promise MEETING

17-20 JUNE 2014

HYDRA, GREECE

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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

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Thursday, 19th of June 2014

14:00 - 14:15 **3rd 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 *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* 0157: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 *Listeria monocytogenes* 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 *Listeria monocytogenes* -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 *Escherichia coli*, (STEC/VTEC), and multiresistant *E. coli* 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
Integrons and antimicrobial resistance genes of multidrug resistant *Escherichia coli* 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

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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.

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Friday, 20th of June 2014

08:00	**Breakfast with Steering Committee** (Martin, Anca, Sonja, Bela, David, Gary, Kieran, Markus, Andreas)
09:30 - 11:00	**09:30 – 09:45** Detection of *Listeria monocytogenes* in a meat processing environment using molecular methods **Andrei Sorin Bolocan, Avelino Alvarez-Ordonez, Kieran Jordan, Anca Ioana Nicolau** **09:45 – 10:00** The impact of strain competition on the fitness and virulence potential of *Listeria monocytogenes* **Evangelia Zilelidou, Evanthia Manthou, Luminita Ciolacu, Martin Wagner, Kathrin Rychli, Panagiotis Skandamis** **Session 4:** **Gene expression and physiological adaptation** **10:00 – 10:15** The linkage between phenotypic/genotypic features and the adherence ability of *Campylobacter jejuni* **Katja Bezek, Jasna Kovač, Beatrix Stessl, Martin Wagner, Peter Raspor, Sonja Smole Možina** **10:15 – 10:30** 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 Makariti, Antonia Printezi, Anastasia Kapetanakou, Nikoleta Zeaki, Panagiotis Skandamis** **10:30 – 10:45** Characterization of the in vitro gene response of chicken cells to *Salmonella* Enteritidis **Ama Szmolka, Marta Matulova, Zoltán Wiener, Béla Nagy, Ivan Rychlik** **10:45 – 11:00** Characterization of the prfA virulence gene cluster in *Listeria monocytogenes* strains of clinical and food origin **Sofia Poimenidou, Marion Dalmasso, Panagiotis Skandamis, Kieran Jordan**
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).

ABSTRACTS

3RD GENERAL ANNUAL MEETING (=5TH PROMISE CONSORTIUM MEETING)
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Prevalence and phenotypic characterization of Listeria monocytogenes, Salmonella spp. and Escherichia coli 0157:H7 isolates from confiscated foods in Greece

Stavros Manios, Anastasia Kapetanakou, Panagiotis N. Skandamis

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The objective of the study was (i) to evaluate the risk of *Listeria monocytogenes*, *Salmonella* spp. and *Eschericia coli* 0157: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* 0157: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% alkyldimethylbenzyl 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* 0157: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 fillets) 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

Estella Prukner-Radovčić[a], Lidija Kozačinski[b], Maja Lukač[a], Sandra Gutić[c], Danijela Horvatek Tomić[a],

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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 of 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

Jana Minarovičová, Janka Koreňová, Eva Kaclíková, Tomáš Kuchta

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

ABSTRACTS

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Modelling of prevalence for foodborne pathogens in EU countries

Jasna Kovač[1], Sonja Smole Možina[1], Gary Barker[2]

[1]Chair of Biotechnology, Microbiology and Food Safety, Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia, [2] Gut Health and Food Safety, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom

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

István Tóth

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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 pathotpyes, *Shigella sonnei, Cirobacter rodentium* and several *Salmonella* serovars including Enteritidis, Typhymurium, 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* 0157 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

Elisa Schornsteiner, Martin Wagner, Stephan Schmitz-Esser

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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*.

ABSTRACTS

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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

Béla Nagy[1], Sonja Smole-Mozina[2], Jasna Kovac[2], Martin Wagner[3], Dagmar Schoder[3], Anja Strauss[3], Sabine Schlager[4], Janine Beutlich[5], Bernd Appel[5], Estella Prukner Radovcic[6], Marija Lusicky[7], Mojca Cimerman[7], Istvan Tóth[1], Renáta Kugler[1] and Ama Szmolka[1]

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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 0157 verotoxigenic E. coli (VTEC), and agreed protocols for non-0157 VTEC].
Results of isolations and of characteristics of the above foodborne pathogens from >1800 confiscated food samples were as follows: 0157 VTEC was not detected. All together 15 strains of non-0157 VTEC (among them one enterohemorrhagic *E. coli* strain of 026: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.
Acknowledgement: Support was provided from EU FP7 PROMISE.

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

Renáta Kugler[1], Ama Szmolka[1], Judit Pászti[2] and Béla Nagy[1]

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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, blaTEM-1, tet(A) predominantly occurred, but in general the prevalence of the virulence genes was low. The identification of genes qnrB, aac(6')-Ib, blaOXA-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 sul3gene 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

Ivan Rychlik, Estella Prukner-Radovcic, Sonja Smole-Mozina, Béla Nagy

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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 kpb) was noticeable.
The originally in *Staphylococcus (S.) aureus* described ß-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.

ABSTRACTS

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

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[1], Irene Rückerl[1], Meryem Muhterem[1], Martin Wagner[1], Gary Barker[2]

[1]Institute of Milk Hygiene, University for Veterinary Medicine, Veterinärplatz 1, 1220 Vienna, [2]Institute of Food Research, Norwich, UK

During the PROMISE project, we sampled food contact material (FCM) and non-food contact materials (NFCM) from a food buissness 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

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

IMMF, Institute of Milk Hygiene, Milk Technology and Food Science, Vetmeduni, Vienna

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, stx*1 and *stx*2 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 *Staphyloccus* 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.

ABSTRACTS

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

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

Andrei Sorin Bolocan[1], Avelino Alvarez-Ordonez[2], Kieran Jordan[2], Anca Ioana Nicolau[1]

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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, particulary when other straind outgrow L. monocytogenes during enrichment, therefore avoiding false negative results. Persistence can also be demonstrated.

Acknowledgements
The present research was supported by the EU 7th Framework Programme under the project PROMISE (project number 265877) and by the **ExcelDOC** Project **(***POSDRU/159/1.5/S/132397***).**

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

Evangelia Zilelidou[1], Evanthia Manthou[1], Luminita Ciolacu[2], Martin Wagner[2], Kathrin Rychli[2], Panagiotis N. Skandamis[1]

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Inter-strain competition, in the same microenvironmet 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.

ABSTRACTS

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 Kovač[1], Beatrix Stessl[2], Martin Wagner[2], 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

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[1]Laboratory of Food Quality Control & Hygiene, Department of Food Science & Human Nutrition, Agricultural University of Athens, Iera Odos 75, 118 55, Athens, Greece, [2]Applied 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.

ABSTRACTS

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 Szmolka[1], Marta Matulova[2], Zoltán Wiener[3], Béla Nagy[1], Ivan Rychlik[2]

[1] Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Hungary, [2] Veterinary Research Institute, Brno, Czech Republic, [3]Semmelweis 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 differring 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-1β 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 G0S2 switch-, and the enolase EN02 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 *prf*A virulence gene cluster in *Listeria monocytogenes* strains of clinical and food origin

Sofia Poimenidou[1], Marion Dalmasso[2], Panagiotis N. Skandamis[1], Kieran Jordan[2]

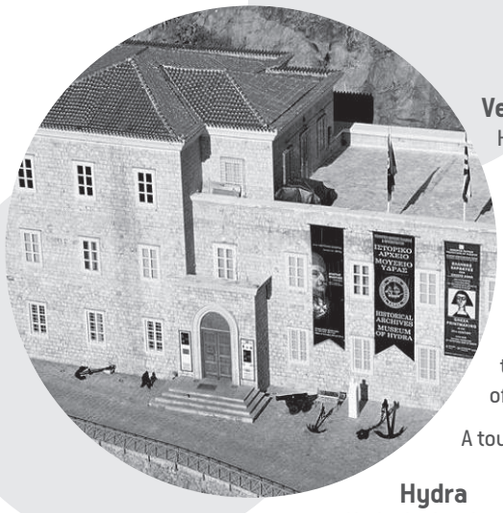
[1] Agricultural University of Athens, Food Science and Human Nutrition Faculty, Lab of Food Quality Control and Hygiene, [2] Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland

The foodborne pathogen *Listeria monocytogenes* can be found in diverse environments, including food processing facilities, farms, and natural environments. Its intracellular life cycle dependents on the *prf*A virulence gene cluster (*ρ*VGC), including *prf*A, *plc*A, *hly*, *mpl*, *act*A, and *plc*B 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 *ρ*VGC structure.

The *ρ*VGC 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 *ρ*VGC loci were alignedusing 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 *prf*A DNA sequences were all silent-mutations as no modifications in the protein sequences were observed for all strains. *act*A and *plc*A 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.

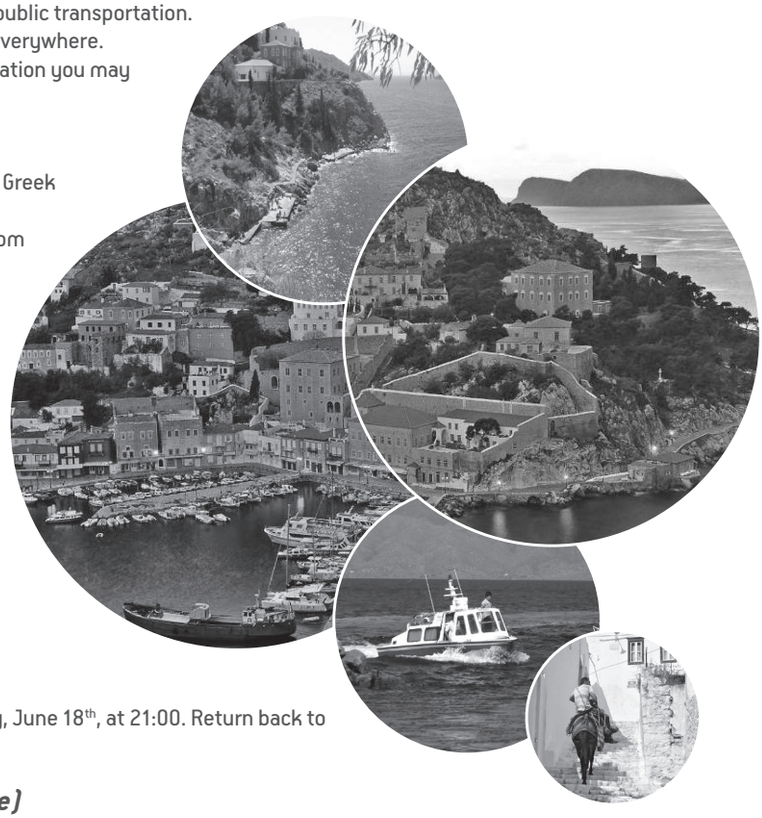
A tour of the Museum will be realized on Thursday, June 19th, at 13.30-14.00.

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

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HYDRA, GREECE

ORGANIZATION

ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ AGRICULTURAL UNIVERSITY OF ATHENS

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