80. All strains included fragments of the non-lytic bacteriophage A118, *L. monocytogenes* ST9 and *L. innocua* also implicated genes of *Listeria* phage 2389. When comparing *L. monocytogenes* ST37 at the beginning and the end of isolation, the loss of a distinct phage region (~33 kbp) was noticeable. The β -lactamase encoding transposon Tn552, was detected in the plasmids of *L. monocytogenes* ST204 (n=1) and ST5 strains (n=6). The transposon Tn554, suspected for benzalkonium resistance, was found in *L. monocytogenes* ST9, one *L. innocua*, and *L. seeligeri*. The *L. monocytogenes* stress-survival islet (SSI-1) was present in ST5, ST204, and ST9.

The results indicate mutations in the *L. monocytogenes* ST5, ST37 phage and/or plasmid genome suspected for better adaption and survival in the particular FPE. Additionally, gene regions for better adaption to disinfectants and acidic stress (Tn554, SSI-1) were found in long term persistent *L. monocytogenes* strains.



EVANGELIA ZILELIDOU

Institution: Agricultural University of Athens | Athen, Greece

Position: PhD student in Food Quality Control and Hygiene at the Agricultural University of Athens

In addition: She will complete her degree in 2015. Her undergraduate studies were held in the Agricultural department of Aristotle University in Thessaloniki, in the sector of Food Science and Technology.

She obtained a joined Master of Science in Food Biotechnology by the School of Biomedical Science of British University of Ulster and the Department of chemistry of the University of Patras.

Her PhD research has focused upon exploring the effect of *L. monocytogenes* inter-strain competition on the behavior of the pathogen (fitness during growth, enrichment, virulence, stress-response to gastric fluid).

COAUTHOR: Evanthia Manthou, Luminita Ciolacu, Martin Wagner, Kathrin Rychli, Panagiotis Skandamis

Growth and virulence of *Listeria monocytogenes* in co-cultivation models.

Interactions that take place between microorganisms could affect their physiological characteristics. Inter-strain competition, in the same microenvironment might influence fitness and virulence potential of different *L. monocytogenes* strains under particular growth conditions.

The study investigated the impact of co-culture on: (i) growth of *L. monocytogenes* strains in nutrient-rich broth, (ii) invasion and intracellular proliferation of *L. monocytogenes* strains using human intestinal epithelial cells.

Growth of eight *L. monocytogenes* strains (serotypes 1/2a, 1/2b, 1/2c, 4b) was determined in single and two-strain mixed cultures (1:1 strain ratio). Resistance to rifampicin and streptomycin was induced for selective strain-enumeration. Populations of $3 \log$ CFU/ml were added to Tryptic Soy Broth and incubated at 10°C . The growth was monitored at regular time intervals for up to 10 days. Based on the growth-data, four strain-combinations were chosen for in vitro virulence assay. Invasion efficiency and intracellular growth after 4h (37°C) was determined in Caco-2 cells for strains in single or mixed cultures, previously incubated for one day at 10°C .

Significant differences in growth kinetics of each strain were observed when grown alone as compared to the same strain in mixed cultures. For instance, strain ScottA showed a 3 fold times reduced growth rate in a mixed culture compared to when cultured alone. Strains that were outgrown by others, did not manage to reach $9 \log$ CFU/ml, contrary to single cultures, suggesting the growth cessation of each strain when its competitor reached the maximum growth levels. The invasion efficiency of one strain (e.g. 15162) was 3-fold higher when grown with strain C5 compared to single culture. The number of intracellular bacteria of ScottA was increased when co-cultured with strain 15162. In contrast, the intracellular growth of strain 6179 was reduced when cocultivated with C5.

Competition between *L. monocytogenes* strains has a strain-dependent effect on fitness and virulence potential of the organism.



ANDREAS FAHRNLEITNER

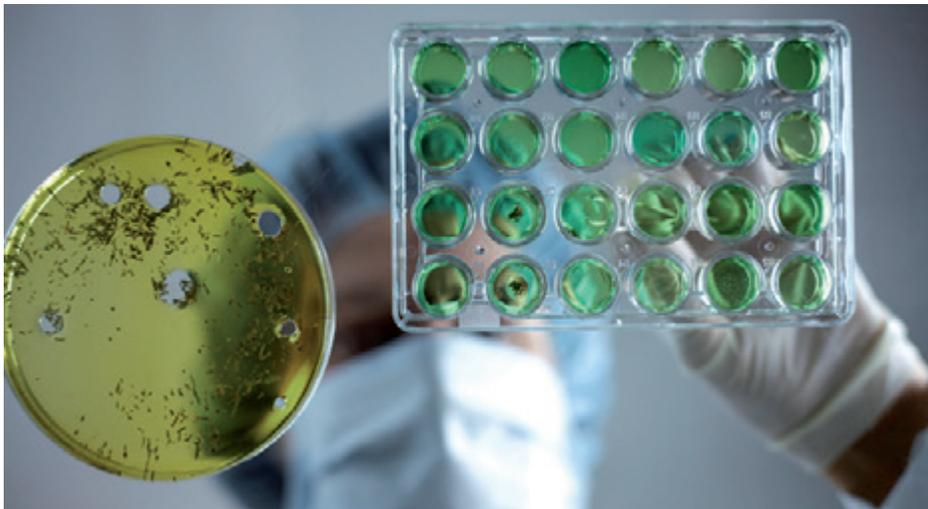
- Institution:** Vienna University of Technology | Vienna, Austria
- Position:** Associate Professor at the Vienna University of Technology and Head of the recently established Interuniversity Cooperation Centre for Water and Health
- In addition:** He is faculty member of the international excellence programme “Vienna Doctoral Programme on Water Resource Systems” (www.waterresources.at) and serves as editor for several international journals/activities. His research interests are microbial diagnostics, pollution microbiology and sanitation, health related water quality, microbial ecology of ground and drinking water resources, and, bio-toxins.

COAUTHOR: GH. Reischer, H. Stadler, J. Drex, Akt Kirschner, S. Cervero-Arago, AP Blaschke & R. Sommer

Opening the „black blox“ of microbial fecal pollution of ground water resources.

Impairment of health-related microbiological water quality is a critical issue since it can cause severe outbreaks or contribute to the background rate of endemic disease. Faecal pollution is considered of paramount importance as contaminating agent, as faecal excreta frequently contain intestinal pathogens in high numbers from human and animal sources. Determination of the microbiological water quality has thus a longstanding tradition and a host of different guidelines, standards or directives has been developed.

However, until recently, monitoring and managing microbial raw water quality at water resources was based on “black box” strategies, because technologies for advanced microbial faecal pollution diagnostics and quantitative pathogen detection were not available. The aim of this presentation is to demonstrate how the puzzle of newly developed approaches and well-established standard methods can be put together in order to evaluate the importance of faecal pollution sources in the catchment (e.g. communal sewage effluents vs. runoff from livestock/wildlife faecal sources), translate gathered information into infection risk levels, and inform water safety management to enable pro-active and sustainable measures.



Evening program

18:30

Departure by bus
to the vineyards of Vienna

**Reception for BacFoodNet and
PROMISE participants**
at the “Heurigen Zawotzky”
Reinischgasse 3, 1190 Vienna

22:00

Departure from the vineyard
and transfer to the Hotels



Visit the promise academy
www.promise-academy.eu



This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 265877.



PROTECTION OF CONSUMERS

by microbial risk mitigation through segregation of expertise



promise

**1st PROMISE STAKEHOLDER EVENT
DUBLIN, IRELAND
20th of November 2013**

REGISTRATION:

The event is free of charge.
Please, register for the stakeholder
event by sending an email to:
Markus Lyson:
lyson@rtd-services.com
Registration until 15th of Nov. 2013.

PROMISE PROJECT

PROMISE is an EU funded research project with the objective to improve and strengthen the integration, collaboration and knowledge transfer between the new and old member states of the European Union and its candidates countries. The goal is to tackle common food safety threats and hence to protect the European consumers.

PROMISE integrates stakeholders like public health authorities and national food safety authorities from the old and new member countries in order to ensure the exploitation of research results into standardization and harmonization efforts and hence to contribute to sustainability of project outcomes.

The PROMISE consortium assesses on both exogenous and indigenous neglected routes of pathogen transmission. Exogenous routes of transmission are those where the source is spatially segregated from the consumer sphere in the EU-27, whereas indigenous routes of transmission are those where a close link between the pathogen eco-niche and a vulnerable food supply chain exists.



www.promise-net.eu



PROMISE EVENT AGENDA

The PROMISE project has a main strategic objective to integrate stakeholders like public health authorities and national food safety authorities from the new and old member countries. The integration is needed to ensure an exploitation of research results into standardization and harmonization efforts.

A further aim is to initiate, maintain and improve a regular communication and dialogue with the respective food industry, including small- and medium sized (SMEs) enterprises, with organisations of standardisation and research and finally with the public, the European consumers.

This stakeholder event presents recent research and knowledge on *Listeria monocytogenes* and its problems occurring in food safety.

- 01:30 pm: Practical implications of recent research on *Listeria monocytogenes*
Prof. Martin Weidmann, Cornell University, USA (keynote address)
- 02:15 pm: Lessons learned from the 2010 Austria outbreak of *Listeria monocytogenes*
Dr. Dagmar Schroder, Vienna, Austria
- 03:00 pm: Detection of *Listeria monocytogenes* in processing facilities across Europe
Dr. Marion Dalmasso, Teagasc, Moorepark
- 03:20 pm: An unrecognised potential source of *Listeria monocytogenes*
Dr. Panos Skandamis, Athens, Greece
- 03:40 pm: Break
- 04:00 pm: Crisis Management with respect to *Listeria monocytogenes*
Dr. Lisa O'Connor, Food Safety Authority of Ireland
- 04:40 pm: Control of *Listeria monocytogenes* at processing facilities
Edward O'Neill, Teagasc, Moorepark
- 05:00 pm: Discussion and Close

PROMISE PROJECT PARTNERS

The PROMISE Consortium comprises twenty partners from fourteen different countries in Europe. The following overview shows their specific know-how and expertises:

University of Veterinary Medicine Vienna – Austria
Coordinator: Prof. Martin Wagner, Institute for Milk Hygiene, Milk Technology and Food Science (www.vetmeduni.ac.at)

Federal Institute for Risk Assessment - Germany
Prof. Bernd Appel, Department of Biological Safety (www.bfr.bund.de)

Institute of Food Research – United Kingdom
Dr. Gary Barker, Application of computational techniques in food safety science (www.ifr.ac.uk)

Agricultural University of Athens - Greece
Panos Skandamis Ph.D., Food Hygiene (www.aua.gr)

Teagasc Moorepark Food Research Centre - Ireland
Dr. Kieran Jordan, Food Safety (www.teagasc.ie)

University of Burgos - Spain
Dr. David Rodriguez-Lazaro, Food Science and Molecular Microbiology (www.ubu.es)

Veterinary Research Institute Brno – Czech Republic
Dr. Ivan Rychlik, Salmonella research (www.vri.cz)

University of Ljubljana - Slovenia
Dr. Sonja Smole Mozina, Department of Food Science and Technology (www.uni-lj.si)

Centre for Agricultural Research of the Hungarian Academy of Sciences – Hungary
Prof. Bela Nagy, Institute for Veterinary Medical Research (www.vmri.hu)

Food Research Institute – Slovak Republic
Dr. Tomas Kuchta, Department of Microbiology and Molecular Biology (www.vup.sk)

University Dunarea de Jos Galati – Romania
Anca Nicolau Ph.D., Faculty of Food Science and Engineering (www.ugal.ro)

Kalite Sistem Laboratuvarlar Grubu – Turkey
Sanim Saner, Founder of KSL (www.ksl.com)

University of Zagreb – Croatia
Prof. Estella Prukner-Radovic, Faculty of Veterinary Medicine (www.unizg.hr)

Austrian Agency for Food Safety and Health, Austria
Christoph Unger, AGES Academy (www.ages.at)

Food Safety Authority – Ireland
Dr. Lisa O'Connor, Food Science (www.fsai.ie)

Hellenic Food Safety Authority – Greece
Angelos Vakalopoulos, Food of Animal Origin Enterprises Control (www.efet.gr)

Turkish Food Safety Authority – Turkey
Serap Nazir, TFSA Board (www.tfsa.tr)

National Sanitary Veterinary and Food Safety Authority – Romania
Liviu Rusu, General Direction of Food Safety (www.ansvsa.ro)

Public Health Authority of the Slovak Republic
Zuzana Sirotná, Department of Medical Microbiology in PHA (www.uvzsr.sk)

RTD Services (DI Andreas Moser) – Austria
Markus Lyson, Management & Dissemination (www.rtd-services.com)



promise **MEETING**
17-20 JUNE 2014
HYDRA, GREECE



This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 265877.



promise MEETING
17-20 JUNE 2014

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3RD ON-TOUR TECHNICAL TRAINING WORKSHOP. WEDNESDAY-THURSDAY
18th - 19th of June 2014

Wednesday, 18th of June 2014

12.00-13.10	Lunch
13:30– 19:00	3rd On Tour-Technical Training Workshop (TTWT) Early stage researcher (ESR; PhDs, junior PostDocs) should take part. Lecture materials should be prepared under guidance of partner UL with respective local partner UoB. Topics for the TTWT have been selected in the Istanbul meeting Topic for the 3rd TTWT: Modelling in the food chain ; Gary Barker, IFR and Panos Skandamis, AUA). Practical materials (protocols) used will be collected (task 4.1.) and compiled in a toolkit called lecture materials (task 4.7, responsible for editing and web upload, partner KSL).
13:30-14:00	Introduction - Why model? (Organization, Interpretation and Discovery) Interactive illustration - "Should we sell?"
14:00-14:30	Concepts - Determinism and Stochasticity (Chance and Uncertainty) Interactive illustration - "Fooled by randomness"
14:30-15:00	Concepts – Probability distribution (Variables, Probability density) Interactive illustration – "How tall are you?"
15:00 - 16:00	Coffee Break
16:00-17:00	Details – Familiar distributions (Modelling prevalence, Taking a sample) Interactive exercise – "Is the sample big enough?" Details – Familiar analyses (Odds, Risks and Risk Factors) Interactive exercise – "Is it dangerous?"
17:00 – 19:00	Modelling microbial growth – Predictive Microbiology
19:00 - 19:30	Departure to Vlychos Beach
19:30	Sit-in at the beach (please bring your swimming suits with you: Tour through the travel experiences collected (Presented by ESR)
21:00	Get together Dinner

Thursday, 19th of June 2014

09:00 - 09:15	Implementation – Tools for food safety modelling
09:15 - 10:00	Implementation – Building distributions (Excel and Random Numbers) Interactive exercise – "Monte Carlo or bust!"
10:00 - 10:30	Coffee Break
10:30 - 12:00	Implementation – Putting distributions together (Risk modelling) Interactive exercise – "The devil is in the tail" Summary
12.15-13.15	Lunch



AGENDA

SCIENTIFIC PROGRAM
3RD GENERAL ANNUAL MEETING (=5TH PROMISE CONSORTIUM MEETING)
19th - 20th of June 2014, Hydra Museum Historical Archives

Thursday, 19th of June 2014

14:00 - 14:15	3 rd GAM Welcome and Introduction (Martin)
14:15 - 15:45	Session 1 Prevalence, genotypic and phenotypic characterization of pathogens in foods from outside EU 14:15 – 14:30 Prevalence and phenotypic characterization of <i>Listeria monocytogenes</i> , <i>Salmonella</i> spp. and <i>Escherichia coli</i> O157:H7 isolates from confiscated foods in Greece Stavros Manios, Anastasia Kapetanakou, Panagiotis N. Skandamis 14:30 - 14:45 Foodborne pathogens in illegal imported food samples confiscated at the Croatian borders Estella Prukner-Radovčić, Lidija Kozačinski, Maja Lukač, Sandra Gutić, Danijela Horvatek Tomić 14:45 – 15:00 Participation of Food Research Institute in EU project „PROMISE“ from the view of monitoring <i>Listeria monocytogenes</i> in cheese production Jana Minarovičová, Janka Koreňová, Eva Kaclíková, Tomáš Kuchta 15:00 – 15:15 Modelling of prevalence for foodborne pathogens in EU countries Jasna Kovač, Sonja Smole Možina, Gary Barker Session 2 Antimicrobial resistance, phages and community analysis 15:15 – 15:30 Lytic bacteriophages isolated from confiscated food of animal origin István Tóth 15:30 – 15:45 Community analysis of <i>Listeria monocytogenes</i> -contaminated and uncontaminated dairy plant floor drains by 16S rRNA amplicon pyrosequencing Elisa Schornsteiner, Martin Wagner, Stephan Schmitz-Esser
15:45 - 16:30	Coffee break
16:30 - 18:00	16:30 – 16:45 Characterization of Shiga (Vero) toxin producing <i>Escherichia coli</i> , (STEC/VTEC), and multiresistant <i>E. coli</i> Isolated from Confiscated Foods of Animal Origin at the Borders of European Union Béla Nagy, Sonja Smole-Mozina, Jasna Kovac, Martin Wagner, Dagmar Schoder, Anja Strauss, Sabine Schlager, Janine Beutlich, Bernd Appel, Estella Prukner Radovcic, Marija Lusicky, Mojca Cimerman, Istvan Tóth, Renáta Kugler, Ama Szmolka 16:45 – 17:00 Integrans and antimicrobial resistance genes of multidrug resistant <i>Escherichia coli</i> and coliform bacteria from foods of animal origin confiscated at the Hungarian borders Renáta Kugler, Ama Szmolka, Judit Pászti, Béla Nagy 17:00 – 17:15 Characterisation of egg laying hen and broiler fecal microbiota in poultry farms in Croatia, Czech Republic, Hungary and Slovenia Ivan Rychlik, Estella Prukner Radovcic, Sonja Smole-Mozina, Béla Nagy





AGENDA

SCIENTIFIC PROGRAM
3RD GENERAL ANNUAL MEETING (=5TH PROMISE CONSORTIUM MEETING)
19th - 20th of June 2014, Hydra Museum Historical Archives

17:15 - 18:00

Session 3

***Listeria* in the food processing environment**

17:15 – 17:30

Key features for the adaption and survival of *Listeria monocytogenes* in the food processing environment

Muhterem-Uyar, Martin Wagner, Stephan Schmitz-Esser, Beatrix Stessl

17:30 – 17:45

Modelling *L. monocytogenes* occurrence in different compartments in a contaminated food business operation: Can environmental sampling support reliability of food testing in practice

Beatrix Stessl, I. Rückerl, Meryem Muhterem, Martin Wagner, Gary Barker

17:45 – 18:00

The effect of hygiene barriers on the reduction of indicator and zoonotic bacteria in food processing companies

Beatrix Stessl, Meryem Muhterem, Lisa Simmer, Sonja Klinger, Martin Wagner

20.30

Dinner

GAM meeting dinner will be enriched with progress reports of PhD and doctoral students.



AGENDA

SCIENTIFIC PROGRAM
3RD GENERAL ANNUAL MEETING (=5TH PROMISE CONSORTIUM MEETING)
19th -20th of June 2014, Hydra Museum Historical Archives

Friday, 20th of June 2014

08:00	Breakfast with Steering Committee (Martin, Anca, Sonja, Bela, David, Gary, Kieran, Markus, Andreas)
09:30 - 11:00	<p>09:30 – 09:45 Detection of <i>Listeria monocytogenes</i> in a meat processing environment using molecular methods Andrei Sorin Bolocan, Avelino Alvarez-Ordóñez, Kieran Jordan, Anca Ioana Nicolau</p> <p>09:45 – 10:00 The impact of strain competition on the fitness and virulence potential of <i>Listeria monocytogenes</i> Evangelia Zilelidou, Evanthia Manthou, Luminita Ciolacu, Martin Wagner, Kathrin Rychli, Panagiotis Skandamis</p> <p>Session 4: Gene expression and physiological adaptation</p> <p>10:00 – 10:15 The linkage between phenotypic/genotypic features and the adherence ability of <i>Campylobacter jejuni</i> Katja Bezek, Jasna Kovač, Beatrix Stessl, Martin Wagner, Peter Raspor, Sonja Smole Možina</p> <p>10:15 – 10:30 Investigating boundaries of survival, growth and expression of genes associated with stress and virulence of <i>Listeria monocytogenes</i> in response to acid and osmotic stress Ifigenia Makariti, Antonia Printezi, Anastasia Kapetanakou, Nikoleta Zeaki, Panagiotis Skandamis</p> <p>10:30 – 10:45 Characterization of the in vitro gene response of chicken cells to <i>Salmonella</i> Enteritidis Ama Szmolka, Marta Matulova, Zoltán Wiener, Béla Nagy, Ivan Rychlik</p> <p>10:45 – 11:00 Characterization of the prfA virulence gene cluster in <i>Listeria monocytogenes</i> strains of clinical and food origin Sofia Poimenidou, Marion Dalmasso, Panagiotis Skandamis, Kieran Jordan</p>
11:00 - 11:30	Coffee Break
11:30 - 12:15	Reporting and overview on deliverables, Financial issues, consortium agreement, other issues (Andreas, Markus)
12:15 - 12:30	Final Symposium Vienna and closing (Martin)
14:00 - 18:00	Meeting with Policy and Sustainability Board (Food safety authorities).





3RD GENERAL ANNUAL MEETING (=5TH PROMISE CONSORTIUM MEETING)
19th - 20th of June 2014, Hydra Museum Historical Archives

Prevalence and phenotypic characterization of *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* O157:H7 isolates from confiscated foods in Greece

Stavros Manios, Anastasia Kapetanakou, Panagiotis N. Skandamis

Laboratory of Food Quality Control and Hygiene, Department of Food Science and Nutrition, Agricultural University of Athens, Greece

The objective of the study was (i) to evaluate the risk of *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* O157:H7 originating from foods of animal or plant origin that are illegally imported to Greece and (ii) to determine the resistance of the isolates to 10 antibiotics and to two commercial sanitizers.

In total 201 samples of animal (58.2%) or plant origin (41.8%) were analyzed according to the corresponding ISO methods for the detection of *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7, as well as for the enumeration of total viable counts (ISO) and *Staphylococcus aureus* (ISO). The isolates were arranged into clusters according to their PFGE pattern and their serogroup was determined following established multiplex-PCR protocols. The resistance of the isolates to 10 antibiotics (amoxicillin/clavulanic acid 10/20 µg, ampicillin 10 µg, chloramphenicol 50 µg, ciprofloxacin 10µg, gentamycin 10 µg, cefotaxime 30 µg, erythromycin 30 µg, rifampicin 2 µg, tetracycline 30 µg, streptomycin 10 µg) was evaluated using the disk diffusion method suggested by the Clinical and Laboratory Standards Institute. In addition, the Minimum inhibitory (MIC) and bactericidal (MBC) concentration of the isolates to P3-triquart (ECOLAB; 30-50% alkyl dimethylbenzyl ammonium chloride, pH=7-8.5) and P3 - oxysan ZS (ECOLAB; 7% peroxyacetic acid, pH=1.0) was determined using the micro-dilution method.

The TVC of the analyzed samples ranged from below the detection limit (DL; 1 log CFU/g) to 7.6 log CFU/g, with average 3.8 ± 1.9 log CFU/g. No *Staph. aureus* was enumerated (DL = 2 log CFU/g), while all samples were found also negative to *Salmonella* spp. and *E. coli* O157:H7. In contrast, 22 samples were found positive to *L. monocytogenes* which were further arranged into eleven PFGE clusters. The majority of the isolates (86.4%) belonged to 1/2a or 1/2c serogroup, while 13.6% of those was 4a, 4b or 4e (acc. Doumith et al., 2004). All isolates were found susceptible to the antibiotics tested, except for cefotaxime where high (strain FQCH_92, isolated from pangasius filets) or intermediate (all the remaining strains) resistance was observed. The MIC of the isolates ranged from 2.5 to 20 ppm of P3-triquart and from 500 to 6250 ppm of P3-oxysan ZS. The MBC for the elimination of 6 log CFU/ml was 3.5–35 ppm for P3-triquart (suggested concentration 2000-5000 ppm), while the corresponding concentration of P3- oxysan ZS was 12500 ppm for all isolates, when the suggested concentration was 1000 to 2500 ppm.

These results suggest that *L. monocytogenes* is one of the major microbial risks deriving from illegally imported foods in Greece. The increased MBC of the isolates to commercial sanitizers raises concerns about the hygienic measures that should be followed to ensure the safety of the consumers.

Foodborne pathogens in illegal imported food samples confiscated at the Croatian borders

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Croatian partner, as a part of PROMISE team, has been involved in investigations carried out under the Work package 1 (WP1) - Analysis of neglected exogenous routes of transmission of foodborne pathogens. In order to assess the level of risk to public and animal health of the uncontrolled imported food of animal origin entering into Republic of Croatia, 100 food samples (dry meat products, fresh and frozen meat samples, dairy product and egg samples) were collected at 7 border points between Croatia and third countries, according to the DoW. Confiscated samples were mainly coming from Bosnia (70 samples), China (8 samples), Serbia (5 samples), Macedonia (7 samples), one from Albania and 10 were of unknown origin. The illegal food were mainly designated for personal use, only the samples confiscated in sea port Rijeka were for market. Final destination for 43 samples were Croatia, for 22 were Austria, for 16 were Germany, for 8 samples were Hungary, for 5 were Slovenia, for 2 were Italy and for one was Switzerland. Final destinations for 3 samples were not clear. Regarding category of the product, altogether 15 products belonged to the dairy products (fresh or hard cheese, salty cream), 29 were described as fresh or fresh frozen chicken, pork or beef meat. The majority of samples, in total 48, were dry meat products (sausages, ham, and bacon). The rest of the samples were eggs (3 samples), fish in the can (2 samples) and dehydrated meat noodles from China. The testing of microbiological quality of samples was carried out with specific attention on the determination of presence of foodborne pathogenic bacteria (*Salmonella*, *Listeria*, *Campylobacter* and MDR *Escherichia coli*). For the bacterial culture techniques, the appropriate ISO methods were chosen. From altogether 100 illegally imported food samples, 24 were negative for bacteria. All examined samples were negative for the presence of the most common pathogenic bacteria as *Salmonella* sp., *Campylobacter* sp. and *Listeria monocytogenes*. Multidrug resistant *E. coli* was found in only one sample. Even if the majority of samples contained only saprophytic or facultative pathogen bacteria, there is still a concern that some of those food items could be found on the local market or be consumed by a larger group of people. The higher number of the samples were confiscated during Christmas and Easter holidays and mainly confiscated dry smoked meat were found to be negative for the presence of investigated bacteria, but in any case, it could be reasonable to continue such monitoring for longer period of time and on larger amount of samples.

Participation of Food Research Institute in EU project „PROMISE“ from the view of monitoring *Listeria monocytogenes* in cheese production

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Food Research Institute has participated in the EU project PROMISE (7 FP) since 2012. Project is based on exchanging the expertise, improving the integration, collaboration and knowledge transfer, dissemination experiences between new member and old member states and candidate countries and integration of stakeholders (food safety authorities) in order to ensure the exploitation of research results into practice. Research activities are focused on investigation of indigenous and exogenous routes of foodborne pathogens transmission.

The aim of our study was monitoring of *Listeria monocytogenes* in sheep cheese producers, who produce Slovak bryndza cheese from unpasteurized milk.





3RD GENERAL ANNUAL MEETING (=5TH PROMISE CONSORTIUM MEETING)
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Modelling of prevalence for foodborne pathogens in EU countries

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One of the important aims of the project PROMISE is to investigate factors that influence uncertainty and variability in prevalence reporting and to develop descriptive models that aid interpretation. As the prevalence of foodborne diseases is thought to be significantly underestimated, there is a need to estimate the effectiveness of the reporting process in order to appreciate the real burden of disease. The prevalence is underestimated due to underreporting and under-diagnosis associated with passive surveillance, incorrect diagnosis and inefficient communication with authorities, as well as with the unregistered cases with mild symptoms that do not seek medical help. Prevalence is normative, characterizing the rate of cases in a population at a specific time and is easy to visualize and compare. It is measured from finite samples and the reported cases behave according to a binomial process; therefore uncertainty in prevalence can be described by beta distributions. Prevalence can be considered as the probability for a binomial process and the uncertainty about prevalence is the probability density of the binomial parameter which has a beta distribution. Existing belief about the prevalence can be systematically updated given the data from successive finite samples. Bayes' theorem is used to express how to rationally change a subjective belief by taking evidence into account. Model parameters affecting the prevalence reporting (by GPs) can be defined in order to construct a probabilistic graphical model. Different parameters affecting the process of reporting are involved in cases of hospitalization and these correspond with a separate branch of the model. Because of the differences in symptoms and severity of the disease caused by different pathogens, as well as the differences in medical and reporting practice among EU countries, some of the parameters depend on the country and others depend on the pathogen. Parameters were modelled using linear regression based on existing data for seven foodborne pathogens in seven EU countries. Only common statistical data available for EU countries was used to model the parameter uncertainty distributions. Monte Carlo simulations of modelled parameters were used to estimate beliefs about prevalence of foodborne pathogens in additional EU countries.

Lytic bacteriophages isolated from confiscated food of animal origin

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During the isolation of food-borne pathogenic bacteria from confiscated food we could not isolate STEC but we were able to isolate lytic phages. The aim of present study was to investigate the isolated phages' bacterial host specificity and to reveal their morphology. Altogether 207 confiscated food samples were tested for the presence of STEC/VTEC and lytic phages, by using Mitomycin C at 0.5µl/ml final concentration as inducing agent and *E. coli* K-12 C600 strain as indicator strain. Lytic phages were isolated from 10 % (21/207) of the food samples. Electron microscopic studies revealed that the lytic phages represented different families including tailed *Myoviridae*, *Syphoviridae* and filamentous *Inoviridae*. The induced phage suspensions frequently contained more than one type of phages. The host specificity of lytic phages was tested by spot assay using *E. coli* strains representing EHEC, EPEC, APEC, UPEC pathotypes, *Shigella sonnei*, *Citrobacter rodentium* and several *Salmonella* serovars including Enteritidis, Typhimurium, Hadar and Infantis. Different lytic patterns were observed and all the tested *E. coli* and *S. sonnei* strains were lysed by at least one phage. Interestingly the isolated lytic phages lysed the EHEC *E. coli* O157 strains tested and the *S. Typhimurium* study strain was also lysed by four phages. Further studies are needed to elucidate the inhibitory effect of phages in standardized isolation procedures of STEC and *Salmonella*.

Community analysis of *Listeria monocytogenes* -contaminated and uncontaminated dairy plant floor drains by 16S rRNA amplicon pyrosequencing

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Controlling *Listeria (L.) monocytogenes* is of great concern for food safety. Floor drains are an important source for contamination and recontamination of food production plants with food-borne pathogens. However, the microbial community of floor drains has only rarely been investigated until now. We hypothesize that the survival of *L. monocytogenes* in floor drains is dependent on the co-occurrence of other microbes. The aim of this study was to characterize the microbial community of drain water- and biofilm in two Austrian dairy plants using Roche/454 pyrosequencing of 16S rRNA gene amplicons. The community composition of three *L. monocytogenes*-contaminated and two -uncontaminated floor drains were analyzed along the time line. In order to compare the community composition of drain water and -biofilm, four and three floor drains from the contaminated and the uncontaminated dairy plant, respectively, were sampled at one time point. All samples were tested for the presence of *L. monocytogenes* using quantitative PCR and cultivation after enrichment in half and full-strength Fraser broth. In total, 24 drain samples including biofilm and drain water samples from the *L. monocytogenes*-contaminated and the uncontaminated dairy plant were sequenced and analyzed using mothur. After quality control 94889 reads remained (approx. 4350 reads per sample). The communities in the floor drains were dominated by three phyla (*Proteobacteria*, *Firmicutes* and *Bacteroidetes*; more than 94.5% of all reads). Already on phylum level, the community composition of most analyzed samples was highly different. The most abundant families were: *Streptococcaceae*, *Lactobacillaceae*, *Flavobacteriaceae* and *Pseudomonadaceae*. In drains from production areas, product-associated bacteria e.g. *Lactococcus* were highly abundant. The presence of *L. monocytogenes* reads was shown, although at low abundance. Here we show first deep insights into the community composition of floor drains which might allow the detection of possible co-occurring taxa which might help controlling *L. monocytogenes*.





3RD GENERAL ANNUAL MEETING (=5TH PROMISE CONSORTIUM MEETING)
19th - 20th of June 2014, Hydra Museum Historical Archives

Characterization of Shiga (Vero) toxin producing *Escherichia coli*, (STEC/VTEC), and multiresistant *E. coli* Isolated from Confiscated Foods of Animal Origin at the Borders of European Union

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In frame of the of EU FP 7th Project "Protection of consumers by microbial risk mitigation" (PROMISE) foods of animal origin confiscated at airports and ground ports of Austria, Croatia, Germany, Hungary and Slovenia were tested for the presence of foodborne pathogens by using standardized guidelines [ISO: for O157 verotoxigenic *E. coli* (VTEC), and agreed protocols for non-O157 VTEC].

Results of isolations and of characteristics of the above foodborne pathogens from >1800 confiscated food samples were as follows: O157 VTEC was not detected. All together 15 strains of non-O157 VTEC (among them one enterohemorrhagic *E. coli* strain of O26:H46) were identified, mainly from food of ruminant origin. Phenotypic and genotypic (microarray) analysis of VTEC indicated an absence of antimicrobial resistance determinants but, several VTEC strains showed an abundance of *E. coli* fitness and virulence genes including *stx* (and less frequently *eae*). In contrast, the 28 strains of multiresistant *E. coli* were characterized by a low numbers of virulence genes but by a higher number of antimicrobial resistance genes and by class 1 integrons.

Regarding Shiga (Vero) toxin producing *Escherichia coli*, (STEC/VTEC), and multiresistant *E. coli*, it is concluded that the above results should not raise major food safety concerns. However, they only represent a small fragment of illegal food import to the EU.

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Integrons and Antimicrobial Resistance Genes of Multidrug Resistant *Escherichia coli* and Coliform Bacteria from Foods of Animal Origin Confiscated at the Hungarian Borders

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The import of contaminated food may represent a food safety risk by the spread of pathogenic and/or multidrug resistant (MDR) bacteria and their determinants for virulence and antimicrobial resistance.

Here we aimed to isolate and characterize MDR *E. coli* and coliform bacteria from food samples from non-Schengen countries confiscated at the Hungarian borders. *E. coli* and coliform colonies were isolated based on their phenotype on Chromocult[®] Coliform selective media. Furthermore, API[®], PCR and 16S rDNS sequencing were used for species identification. Resistance phenotypes were determined by disc diffusion method for 18 antimicrobials with animal and human clinical relevance. Corresponding antimicrobial resistance and virulence gene patterns were identified using PCR microarray systems AMR05 and Ec03 respectively. The gene cassette arrangements of the integrons were defined by amplicon sequencing.

From the total of 207 confiscated food samples 833 coliform isolates were collected. Among them 17 (13 *E. coli* and 4 coliforms identified as *Enterobacter* spp.) showed resistance to at least three different antimicrobial classes thus were designated as MDR. The 17 strains represented 14 different food samples. Resistance genes *strA*, *strB*, *sul2*, *bla*TEM-1, *tet*(A) predominantly occurred, but in general the prevalence of the virulence genes was low. The identification of genes *qnrB*, *aac*(6')-Ib, *bla*OXA-7 in some of the isolates indicated the presence of certain emerging antimicrobial resistance plasmids. Class 1 integrons were found in 10 of the 17 MDR isolates (9 *E. coli*, 1 coliform), and in the majority of them the *sul1* gene was absent from their 3' conserved segment (CS). Interestingly, in one of the pork samples we detected a non-typical class 1 integron carrying the *sul3* gene on its 3'CS.

Above results showed that these illegal foods may frequently carry MDR *E. coli* and coliform bacteria with some unusual or new antimicrobial resistance traits.





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Characterisation of egg laying hen and broiler fecal microbiota in poultry farms in Croatia, Czech Republic, Hungary and Slovenia

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Poultry meat is the most common source of proteins of animal origin for human consumption worldwide. However, extensive breeding of animals in close proximity has led to their colonisation with microbiota which have zoonotic potential and/or antibiotic resistance. In this study we were therefore interested in the fecal microbiota composition and antibiotic resistance gene prevalence by culture-independent protocols such as real-time PCR and 16S rRNA gene pyrosequencing in both egg laying hens and broilers originating from 4 different Central European countries. tet(B), sul1 and strA were the most prevalent genes being present in approx. 1 out of 1,000 bacteria. The prevalence of sul2 and tet(A) in poultry fecal microbiota was 3 and 9 times lower than that of tet(B), respectively. cat was the least prevalent being present in less than 3 out of 10,000 bacteria. Correlation analysis of microbiota composition and the prevalence of strA, tet(A), tet(B), sul1, sul2 or cat genes showed positive correlations for 9 bacterial families with Enterobacteriaceae, Staphylococcaceae, Turicibacteraceae, Enterococcaceae and Aerococcaceae being associated the most with the prevalence of these genes. The core of chicken fecal microbiota was formed by 17 different families. Rather unexpectedly, representatives of Desulfovibrionaceae and Campylobacteraceae, both capable of hydrogen utilisation in complex microbial communities, belonged among this core of microbiota families. Understanding such metabolic bacterial mutualisms in complex microbiota systems may allow for interventions which may result in the replacement of Campylobacteraceae by Desulfovibrionaceae and a reduction of Campylobacter colonisation in broilers, carcasses, and consequently poultry meat products.

Key features for the adaption and survival of *Listeria monocytogenes* in the food processing environment

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The food industry is confronted with the problem of *Listeria monocytogenes* persistence in the processing equipment and environments. Several studies described the problem of *L. monocytogenes* processing plant re-colonization after ineffective eradication measures.

The hypothesis of the actual study is that persistent *L. monocytogenes* and *Listeria* spp. isolates are better adapted to the food producing environment (FPE), show a higher adhesion on food processing equipment, and are able to survive on sanitized equipment due to special genetic strain features. The possibility of horizontal gene transfer between *Listeria* spp. isolates established in the same food processing environment is given due to spatial proximity.

Therefore, the whole genome of 15 *L. monocytogenes* and *Listeria* spp. strains was sequenced applying the Illumina sequencing technique. The strain set comprised non-persistent and persistent *Listeria* spp., isolated within a discrete timeframe (June 2010-February 2013) from the same dairy FPE. The *Listeria* spp. isolates were previously characterized by pulsed-field gelelectrophoresis (PFGE) and multi-locus sequence typing (MLST).

Preliminary results showed that the *L. monocytogenes* core-genome of the 12 selected strains was highly syntenic. Differences were seen in mobile elements of the accessory genome (plasmids, phage regions, and transposons). Eight *L. monocytogenes* and two *L. innocua* strains indicated the presence of 15-90 kbp plasmids. The long-term *L. monocytogenes* persistent strains, assigned to genetic lineage I [sequence type (ST) 5] and lineage II (ST204), harbored a plasmid comparable to plm80. All strains included fragments of the non-lytic bacteriophage A118, *L. monocytogenes* ST9 and *L. innocua* also implicated genes of *Listeria* phage 2389. When comparing *L. monocytogenes* ST37 at the beginning and the end of isolation, the loss of a distinct phage region (~33 kbp) was noticeable.

The originally in *Staphylococcus (S.) aureus* described β -lactamase encoding transposon Tn552, was detected in the plasmids of *L. monocytogenes* ST204 (n=1) and ST5 strains (n=6). Interestingly, in one *L. monocytogenes* ST5 representative, isolated at the end of the study, the transposon Tn552 was absent. Furthermore, the transposon Tn554, suspected for benzalkonium resistance, was found in *L. monocytogenes* ST9, one *L. innocua*, and *L. seeligeri*. The *L. monocytogenes* stress-survival islet (SSI-1) was present in ST5, ST204, and ST9.

The results indicate mutations in the *L. monocytogenes* ST5, ST37 phage and/or plasmid genome suspected for better adaption and survival in the particular FPE. Additionally, gene regions for better adaption to disinfectants and acidic stress (Tn554, SSI-1) were found in long term persistent *L. monocytogenes* strains.





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Modelling *L. monocytogenes* occurrence in different compartments in a contaminated food business operation: Can environmental sampling support reliability of food testing in practice?

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During the PROMISE project, we sampled food contact material (FCM) and non-food contact materials (NFCM) from a food business operator (FBO) contaminated with *L. monocytogenes* (Task 2.2.). Food processing environment was tested at IMMF, and more than 1,284 data were provided to the consortium (Task 2.3). In total, more than 9,000 data points from food lot control were collected during 2011-2013.

We recorded major changes in the quality management operations such as changes of quality managers, changes in sanitation regime, internal recall actions etc along a timeline of three years. In a modeling approach, we tried to determine how efficient the management operations were with regard to the occurrence of *L. monocytogenes* in different ecological habits. We found that occurrence rates remained quite the same on NFCM, whereas on FCM that FBO was more successful to combat the contamination.

The effect of hygiene barriers on the reduction of indicator and zoonotic bacteria in food processing companies

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The aim of the study was to determine the efficiency of hygiene sluices on the reduction of hygiene indicator bacteria (aerobic mesophilic bacteria, coliforms, Staphylococci) and zoonotic agents (*Listeria monocytogenes*, *Salmonella* spp.,) in food processing companies. Generally, hygiene sluices are efficient barriers against the transmission of spoilage and foodborne pathogen bacteria. Our hypothesis is that incorrect usage and inadequate disinfection is often ignored by the quality management boards (QMB) of food processing companies. Especially, when high-tech hygiene locks are used, staff member consider themselves and their working clothes and shoes to be efficiently cleaned and disinfected. In our approach we included five food processing facilities located in Upper- and Lower Austria including three slaughterhouses and two RTE-food processors. Samples were taken before and after sanitation comprising personal fingerprints on Baird Parker agar, swab samples from shoes and the hygiene lock, and water residues present in the lock. The swab samples were enumerated for aerobic mesophilic - and coliforms counts on Plate Count - and Violet Red Bile agar, respectively. *E. coli* isolates were confirmed by a multiplex PCR including *eae*, *rfbE*, *hlyA*, *fliC*, *stx1* and *stx2* targets. The presence/absence of *Salmonella* spp. was tested after a pre-enrichment step in Buffered Pepton Water on Rappaport-Vassiliadis (MSRV) medium. *Salmonella* spp. isolates were PCR confirmed targeting the *invA* gene. To determine *L. monocytogenes* samples were enriched in Half Fraser broth. After 24h at incubation temperature of 37°C the enrichments were streaked on selective *Listeria* agar media (Agar acc. to Ottaviani and Agosti and Palcam agar) and transferred to Fraser enrichment. *L. monocytogenes* were subtyped by Serogroup PCR and pulsed-field gelelectrophoresis (PFGE) to determine possible routes of contamination.

Analyzing the check list data solely in one RTE-food producing facilities no evident failures in the hygiene barrier area were perceived. Missing liquid in the foot bath or failures in hand disinfection were probably minimizing the hygiene measures. During the control of hand disinfection no coagulase positive *Staphylococcus* could be detected. Nevertheless, an effective reduction on coagulase-negative *Staphylococci* after disinfection was not measurable, especially for the slaughterhouse working staff. Coliform bacteria and *L. monocytogenes* on shoes could not be reduced after passing the hygiene locks. In detail, 29% of the shoe samples were detected *L. monocytogenes* positive before and after the foot bath. Most of *L. monocytogenes* isolates were found on shoes of two slaughterhouses and one RTE-food company working staff. *L. monocytogenes* positive swab samples taken from the shoe bath were correlating with positive results from shoe samples. Subtyping revealed that the slaughterhouse A harbored *L. monocytogenes* serogroup 1/2a, 3a and 4b, 4d, 4e (n=21) resulting in five PFGE pulsotypes. In slaughterhouse B *L. monocytogenes* serogroup 1/2a, 3a, 1/2c, 3c and 4b, 4d, 4e (n=11) was present, representing five PFGE pulsotypes. In one RTE-food producing company genetic lineage II (1/2a, 3a; 1/2c, 3c; n=10) strains were over represented, resulting in four PFGE profiles. One shoe sample each was found positive for *E. coli* (*rfbE* positive) and *Salmonella* spp. after sanitation in slaughterhouse B and RTE-food producer C, respectively.

Our data reveal that hygiene locks, if not efficiently working, could be a reservoir for human pathogen bacteria and *L. monocytogenes* in-house clones. The latter could be spread into the food processing environment from an area which is superficially regarded as the cleanest area in the whole food processing facility.



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Detection of *Listeria monocytogenes* in a meat processing environment using molecular methods

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L. monocytogenes, a major food borne pathogen, can persist in the processing environment of ready-to-eat processing facilities, but the conditions that enable its colonisation and persistence are not fully understood.

The aim of this study was to determine the establishment of *L. monocytogenes* in a ready-to-eat meat processing facility in Romania, and to determine if molecular methods can be used to improve its detection.

One hundred and twenty seven samples from a meat product processing facility were collected in July 2012, December 2012, April 2013 and August 2013, with 145 ± 5 days between each sampling occasion. With the exception of first sampling, approximately 50 samples were taken from non-food contact surfaces (NFCS), food contact surfaces (FCS), raw materials (RM) and RTE meat products (RTE-MP) at each sampling time. To determine the presence of *L. monocytogenes*, all the samples were analysed by the ISO 11290-1 method and by PCR of the enriched culture.

The examination by the ISO method showed that 14.9% of the environmental samples were contaminated by *L. monocytogenes*, while the RTi-PCR revealed a prevalence of 41.7%. The NFCS were less contaminated than FCS by RTi-PCR method, but for both categories of surfaces the percent of positive sample was high (33.3% and respectively 55.1%). From the samples positive by RTi-PCR, but negative by ISO, 30 strains displaying different colony characteristics than *L. monocytogenes* were isolated from 30 different samples. The 16S rRNA gene of these strains was sequenced. The sequencing revealed that the strains were *L. welshimeri* and *L. innocua*, species that have been shown to have the ability to grow faster than *L. monocytogenes* on enrichment and plating media such as Fraser broth and chromogenic agars, or to inhibit *L. monocytogenes* growing and thus give false negative results.

Thirty four strains of *L. monocytogenes* were submitted to pulsed field gel electrophoresis (PFGE) using the International PulseNet protocol. One of the pulsotypes, T3, was found on all the sampling occasions, while the types T1 and T11 were present on just two sampling occasions and T2, T4, T5, were only found on one sampling occasion. Thus, the isolates can be sub-divided into three types, persistent (T3- 79.41% of the isolates), sporadic (T1) and supposed as non-persistent (T2, T4, T5).

The study proved that molecular methods can be used successfully to investigate the presence of *L. monocytogenes* in processing environments, particularly when other strain outgrow *L. monocytogenes* during enrichment, therefore avoiding false negative results. Persistence can also be demonstrated.

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The impact of strain competition on the fitness and virulence potential of *Listeria monocytogenes*

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Inter-strain competition, in the same microenvironment affects the behavior of microorganisms. The coexistence of different *L. monocytogenes* strains under particular growth conditions might influence their survival and virulence potential. The aim of this work was to study the impact of co-culture on: (i) growth of *L. monocytogenes* strains in nutrient-rich broth, and (ii) invasion and intracellular proliferation of *L. monocytogenes* strains using human intestinal epithelial cells.

Growth of eight *L. monocytogenes* strains (serotypes 1/2a, 1/2b, 1/2c, 4b) was determined in single and two-strain mixed cultures (1:1 strain ratio). Resistance to rifampycin and streptomycin was induced for selective enumeration of each strain. Populations of 3 log CFU/ml were added to Tryptic Soy Broth (TSB) and incubated at 10°C. The growth was monitored at regular time intervals for up to 10 days. Based on the growth-data, four strain-combinations were chosen for *in vitro* virulence assay. Invasion efficiency and intracellular growth after 4h (37°C) was determined in Caco2 cells for strains in single or mixed cultures, previously incubated for one day at 10°C. Significant differences in growth kinetics of each strain were observed when grown alone as compared to the same strain in mixed cultures. For instance, strain ScottA showed growth rate of 0.35 day⁻¹ when cultured alone, and a growth rate of 1.5 day⁻¹ in a mixed culture. Strains that were outgrown by others, did not manage to reach 9 log CFU/ml, contrary to single cultures, suggesting the growth cessation of each strain when its competitor reached the maximum growth levels. The invasion efficiency of one strain (e.g. 15162) was 3 fold higher when grown with strain C5 compared to single culture. In contrast, the number of intracellular bacteria of ScottA was reduced when co-cultured with strain 15162. The intracellular growth of strain 6179 was reduced when cocultivated with C5.

Competition between *L. monocytogenes* strains has a strain-dependent effect on fitness and virulence potential of the organism.





3RD GENERAL ANNUAL MEETING (=5TH PROMISE CONSORTIUM MEETING)
19th - 20th of June 2014, Hydra Museum Historical Archives

The linkage between phenotypic/genotypic features and the adherence ability of *Campylobacter jejuni*

Katja Bezek^{1,3}, Jasna Kovačič, Beatrix Stessl², Martin Wagner², Peter Raspor^{1,3}, Sonja Smole Možina^{1*}

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Despite their fastidious growth requirements, campylobacters have developed an adaptation strategy that enabled them to become and remain one of the most common causes of acute bacterial gastroenteritis world-wide. The ability of attachment and biofilm formation has been suggested to be one of the key elements of bacterial persistence within the unfavourable environmental conditions. In this study, biofilm formation ability on polystyrene was assessed for 45 *C. jejuni* strains isolated from different sources in Slovenia. The quantification of biofilm formation was evaluated in Mueller Hinton broth after 48 h incubation in flat bottom polystyrene microtiter plates using crystal violet (CV) staining. The amount of the bound CV is proportional to the adhered bacterial biomass; therefore the biofilm could be quantified based on the colour intensity. The colorimetric approach was used due to its suitability for larger screening assays, which enabled rapid evaluation of biofilm formation ability. Tested strains were ranked according to the absorbance intensity (OD_{595}) of the bound dye. The linkage between biofilm formation potential and other phenotypic and genetic features were screened accordingly. On the basis of previously obtained data of antibiotic resistance profiles, multilocus sequence (MLST) and PFGE types of tested *C. jejuni* strains, we have found that strains belonging to certain MLST clonal complex and PFGE types have better ability to adhere, compared to all other genotypes together ($p=0.034$ and $p=0.028$, respectively). Furthermore, the comparative analysis revealed that strains originating from animal sources have a significantly better attachment ability, compared to the strains originating from humans ($p=0.017$). On the other hand, no linkage between adherence ability and antibiotic resistance could have been observed. These findings provide useful information on some of the key environmental and intrinsic elements influencing the pathogen's biofilm formation ability, which can contribute to better control and understanding of its epidemiology.

Campylobacter jejuni, molecular typing, MLST, PFGE, adherence, antibiotic resistance

Investigating boundaries of survival, growth and expression of genes associated with stress and virulence of *Listeria monocytogenes* in response to acid and osmotic stress

Ifigenia P. Makariti¹, Antonia Printezi¹, Anastasia E. Kapetanakou¹, Nikoletta Zeaki², Panagiotis N. Skandamis¹

¹Laboratory of Food Quality Control & Hygiene, Department of Food Science & Human Nutrition, Agricultural University of Athens, Iera Odos 75, 118 55, Athens, Greece, ²Applied Microbiology, Department of Chemistry, Faculty of Engineering, Lund University, Lund, Sweden

Listeria monocytogenes is capable of elucidating adaptive response to adverse conditions, which may harden the organism against lethal stresses. Nevertheless, transcriptional changes underpinning stress responses close to conditions marginal for growth are poorly evaluated. Present study aims to correlate the changes in expression of stress- and virulence-associated genes of *L. monocytogenes* following habituation under suboptimal pH and NaCl, with the survival under extreme acid stress.

Tryptic Soy Broth, supplemented with 0.6% Yeast Extract (TSBYE) with various combinations of pH (4.6-6.4) and NaCl (2-10%w/v) was prepared in triplicate, inoculated with two *L. monocytogenes* strains (C_5 , 6179) separately and stored at 7°C for thirteen days. Growth followed by survival (log reductions, $D_{pH:2.0}$ values) against severe acid stress (TSBYE, pH 2.0 adjusted with HCl) were assessed on day 2, 4, 6, 8, 10 and 13. Relative transcription of *gad2*, *sigB* and *prfA*, compared to control (pH 7.2, day 0) were estimated with quantitative RT-PCR.

Our findings pointed out the inter-strain variation governing growth inhibiting conditions ($pH \leq 5.0$ and $NaCl \geq 6\%$), where C_5 was less affected (a reduction of 2.0- 3.0 log CFU/ mL) than 6179 which was reduced by 4.0- 6.0 log CFU/ mL at the end of storage. Nevertheless, the higher the habituation at the growth permitting ($pH \geq 5.5$; $NaCl \leq 4\%$ w/v) or growth inhibiting conditions, the higher the acquired acid resistance or sensitization, respectively. At day 2, *gad2* increased relative transcriptional levels (Fold Changes >30) are more related to elevated acid resistance (2-4 log reductions), manifested also by increased D-values (biphasic inactivation curve, D_1 : 0.8-5min). At day 6 both *gad2* and upregulation of *sigB* were correlated to low log reductions and high $D_{pH:2.0}$ -values against severe acid stress, while *sigB* upregulation boundaries, regarding pH and NaCl combinations, coincided with growth boundaries. Regarding virulence, the increased transcriptional levels of *prfA* at day 2 was observed for adverse pH-NaCl combinations, while prolonged stay in suboptimal conditions as well as exposure to severe acid stress resulted in general activation of the virulence regulator.

Such data could definitely contribute in designing safe intervention strategies and additionally integrate -omics aspects in quantitative microbial risk assessment.



3RD GENERAL ANNUAL MEETING (=5TH PROMISE CONSORTIUM MEETING)
19th - 20th of June 2014, Hydra Museum Historical Archives

Characterization of the *in vitro* gene response of chicken cells to *Salmonella* Enteritidis

Ama Szmolka¹, Marta Matulova², Zoltán Wiener³, Béla Nagy¹, Ivan Rychlik²

¹ Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Hungary, ² Veterinary Research Institute, Brno, Czech Republic, ³Semmelweis University, Department of Genetics, Cell- and Immunobiology, Budapest, Hungary

Salmonella Enteritidis (SE) is one of the most frequently reported causative agent of human gastroenteritis, originating mainly from poultry. Pathogenesis of SE infection in poultry is well-elucidated, but the complexity of the host cell response, and its relation to differing pathogenic potential of various strains is much less understood. Therefore we intended to provide a genome-wide comparative characterization of the gene expression profiles of chicken cells to wild type strains and virulence-related mutants of *Salmonella* Enteritidis.

Freshly isolated chicken embryo fibroblast (CEF) cells co-incubated with *Salmonella* for 4 hrs were used to model gene response of young chickens to *Salmonella* infection and to measure the invasiveness of wild type strains SE147, SE11 and non-motile mutants of SE11 lacking the *fliD* gene and/or the virulence plasmid. Agilent custom 8×15K microarray was designed to profile the expression of 13741 chicken genes, with emphasis to those related to immune response. Significant gene expression changes with fold change ≥ 3 (in total of 31 genes) were verified by real-time PCR.

Expression profile of infected CEF cells resulted in 314 genes significantly misregulated by the infection with the wild type strain SE147 (206 up-/108 down-regulations) while only 135 genes were significantly expressed as a result to SE11 infection (74 up-/61 down-regulations). There were 100 genes induced by both wild strains, among them CSF3 (colony-stimulating factor), IL-1 β and IL-8 showing the highest upregulations.

In contrast to this, infection with non-motile mutants lacking *fliD* gene and/or the virulence plasmid, did not cause any significant change in host gene expression. However real-time PCR results indicated that the cell cycle-related GOS2 switch-, and the enolase ENO2 genes were highly induced by the mutant strains, indicating that the reduced invasiveness of the mutants might have stimulated cell division and/or metabolism of the host cells.

Results suggest that *fliD* gene plays a key role in the invasiveness of *Salmonella* strains, and could be considered as an important modulator of the chicken response to *Salmonella* infection.

This work was supported by the EU FP6 NoE MedVetNet and EU FP7 PROMISE. Ama Szmolka is a holder of János Bolyai Research Scholarship of Hungarian Academy of Sciences.

Characterization of the *prfA* virulence gene cluster in *Listeria monocytogenes* strains of clinical and food origin

Sofia Poimenidou¹, Marion Dalmasso², Panagiotis N. Skandamis¹, Kieran Jordan²

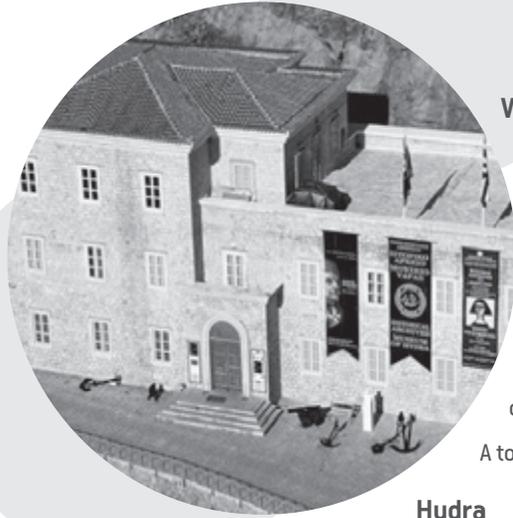
¹ Agricultural University of Athens, Food Science and Human Nutrition Faculty, Lab of Food Quality Control and Hygiene, ² Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland

The foodborne pathogen *Listeria monocytogenes* can be found in diverse environments, including food processing facilities, farms, and natural environments. Its intracellular life cycle depends on the *prfA* virulence gene cluster (*pVGC*), including *prfA*, *plcA*, *hly*, *mpl*, *actA*, and *plcB* genes. Studies have reported variations in virulence among *L. monocytogenes* strains, the causes of which (but the underlying aetiology remains) still need to be uncovered. This study aimed at identifying the correlations of the origin or serotype of *L. monocytogenes* strains with *pVGC* structure.

The *pVGC* was sequenced for 24 strains; 12 from Greece and 12 from Ireland. For each country, 6 strains (3 serotypes 1/2a and 3 serotypes 4b) originated from food processing environments and 6 strains were clinical isolates (3 serotypes 1/2a and 3 serotypes 4b). Gene and protein sequences of the *pVGC* loci were aligned using MUSCLE, and maximum likelihood phylogenetic trees were created using PhyML. The phylogenetic trees for each virulence gene showed two main clusters, one of 1/2a strains and the other of 4b strains. Variations in DNA sequences were not specific to the regional or environmental origins of the strains, illustrating the cryptic acquisition and expression of virulence in *L. monocytogenes*. The mutations in *prfA* DNA sequences were all silent-mutations as no modifications in the protein sequences were observed for all strains. *actA* and *plcA* exhibited the highest diversions among strains in DNA and protein sequences.

Strain-specific virulence determination provides new insights into *L. monocytogenes* virulence and epidemiology, and allows a better risk assessment of the pathogen.

GENERAL INFORMATION



Venue

Hydra Museum Historical Archives
Located in Hydra's main port, Tel. +30 22980 52355/54142

The Hydra Museum Historical Archives (IAMY) was founded in 1918. It was housed in a building donated to the state by Gikas N. Koulouris. The mayor of the island at that time, Anthony Lignos, discovered a large part of this archive in the monastery of the Virgin Mary, which he then categorized.

In 1972 the building was demolished and the new imposing building was built. The inauguration took place in 1996 and since then it has been open daily. The museum is a real treasure of the history of the island of Hydra.

A tour of the Museum will be realized on Thursday, June 19th, at 13.30-14.00.

Hydra

Hydra looks glamorous like an art painting, with grey, white and blue colours above the blue of the sea, an exemplar of architectonics and aesthetics.

There is one main town, known simply as "Hydra port". It consists of a crescent-shaped harbour, around which expands a strand of restaurants, shops, markets, and galleries that cater to tourists and locals. Steep stone streets lead up and outwards from the harbor area. Most of the local residences, as well as the hotels and guesthouses of the island are located on these streets.

Transportation

Cars are prohibited on the island. Donkeys, and water taxis provide public transportation. The inhabited area, however, is so compact that most people walk everywhere. Your hostess will be pleased to assist you with any sort of transportation you may require.

Lunches *(included in the accommodation package)*

During lunch breaks participants will have the opportunity to taste Greek cuisine at the following taverns:

- Gitoniko (Γειτονικό), on June 18th, 2014, at 12.00 (4 min. on foot from the Meeting Venue).
- Ta Gefiria (Τα Γεφύρια), on June 19th, 2014, at 12.15 (4 min. on foot from the Meeting Venue).

Sit-in at the beach

On Wednesday, June 18th, at 19.30 a Sit-in at the beach will take place: Tour through the travel experiences collected (Presented by ESR), at the VLYCHOS beach (please bring your swimming suits with you). A Speed boat will depart to Vlychos at 19.00 from the port, opposite Metropolis & the Clock. Vlychos beach could also be reached by foot, approx. 40-45 minutes walking distance, for those wishing to exercise. Duration trip by Speed boat: 10 minutes.

A get together dinner *(included in the accommodation package)*

A get together dinner will follow at the tavern ENALIO on Wednesday, June 18th, at 21:00. Return back to Hydra port, the same way, by speed boat.

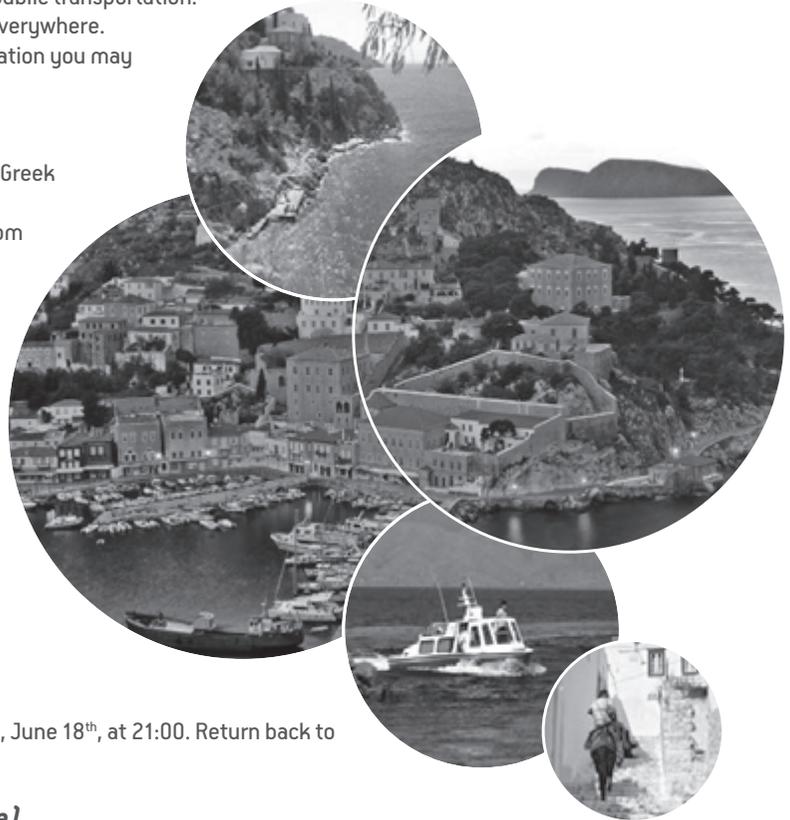
Formal dinner *(included in the accommodation package)*

The formal dinner will be held at SUNSET Restaurant, on Thursday, June 19th, 2014, at 20.30. It is a restaurant at Kanonia area with a nice view of Hydra, where you can enjoy the wonderful sunset. SUNSET Restaurant is located approx. 15 min. walking distance from the Meeting's Venue.

Dietary Restrictions

Kindly inform Mrs. Liana Eliopoulou at the secretariat desk, should you have any dietary restrictions or special requirements.

Note: Drinks are not included in any of the meals mentioned above.





promise MEETING
17-20 JUNE 2014
HYDRA, GREECE

ORGANIZATION



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PROTECTION OF CONSUMERS

by microbial risk mitigation through combating segregation of expertise

**Joint PROMISE and BACFOONET
International Conference in Vienna, Austria
17th - 19th November 2014**



JOINT CONFERENCE

In Europe concerns about food are moving away from issues of ensuring an adequate supply and choice of products towards issues of food safety, animal and plant welfare, labelling and traceability. Mitigation of pathogen bacteria as well as persistent bacteria on foods and processing sites are of great concern in the food industry causing continuous recontamination and safety problems.

Two EU-funded projects, PROMISE and BacFoodNet (www.bacfoodnet.org), deal with the objective to tackle common food safety threats and hence to protect the European consumers. They decided to jointly organise a common international conference on „Persistent lifestyles of food-borne pathogens and its consequences“.

On 17th November 2014 PROMISE will present their results regarding microbial risk mitigation.

From 18th to 19th November 2014 BacFoodNet will share their experiences on mitigating bacterial colonization on foods and foods processing surfaces.



 This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n°265877.

www.promise-net.eu

CONFERENCE PROGRAM

- 10:45 Official Opening of the meeting by Wolfgang Hermann
- Session 1: Do routes of scant attention for the transmission of food-borne pathogens exist?**
- 11:00 „Behavior of pathogenic bacteria in the food chain: *Listeria monocytogenes* as a case study“
Luca Cocollinn, DISAFA University of Turin
- 11:30 „*Listeria monocytogenes* in food confiscated at airport Vienna“
Martin Wagner, University of Veterinary Medicine Vienna
- 11:50 „Neglected routes of macro transmission of food-borne pathogens: Do black markets exist?“
Anca Nicolau, Universitatea Dunarea de Jos, Galati
- 12:10 „Food-borne viruses in food confiscates sampled at Airport Bilbao“
David Rodriguez Lázaro, Instituto Tecnológico Agrario de Castilla y León
- 12:30 „Novel virulence features in *L. monocytogenes* isolated from food from a Romanian black market“
Kathrin Rychli, University of Veterinary Medicine Vienna
- 12:50 „Food travelling globally: VTEC types and antibiotic resistance markers“
Bela Nagy, Hungarian Academy of Sciences, Budapest
- 13:10 „Is internet cheese a matter of concern?“
Dagmar Schoder, University of Veterinary Medicine Vienna
- 13:30 *Lunch break*
- Session 2: *L. monocytogenes* as a model of a saprophyte in food processing environments**
- 14:30 „EFSA Baseline study for *L. monocytogenes* in European food processing environments“
Marios Georgadis, European Food Safety Authority, Parma, Italy
- 15:00 „Prevalence of *L. monocytogenes* in European food processing environments“
Jordi Rovira, University of Burgos, Spain
- 15:20 „Dynamics of *L. monocytogenes* contamination in farmhouse cheese making“
Kieran Jordan, TEAGASC Research Center, Cork
- 15:40 „Monitoring pathogens colonizing a Spanish meat processing plant“
Beatriz Melero, University of Burgos, Spain
- 16:00 „Composition of the microbiome in water and biofilms of *L. monocytogenes* positive drains“
Stephan Schmitz-Esser, University of Veterinary Medicine Vienna

16:20 *Coffee Break*

Session 3: The competition of pathogens in their natural or artificial habitat

- 16:50 „A global look on emerging food-borne disease“ (not confirmed)
 Hilde Kruse, World Health Organisation, Copenhagen
- 17:20 „Evolution of *L. monocytogenes* persistence in a food processing environment“
 Meryem Muhterem, University of Veterinary Medicine Vienna
- 17:50 „Growth and virulence of *Listeria monocytogenes* in co-cultivation models“
 Evangelia Zilelidou, Agricultural University of Athens
- 18:20 „Opening the black box of microbial fecal pollution of ground water resources“
 Andreas Fahrleitner, Vienna University of Technology
- 19:00 Concluding remarks by Martin Wagner and evening reception at AGES

The program of the next two days of 18th and 19th of November can be found on the BacFoodNet website.
(www.bacfoodneet.org)

Registration info:

Registration has to be done via the website of AGES and the link is the following:
<http://www.ages.at/ages/ages-akademie/programm-detail/kalender/event/83/aktuell/>

The conference is free of charge.

For any questions, please, contact Ms. Silvia Prock: prock@rtd-services.com

Sensible data: A challenge for Risk Communication?

A risk communication workshop on sensible data produced by the PROMISE consortium with involved research partners and Food Safety Agencies

Combined illegal food import and policy- and decision-makers workshop

18th to 19th December Vienna, Austria

Program, 18th Dec. 2014



12:00 Get together at the Agency

Part 1: Risk communication in a global context

Session 1: Introduction into risk communication of sensible data

13:00 „Risk communication: more channels, more challenges and less control“
Patrick Wall, Professor, Coordinator of Foodrisc consortium www.foodrisc.org,
University College of Dublin, Ireland

13:45 „EFSA’s experiences and vision of Risk Communications“
Shira Tabachnikoff, International Cooperation Advisor, Communications &
External relations Department, EFSA, Parma, Italy

14:20 „Systematics and impact of border controls in the European context“
Ulrich Herzog, Head of Department Consumer Affairs,
Ministry of Health, Vienna, Austria

14:45 Coffee Break

Part 2: RC and sensible data: ILLEGAL FOOD IMPORT WORKSHOP

Session 2: Characterization of isolates collected during the PROMISE project from passenger’s luggage: Have superbugs been found?

15:15 „What PROMISE has found: Plotting data from PROMISE results against prevalences
of food-borne pathogens from national surveys“
Stavros Manios, Agricultural University of Athens, Greece

15:35 „Putative transmission of animal disease associated agents“
Friedrich Schmoll, Austrian Agency for Health and Food Safety, Vienna, Austria

15:55 „Hazard identification of Salmonella: PROMISE isolates collected from travellers luggage“
Burkhard Malorny, Federal Institute for Risk Assessment, Berlin, Germany

16:15 „Types of *Listeria monocytogenes* collected from passengers luggage“
Martin Wagner, University of Veterinary Medicine, Vienna, Austria

Program, 18th / 19th Dec. 2014

- 16:35 „VTEC and MDR resistant *E. coli*: Final outcomes“
Bela Nagy, Hungarian Academy of Sciences, Budapest, Hungary
- 16:55 „Food-borne viruses and Staphylococcus aureus found in confiscates sampled during the PROMISE project“
David Rodriguez-Lazaro, University of Burgos, Spain
- 17:15 „Pathogens isolated from samples collected from Turkish markets“
Samim Saner, Kalite Sistem Laboratories, Istanbul, Turkey
- 17:35 „WORKSHOP DISCUSSION: PROMISE WP1 data: What needs to be told to the public?“
PROMISE Coordinator and Workshop participants**
- 18:00 *Concluding remarks by Martin Wagner and evening reception.*

Session 3: Risk communication: May novel markers be identified from isolates collected from food processing environments?

- 08:30 „*L. monocytogenes* in the Austrian environment: soil as a source of contamination in food operations“
Beatrix Stessl, University of Veterinary Medicine, Vienna, Austria
- 08:50 „What could explain contamination in a Romanian food processing environment?“
Andrei Balocan & Anca Nicolau, Danube University of Galati, Galati, Romania
- 09:10 „No neglected sources of pathogens in small and medium-sized food factories in Slovakia?“
Tomas Kuchta, Food Research Institute, Bratislava, Slovakia
- 09:30 „The interesting genetic nature of *L. monocytogenes* MLST type 121“
Stephan Schmitz-Esser, University of Veterinary Medicine, Vienna, Austria
- 09:50 „A 3-year hygiene and safety monitoring of a meat processing plant which uses raw materials of global origin“
Panos Skandamis, Agricultural University of Athens, Greece
- 10:10 Coffee Break

Program, 19th Dec. 2014

Part 3: WORKSHOP WITH POLICY- AND DECISION MAKERS: the perspective of Food Safety Authorities

Session 4: Risk communication: Experiences by European Food Safety Authorities and from an industrial perspective

- 10:40 „Risk communication - the balance of speed & accuracy“
Lisa O'Connor, Irish Food Safety Authority, Dublin, Ireland
- 11:00 „Problems of communicating risk in new member states“
Gina Popovici, Romanian Food Safety Authority, Bucharest, Romania
- 11:20 „Risk communication in Slovakia“
Suzana Sirotna, Public Health Institute, Bratislava, Slovakia
- 11:40 „Risk communication in cases of contamination: an industrial perspective“
Peter Raspor, University of Primorska, Slovenia

Session 5: Visualisation of risks

- 12:00 „Risk communication - General aspects and an example concerning the visualisation of data“
Guido Correia-Carreira, Federal Institute for Risk Assessment, Berlin, Germany
- 12:20 „Risk maps for RC in Austria concerning microbiological hazards in food“
Monika Matt, Austrian Agency for Health and Food Safety, Vienna, Austria
- 12:40 „WORKSHOP DISCUSSION: PROMISE WP2 and WP3 data: What needs to be told to the public?“
PROMISE Coordinator and Workshop participants**
- 13:10 Lunch Break

Session 6: PROMISE Recommendations and Final Outcomes

- 14:10 „The PROMISE Academy: Faces and Frames“
Duru Eroglu, Kalite Sistem Laboratories, Istanbul, Turkey**
- 14:30 *The PROMISE project: the end of a journey (final deliverables, publications, materials, reporting, etc.)
Andreas Moser, Martin Wagner, Sabine Ecker (PROMISE Management team)*

Contact & Information

For more information about the PROMISE project, please, visit:

<http://www.promise-net.eu> (General project information and main results, partner profiles, events)

<http://www.promise-academy.eu> (Platform with all relevant presentations, case studies, training material and deliverables)

Main contact persons regarding PROMISE:

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PROMISE Project Communications:

DI Andreas Moser MES, MBA, rtd services, Innsbruck, Austria
email: moser@rtd-services.com



Promise - A promising food-future for Europe

With increasing mobility of the people food quality and safety is getting more and more important. Across Europe food borne illness is a major component in considerations of public health. In 2006 and 2007 only at Frankfurt airport 22 tonnes of illegal imported food was confiscated. This food could be infected with pathogens which endanger the health of the European population.

The EU supported project „PROMISE“ focuses on common food safety threats and hence is protecting European consumers. Since the beginning of 2012 19 partners and food authorities from whole Europe work together to identify the entry points of illegal food imports and their threats. The overall goal of the project is also to improve and strengthen the integration, collaboration and knowledge transfer between the new and old member states of the European Union and its candidate countries. The collaborative work plan assures an exchange of expertise through regional training and dissemination actions to tackle common food safety threats. But the project also wants to integrate stakeholders such as public health, national food safety authorities, food industry and the public from the old and newer member countries in order to ensure the exploitation of research results. The coordinator of the project is Martin Wagner from the Veterinary University of Vienna, Austria. The project is financed with 3 Million Euros by the EU till 2014.

Training workshop for young researchers in Dublin

As personal contact and communication is crucial for the success of such big research projects, the team members met in Dublin (Ireland) at the end of November 2013. Besides a general Consortium meeting, a specific training workshop for young researchers and the first specific stakeholder event was organized. Young researchers from different countries, the future-scientists of tomorrow, got the possibility to take part in the technical workshop concerning novel sequencing technologies and phylogenetics. Together with experienced senior researchers they were trained on methods, techniques and practical knowledge of detecting food pathogens. Creative results were achieved for example by building bacteria cell walls and most important, networking and knowledge transfer was enforced.

First stakeholder event

In order to present the activities and results of PROMISE to a wider public like health authorities, consumer organizations and other relevant stakeholders, the host team of the Agriculture and Food Development Authority, Ireland (TEAGASC), organized the first specific stakeholder event, which was simultaneously offered as a webinar throughout Europe. More than 100 participants listened to very interesting presentations and talks on food safety issues due to pathogenic organisms, in particular *Listeria monocytogenes*. This event took place in cooperation with the annual conference of the *safefood Listeria* Knowledge Network. The key-note speech was given by the well-known *Listeria* expert Martin Wiedmann from the Cornell University, Ithaca, NY, USA. His talk was about practical implications of recent research on *Listeria monocytogenes*.

Contact:

DI Andreas Moser

RTD Services, Austria - <http://rtdservices.wordpress.com>

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www.promise-net.eu



Forscher spüren reisenden Bakterien hinterher

KARIN KRICHMAYR, 19. März 2013, 19:21



foto: standard/christian fischer
Exotisches Sammelsurium in der Tiefkühltruhe für beschlagnahmte Waren: Dieses verkohlte Tier ist nicht mehr identifizierbar.



foto: standard/christian fischer
Zollkontrolle am Wiener Flughafen: Essbare Souvenirs sind beliebt, dürfen jedoch nur eingeschränkt importiert werden. Tierisches ist meist tabu.

In vielen Koffern finden Wiener Zollfahnder illegale Mitbringsel - Beschlagnahmtes landet im Labor, um herauszufinden, welche Keime um die Welt reisen

Unzählige Plastiksackerln quellen aus der Reisetasche des jungen Mannes. Geduldig löst er die Knoten und schält Schicht um Schicht den Inhalt heraus. Der strenge Geruch lässt schon von weitem darauf schließen, dass sich Organisches darin befindet: Fisch, geräuchert und getrocknet, in allen Variationen. "Sehr lecker", merkt der Mann an. Der Zollbeamte, der einen Blick in jede Verpackung wirft, bleibt skeptisch. Unter den Sackerln kommen Dosen und Gläser mit Hülsenfrüchten, Reis, eingelegtem Gemüse hervor.

"Das Essen ist in Nigeria einfach besser und billiger als in den Afro-Shops hier", rechtfertigt sich der Mann in lupenreinem Wienerisch. Er ist von Lagos über Istanbul zurück nach Wien geflogen - und somit in einem der "Toprisikoflüge" nach Österreich gekommen, wie es im Jargon der Zollfahnder heißt. "Istanbul ist ein Knotenpunkt für Reisende aus Nicht-EU-Staaten", sagt Gerhard Heyduk, zuständig für die Abfertigung von Touristen und anderen Reisenden am Flughafen Schwechat.

Auch an diesem Vormittag passen die Beamten besonders die Passagiere türkischer Maschinen ab. In einem Raum direkt vor dem Ausgang in die

Flughafenhalle werden Koffer und Taschen geöffnet oder durch die Röntgenanlage geschickt. Gesucht werden nicht nur unverzollte Zigarettenstangen und Hochprozentiges, sondern auch tierische Produkte aller Art.

Denn die Einfuhr von Fleisch, Milchprodukten und Honig im Reiseverkehr ist streng reglementiert. Aus den meisten Ländern darf gar nichts Tierisches mitgebracht werden, und auch sonst sind nur sehr geringe Mengen erlaubt. So soll die Verbreitung von Krankheitserregern und schädlichen Keimen wie zuletzt dem Ehec-Erreger verhindert werden. Den eingeschleppten Keimen sind auch die Forscher von der Veterinärmedizinischen Universität (Vet-Med) in Wien auf der Spur. Sie nehmen die konfiszierten Lebensmittel mit und untersuchen sie im Labor.

Die eingepackten Fischspezialitäten aus Nigeria gehören nicht dazu. Bis zu 20 Kilo Fisch dürfen mitgenommen werden. Trennen muss sich der Mann mit dem großen Essvorrat allerdings von ein paar Fleischspießen, eingerollt in fettiges Zeitungspapier. "Das kriegt man gar nicht in Österreich. Ich wollte es gleich essen", protestiert er. Dann kramt der Beamte auch noch in Folie gehüllte hellgelbe

Bällchen hervor. Er riecht daran und fragt: "Ist das ein Milchprodukt?" Erst nach Hinzuziehen von Kollegen und dem anwesenden Grenztierarzt kann bestätigt werden, dass es sich bei der unbekanntem Masse um eine Speise aus Maniokstärke handelt und nicht etwa um eine Art Käse.

Gefundenes Fressen

Dass Touristen mit Taschen und Koffern voller illegaler Lebensmittel um die Welt reisen, ist nichts Ungewöhnliches: Allein am Flughafen Wien wurden 2012 7795 Kilogramm tierischer Produkte beschlagnahmt. Bei 1850 Aufgriffen sind das mehr als vier Kilo im Schnitt. Für die Lebensmittelhygieniker von der Vet-Med sind die Fleisch- und Milchprodukte, die normalerweise vernichtet werden, ein buchstäblich gefundenes Fressen.

"Für den Handel gibt es genau geregelte Kontrollen. Wir aber wollen herausfinden, welche Keime über den Reiseverkehr importiert werden. Diese Routen sind bisher kaum beforscht", sagt Martin Wagner vom Institut für Milchhygiene an der Vet-Med. Wagner leitet das mit drei Millionen Euro dotierte EU-Forschungsprojekt Promise, an dem 20 Partner aus ganz Europa teilnehmen.

Seit dem Projektstart 2012 werden an verschiedenen Flug- und Seehäfen sowie an Grenzübergängen mit regem Schwarzmarkthandel Proben genommen. Am Wiener Flughafen stehen neben Flügen aus der Türkei auch Reisende aus Asien, dem Nahen Osten, dem arabischen Raum einschließlich Nordafrikas im Fokus. Insgesamt 600 Proben aus 35 Ländern - zu einem überwiegenden Teil aus der Türkei - untersucht die Wiener Forschergruppe, europaweit sollen bis Juni in Summe 2500 Proben gesammelt werden.

Bei den Zollkontrollen halten sich die Wissenschaftler im Hintergrund. "Am besten ist es, wenn die Proben so frisch wie möglich oder konserviert sind", sagt Projektmitarbeiterin Anja Strauß. Sie steht mit einer Kollegin in einem Nebenraum und freut sich über die gerade eingelangten Fleischspieße. Zuerst aber müssen die unerlaubt importierten Waren gewogen werden. Vier Euro pro Kilo "Vernichtungsgebühr" sind von den gestellten Essensschugglern fällig. Dann bekommt das Fleisch gleich ein Pickerl verpasst - damit es nicht in den Tiefkühltruhen landet, deren Inhalt vernichtet wird, sondern im Probenkühlschrank.

In den Kühltruhen eröffnet sich ein exotisches Sammelsurium der Beute: Entenfüße, Zungen, Schnecken, hundertjährige Eier, Würste mit undefinierbarem Inhalt, notdürftig eingewickelt oder in knallbunten Verpackungen mit asiatischen Schriftzeichen. Die Beamten erzählen von 72 Kilo Käse, die bei einem Passagier gefunden wurden, von gefrorenen Tauben, getrocknetem Stachelschwein und Antilope. An diesem Tag sorgt ein angekohltes Tier in der Truhe für Aufsehen, das Ähnlichkeit mit Geflügel hat. "Es hat Fell, Klauen und breite Rippen", stellt der Grenztierarzt bei einem ersten Blick fest. Was es wirklich ist, kann niemand sagen.

Salmonellen, Ehec, Listerien

Mehr als eine Tonne tierischer Lebensmittel haben die Vet-Med-Forscher in Hochsicherheitscontainern in Labors der Gesundheitsbehörde Ages verfrachtet. Dort werden unter strengen Auflagen Bakterien isoliert: Salmonellen, Ehec-Stämme, Listerien, Campylobacter sowie Staphylokokken und Escherichia coli, die sich als besonders resistent gegen Antibiotika erweisen. Die Bakterien werden dann genauen Analysen unterzogen. Erste Zwischenergebnisse hätten gezeigt, dass vor allem frische Produkte, die nicht aus dem Handel stammen, stark mit gesundheitsschädlichen Bakterien wie Escherichia coli und Staphylokokken belastet waren. Andere Keime wurden in der für sie typischen Umgebung gefunden, also Salmonellen auf rohem Geflügel und Listerien in Käse.

"Bisher haben wir noch keine Anzeichen für ein Bedrohungsszenario gefunden", fasst Martin Wagner die vorläufigen Ergebnisse für Österreich zusammen. Fälle von Tierseuchen, die sich auf eine importierte Wurstsemmel oder Ähnliches zurückverfolgen ließen, hätten gezeigt, dass sich Erreger sehr wohl über globale Touristenströme verbreiten. Im Zuge des EU-Projekts soll eine

Stammdatenbank aufgebaut werden, Richtlinien für Risikomanagement und Informationskampagnen sollen erarbeitet werden.

Am Flughafen sind die Beamten weiter dabei, Leuten zu erklären, dass sie ihr Faschiertes oder ihre abgepackten Hotdogs nicht mitnehmen - und auch nicht auf der Stelle verzehren - dürfen. Gerhard Heyduk bleibt gelassen: " Am Nachmittag werden wir noch mehr finden." Womit die vermutlich hohe Dunkelziffer wieder ein wenig kleiner wird. (Karin Krichmayr, DER STANDARD, 20.03.2013)

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