

## **SESSION 1**

ORAL PRESENTATIONS

## ANTIBIOTIC RESISTANCE MONITORING IN AUSTRIA

J. Köfer

(Österr. Agentur f. Gesundheit u. Ernährungssicherheit GmbH, Austria)

Austria has set up an antibiotic resistance monitoring programme according to the guideline 2003/99 in 2004. The antibiotic resistance of zoonotic pathogens (*Salmonella enterica*, *Campylobacter* spp.) and of so-called indicator bacteria (*Enterococcus* spp. or *E. coli*) are from slaughter pigs, cattle, broilers and bulk milk of cattle are tested on a continuous basis. The REMOST-programme consists of a sampling system, which indicates where, how and when samples are to be taken, an analysis system for the continuous analysis of data and a catalogue of measures based on these modules. Isolation of the bacterial strains is done by streaking the material to be tested (faeces, meat, milk) on different agar media. After biochemical verification of suspect colonies, the resistance behaviour is tested using the SENSITITRE<sup>®</sup> system. The test results are published on an annual basis and are fed into a central database, which is linked to the VETGIS<sup>©</sup> Styria geographical information system.

Tab. 1: Occurrence of resistance among *C. jejuni* from broilers (n = 93) and cattle (n = 87); faeces

AB	resistant (in %)	KI (95%)	Distribution (%) of MIC's (mg/l)														
			0,008	0,015	0,03	0,06	0,12	0,25	0,5	1	2	4	8	16	32	64	
CIP	broilers	52,7	[42.1-63.1]				16,1	22,6	3,2	2,2	1,1	2,2	3,2	28,0	17,2	4,3	
	cattle	26,4	[17.6-37]				14,9	37,9	13,8	3,4	2,3	1,1	1,1	4,6	18,4	2,3	
ERY	broilers	2,2	[0.3-7.6]				1,1	4,3	28,0	39,8	17,2	1,1	1,1	3,2	2,2	2,2	
	cattle	0,0	[0-4.2]				1,1	2,3	16,1	52,9	24,1	2,3					
NAL	broilers	44,1	[33.8-54.8]										32,3	8,6	6,5	8,6	44,1
	cattle	29,9	[20.5-40.6]										8,0	36,8	17,2	2,3	5,7
TET	broilers	38,7	[28.8-49.4]				1,1	16,1	21,5	15,1	1,1	1,1	3,2	2,2	7,5	31,2	
	cattle	11,5	[5.7-20.1]				3,4	8,0	24,1	36,8	5,7	4,6	1,1	2,3	1,1	1,1	10,3

## METHODS USED TO IDENTIFY VIRULENCE FACTORS OF BACTERIA

**J.E. Olsen**

(Department of Veterinary Pathobiology, Royal Veterinary and Agricultural University, Denmark)

This presentation gives an overview of the methods used to identify bacterial virulence factors. It will briefly touch on studies of virulence factors in a historical perspective and then discuss benefits and pitfalls of the common approaches used to screen for virulence factors and to demonstrate the role of such factors in host pathogen interaction.

Historically, virulence factors have mostly been identified in an empirical fashion and by comparative assumptions (if a toxin is the cause of disease in *C. botulinum*, it may also be the case in *C. perfringens*). More lately, we have developed methods for systematic screening for virulence associated genes, based on random mutagenesis, comparative genomics and expression analysis, just as we can perform detailed analysis on individual genes and gene-products by a number of sophisticated methods.

In transposon mutagenesis, knock out is performed in genes by a random approach. Screening of mutants has traditionally been cumbersome, since each mutant needed to be tested individually. Methods, where each mutant is tagged by a random sequence are, however, now available, which enables a much easier screening approach. In comparative genomics, genomes are either screened for motif-sequences or for full genes with homology to known virulence factors in other bacteria. Identified genes are further characterized by more specific approaches, such as site specific mutagenesis. More recently, expression analysis, using a number of techniques such as *in vivo* expression technology, *gfp*-labelled promoter fusions and DNA array have been used to identify virulence genes. By this approach it is assumed, that any gene that is differentially up regulated during infection may be a virulence gene. The precise role and the actual proof still need to be determined by more cumbersome techniques.

A molecular Koch's postulate has been suggested for proving the involvement of a gene in virulence: The gene should be present in all virulent isolates and knock-out mutation should cause attenuation. It should be proved that attenuation is not caused by down stream or random effects, and complementation should restore virulence. Preferably, an *in vivo* role should be established.

## **TRANSCRIPTIONAL ANALYSIS AND BIOLOGY IN INTESTINAL COLONISATION OF THE CHICKEN BY SALMONELLA AND CAMPYLOBACTER**

**P. Barrow, M. Jones, P. Adams, C. Woodall, M. Watson, D. Maskell**

(Institute for Animal Health, United Kingdom)

Salmonella and Campylobacter jejuni colonise the chicken intestine very well. Experimental infections were set up in which newly hatched chickens were infected and the caecal contents harvested and bacterial RNA produced for conversion to labelled cDNA for hybridisation to a whole genome *S. Typhimurium* microarray. Patterns of gene expression were compared with in vitro late log phase grown bacteria. The Salmonella data suggested that bacteria in the caecal lumen had adapted to using short chain carbon sources and most of the bacterial cells were not actively growing. There was microscopical support for this. *C. jejuni* showed evidence of adaptation to reduced oxygen and nutrient concentrations. Interactions with avian macrophages and in vivo were measured with a chicken immune gene array and indicated a strong pro-inflammatory response to *S. Typhimurium* infection.

## GENOMIC DIVERSITY WITHIN THE GENUS *LISTERIA*

Ph. Glaser

(Institut Pasteur, France)

*Listeria monocytogenes*, an environmental bacterium, is the causative agent of food-borne listeriosis. The clinical features of listeriosis include septicemia, meningitis, abortion, perinatal infections and gastroenteritis. *L. monocytogenes* is isolated from a broad range of vertebrates including mammals, birds and amphibians. Not all strains of *L. monocytogenes* seem to be equally capable of causing disease, as serovar 4b isolates are responsible of all major food-borne outbreaks and most sporadic cases. *L. ivanovii*, the second pathogenic *Listeria* species has a host-specificity for ruminants. Evolution within the genus *Listeria* was tracked by different means to understand the diversity in its interactions with the host. After sequencing the genome of the laboratory strain EGDe responsible for a listeriosis outbreak in a laboratory animal house and of an isolate of the closely related non-pathogenic species *L. innocua* we have recently determined the genome sequence of a *L. monocytogenes* serovar 4b strain responsible of an epidemic event in France and of a *L. ivanovii* strain responsible of several cases of abortion in a yew heard in Spain. Recently, TIGR has released three *L. monocytogenes* genome sequences. We then applied genome comparisons in order to better characterize functions required for intracellular multiplication and traits related to the specific epidemiologic features of a strain. Based on the comparison of *Listeria* sequences we constructed a focused DNA-array, which was used to characterize a collection of *Listeria* strains originating from environmental and food samples and from clinical cases. The correlation of genomic, phylogenetic and epidemiological properties of the strains allowed to analyze gene flux within the species and to identify lineage specific markers.

## **SESSION 2**

ORAL PRESENTATIONS

## GENETIC PROFILES OF *L. MONOCYTOGENES* ISOLATED ALONG THE FOODCHAIN

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*L. monocytogenes*, a major foodborne pathogen, is mainly attacking the food chain by contaminating the food products and food production environments at latter stages of food processing. However, in a minor number of cases, a hazard may also occur from contamination of raw products in the pre-harvest area, most likely when the technological barriers used are not sufficient to reduce or eliminate the pathogen from the food processing chain.

In the recent years, we have both studied the impact of *Listeria* infections in ruminants and fecal *Listeria* raw milk contamination on on-farm dairying, the impact of *Listeria* contamination on cheese processing in small and large cheese-making plants, where we were especially interested in a prediction of the length of periods in which persistent *Listeria* are detectable from the cheese and the food processing environment. A recent research was focused on the *Listeria* contamination of different vegetables and salads produced in a plant in Poland, the survival of *Listeria* during storage of Brussels spouts and other products and the genetic relatedness of the isolates recovered from the products and the plant interiors.

Furthermore we investigated the *Listeria* contamination status of ready-to-eat (RTE) products purchased from delicatessen shops and supermarkets located in the Vienna region and we determined the *Listeria* contamination status of RTE products stored in household refrigerators. This study was based on a risk classification approach. The household investigations focused also on the occurrence of *L. monocytogenes* in hygiene samples, fecal samples from pets and samples from vacuum cleaners. In any of these studies, we typed the isolated *L. monocytogenes* with Pulsed field gel electrophoresis and other molecular tools. The molecular typing was a valuable tool to confirm the contamination and infection chains, to examine changes in the prevalence of particular *Listeria* types and to conclude whether a contamination occurred more likely during raw food production, food processing or food handling.

**IN SILICO ANALYSIS OF PROTEIN SECRETION SYSTEMS IN *LISTERIA MONOCYTOGENES* EGDE: NEW INSIGHT IN THE ORIGIN OF BACTERIAL VIRULENCE AND/OR BIOFILM FORMATION**

**M. Desvaux and M. Hébraud**

(INRA Institut National de la Recherche Agronomique, France)

Consistent information about protein secretion in Gram-positive bacteria is essentially restricted to the model organism *Bacillus subtilis*. While scattered information is available for some pathogenic Gram-positive bacteria, namely *B. cereus*, *Staphylococcus aureus*, *Clostridium difficile*, *Mycobacterium tuberculosis* and Group A *Streptococci*, little is known about the protein secretion systems present in *Listeria* species. *L. monocytogenes*, a food-borne pathogen responsible of listeriosis, is particularly problematic in the food industry because of its adaptation to a wide range of environmental conditions. Following bibliographic and genomic analyses, the protein secretion systems present in *Listeria* spp. were investigated. As an usual feature of Gram-positive bacteria, ABC (A<sub>T</sub>P B<sub>i</sub>nding C<sub>assette</sub>) exporters are not involved in protein secretion. Members of the MscL family (L<sub>arge</sub> c<sub>onductance</sub> M<sub>echano</sub>s<sub>ensitive</sub> i<sub>on</sub> c<sub>hannel</sub>) and a Tad (T<sub>ight</sub> a<sub>d</sub>herence) export apparatus are most likely absent. It appears protein secretion relies essentially on the Sec pathway (S<sub>ecretion</sub>). The Tat pathway (T<sub>win</sub>-a<sub>rginine</sub> t<sub>r</sub>anslocation) seems present in all but one sequenced *Listeria*. In addition, a FEA (F<sub>lagella</sub> E<sub>xport</sub> A<sub>pparatus</sub>), a FPE (F<sub>imbrilin</sub>-P<sub>rotein</sub> E<sub>xporter</sub>), some holins and a Wss (W<sub>XG100</sub> s<sub>ecretion</sub> s<sub>ystem</sub>) are systematically present. Because a translocated protein can either be displayed on the bacterial cell surface, secreted into the extracellular milieu, or even injected directly into a host cell, these systems promote interaction of a bacterium with its surrounding. Thus, this investigation provides new insight into biofilm formation and virulence of *Listeria* spp. as well as information about protein secretion systems in Gram-positive bacteria different from the model organism *B. subtilis*.

## FACTORS INVOLVED IN THE ATTACHMENT AND BIOFILM FORMATION OF *SALMONELLA*

C. Gamazo, B. García, C. Latasa, C. Solano, Í. Lasa

(University of Navarra, Spain)

*Salmonella enterica* produces at the air-broth interface on the rich medium LB at room temperature a pellicle whose matrix is mainly composed of curli fimbriae, cellulose and a still unidentified polysaccharide. Bacterial adherence and invasion assays to eukaryotic cells and *in vivo* virulence studies of cellulose deficient mutants indicated that, at least under our experimental conditions, the production of cellulose is not involved in the virulence of *S. Enteritidis*. However, cellulose deficient mutants were more sensitive to chlorine treatments, suggesting that cellulose production and biofilm formation may be an important factor for the survival of *S. Enteritidis* on surface environments. In our ongoing investigation of the factors involved in the *S. Enteritidis* biofilm formation process, we have investigated the role of two novel surface proteins of *S. Enteritidis* with structural homology to Bap of *S. aureus*. Our work showed that the biofilm matrix of *S. Enteritidis* included a novel surface protein, in which absence *S. Enteritidis* is unable to produce the pellicle on the air-broth interface on LB and showed a lower capacity for invading murine intestine epithelial cells and organ colonization.

## **SURVIVAL OF SALMONELLA SEROVARS ON BEEF CARCASSES**

***G. M. Knudsen, S. Aabo and J. E. Olsen***

(Department of Veterinary Pathobiology, The Royal Veterinary and Agricultural University and Department of Microbial Food Safety, Danish Institute of Food and Veterinary Research, Denmark)

*Salmonella* are important zoonotic pathogens of great health and economic importance. There are more than 2500 serovars, many of which differ in pathogenicity and survival capability in the environment. We have investigated the survival of several serotypes on beef cuts over a 14-days period at 3°C. A reduction in the CFU was found for all the tested serotypes. The reductions varied from a 30-fold reduction for *S. Typhimurium* DT104 to a more than 1000-fold reduction for *S. Infantis* and *S. Newport*. This experiment indicates that storage at low temperature of beef carcasses can be used as a reduction strategy for *Salmonella* spp. in beef. To investigate molecular mechanisms to resist low temperature stress, mutants in the *otsA*, *rpoS* and *clpP* genes are under investigation. A mutant in the Clp proteolytic complex *S. Typhimurium* C5 was tested for growth and survival at low temperature. Both wildtype and mutant survived 20 days at 5°C. Both at 10 and 15°C, however, the  $\Delta clpP$  mutant was severely affected in ability to form colonies compared to the wildtype. This indicates that the Clp proteolytic complex is essential for growth at 10 and 15°C but not survival at 5°C.

**STRESS SURVIVAL MECHANISMS IN *CAMPYLOBACTER* SPP.*****K. Jordan and C. Murphy***

(Teagasc, Moorepark, Fermoy Co. Ireland)

*Campylobacter* spp. are sensitive to environmental influences and do not appear to survive well in the environment. They have been shown to lack most of the survival mechanisms, such as the stationary phase RpoS mechanism, commonly found in other pathogens. However, they continue to be the greatest cause of foodborne illness worldwide. This implies that they have developed survival mechanisms that have not yet been identified.

We have identified two survival mechanisms in *Campylobacter jejuni*, an adaptive tolerance response (ATR) and production of an extracellular component giving stress protection. Using proteomics and genomics, the ATR of *Campylobacter jejuni* has been studied in detail at the protein and gene expression levels. The results showed the increased expression of several survival mechanisms, including the heat shock response and a two-component regulator.

## **EFFECT OF INDUSTRIAL AND NATURAL DISINFECTANTS ON SPOILAGE, PATHOGENIC AND TECHNOLOGICAL STRAINS GROWN ALONE OR IN MIXED CULTURE IN BIOFILM**

**I. Lebert, S. Leroy, R. Talon**

(Institut National de la Recherche Agronomique, France)

Traditional dry sausages rely on natural contamination by environmental flora. This contamination occurs during slaughtering, increases during manufacturing and is composed of useful microorganisms for the fermentation and flavour of sausages, but also spoilage and pathogenic flora. The aim of this study was to develop targeting disinfectant solutions bactericide for pathogen and spoilage flora while preserving technological flora.

Natural (*Satureja* essential oil) and industrial (Brillo, Johnson Wax Professional and an alkaline solution, Ecolab) disinfectants were tested on bacteria in monoculture for technological strains (*Staphylococcus equorum*, *S. succinus* and *Lactobacillus sakei*) and in a mixed culture of *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas fragi* and *Escherichia coli*. Cultures were performed in biofilm in glass fibre filters (incubation 20°C - 24 h) before application of the disinfectant (1 h - 16°C).

The alkaline solution with 10% Na<sub>2</sub>SO<sub>4</sub> was efficient to decrease spoilage bacteria (5.1 log), maintained the technological flora but did not reduce the pathogens. The essential oil (2 %) was highly efficient on spoilage strains (5 log) and *S. equorum* (4 log), moderately on pathogens (2.3 log), not efficient on *S. succinus* and *L. sakei* (0.5 log). The disinfectant Brillo (3 %) eliminated all the strains.

These results show the difficulties to obtain a disinfectant efficient simultaneously on spoilage and pathogenic bacteria and preserving technological flora. Essential oils could be a good alternative only if contamination by pathogenic bacteria is low during traditional sausages manufacturing.

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## **WORKSHOP 1**

Workshops on modelling of stress adaptation, survival, biofilms, resistance to disinfectants with introductory discussion papers

ORAL PRESENTATIONS

## EXPOSURE OF SALMONELLA AS A FORCE FOR BACTERIAL EVOLUTION

**T. Humphrey**

(University of Bristol, United Kingdom)

The history of human and animal infections with *Salmonella* spp. is characterised by frequent changes in either phage type and/or serotype. A possible exception to this is the international pandemic of egg-associated *Salmonella* Enteritidis infection which has lasted for over 20 years. However, even within that serotype there have been marked changes in phage types found to be prevalent in either human cases or food animals. In the last 3 – 4 years, many parts of Europe have seen the appearance of phage types of *S. Enteritidis*, previously very uncommon. This is in contrast to the majority of the period of the *S. Enteritidis* pandemic in Europe where human cases predominated by PT4. The reasons for the appearance of the new phage types is not yet fully understood but maybe associated with exposures to hostile environments, which drive changes in the expression of cell surface structures. Work at Bristol has demonstrated that repeated exposure to conditions such as high temperature or desiccation can markedly change *Salmonella* genotype. Similarly, exposure to farm biocides bring about change and may also result in resistance to antibiotics. These and other matters will be discussed.

## **ANALYSIS OF *SALMONELLA TYPHIMURIUM* STRESS RESPONSE USING THE PROMOTER ARRAY AND MICROARRAY ANALYSIS**

**I. Rychlik, K. Papezova, M. Crhanova, D. Gregorova**

(Veterinary Research Institute, Czech Republic)

*Salmonella typhimurium* is exposed to a number of stressful environments during its life cycle within and outside its various hosts. During intestinal colonisation *S. typhimurium* is successively exposed to acid pH in the stomach, to the detergent-like activity of bile, to decreasing oxygen supply, to the presence of multiple metabolites produced by the normal gut microflora and finally it is exposed to cationic antimicrobial peptides present on the surface of epithelial cells. It is therefore of a general interest to monitor expression of both virulence and stress-related genes under such conditions. To achieve this two different approaches were used. Using transcriptional promoter fusions we found that expression profiles of SPI1 gene promoters related to the invasion of the epithelial cell were nearly identical. These promoters were active in LB pH 7.4 with or without butyrate but were suppressed by acidification, bile salts, and in minimal medium. SPI2 gene promoters were characterised by their expression in LB pH 7.4 and minimal medium, although their expression profiles were not as uniform as those of invasion-related genes. Subsequent microarray analysis of *S. typhimurium* starved in minimal medium and comparison with *S. typhimurium* transcription in LB supplemented with bile salts or polymyxin allowed identification of new stress-induced genes. These included *dps*, *ymdF*, *STM1513*, *osmC*, *rpsV* and *yciG* which were induced under all tested conditions. Interestingly, *ymdF*, *STM1513* and *yciG* encode for highly similar peptides 54 - 60 aminoacids in size indicating that they form a new stress induced protein family.

## **WORKSHOP 2**

Workshops on modelling of stress adaptation, survival, biofilms, resistance to disinfectants with introductory discussion papers

ORAL PRESENTATIONS

## **DO WE NEED VIRULENCE DATA FOR QUANTITATIVE RISK ASSESSMENT**

**S. Aabo**

(Department of Microbial Food Safety, Danish Food and Veterinary Research Institute, Denmark)

It is currently stated in many applications for research funding that virulence data are essential for quantitative risk assessment. This workshop has been designed to evaluate this statement and to enlighten which kind of data is needed.

After a brief introduction to quantitative risk assessment, the participants will be asked to defend, reject or refine the following statement:

“Virulence data are not essential for quantitative risk assessment of food borne pathogenic bacteria”

The outcome of the workshop will most probably be a refined sentence that will form a recommendation of which kind of virulence data that are essential for quantitative risk assessment.

## **SESSION 3**

ORAL PRESENTATIONS

## NITROGEN BASED TREATMENT FOR SANITATION OF PATHOGEN CONTAMINATED MANURE

### B. Vinnerås

(National Veterinary Institute, Sweden)

During a zoonotic out-brake the normal procedure is to sanitize the stables. The manure also has to be handled in a proper and safe manner. Present recommendation for manure treatment is addition of 3% of lime combined with mixing during one consecutive week. This kind of manure treatment holds several problems. Initially it is the health of the farmer, mainly due to the risk of lime dust and from handling the treated manure that holds a pH over 11. The high pH results in formation and precipitation of carbonates and a loss of ammonia as air emission. The objective of this study was to find a simple, safe and efficient treatment method for contaminated manure, using *Salmonella typhimurium* as a model organism.

#### **Material and Methods**

Urea and ammonia were added in concentrations corresponding to 0.5% and 1% ammonia nitrogen to pig manure at 4°C and 14°C. The reduction over time of *S. typhimurium* was monitored and compared to storage only. *S. typhimurium* was added to the manure in a concentration corresponding to  $10^7$  cfu, ml<sup>-1</sup>.

#### **Results & Discussion**

The addition of urea resulted in an increase of pH from pH 7 to between pH 8.5 and pH 9.3 whilst the ammonia addition resulted in a pH increase to between pH 9.5 and pH 10.3.

The addition of urea and ammonia resulted in a rapid increase in the decimal reduction time for *S. typhimurium* (Table 1)

**Table 1**, the decimal reduction in days of *S. typhimurium* depending of added concentration of urea and ammonia compared to storage in pig manure

<b>Treatment</b>	<b>Temperature</b>	<b>Decimal reduction</b>
Storage	4°C	No reduction
Storage	14°C	25 days
Urea 2%	4°C	7 days
Urea 2%	14°C	3 days
Ammonia 1%	4°C	5 days
Ammonia 0,5%	4°C	5 days
Ammonia 0,5%	14°C	32 h

Ammonia based sanitation of pathogenic contaminated manure is an efficient treatment. The ammonia can be added either in the form of liquid ammonia or as urea granulates. By covering the surface of the manure, assuring only small ammonia losses, the added ammonia will remain in the manure after the treatment increasing the fertilising value of the material and thereby keeping the cost for the sanitation low.

#### **Acknowledgment**

The study was financed by the Swedish board of Agriculture

## **IN VITRO TOXICITY OF *ESCHERICHIA COLI* SHIGA TOXIN VARIANTS FOR VERO CELLS**

**L. De Baets, J.P. Hernlsteens, H. De Greve, H. Imberechts**

(CODA – CERVA, Belgium)

Shiga toxin producing *E. coli* (STEC) are associated with gastrointestinal disease and with hemolytic uremic syndrome in humans. Ruminants are considered a reservoir for human infection and transmission may occur via consumption of contaminated food, through direct contact with infected animals, with contaminated swimming water, or through person-to-person contact. The major virulence factor of EHEC is the Shiga toxin (Stx), whose profound and irreversible cytopathic effect on Vero cells was first reported by Konowalchuck in 1977. Many variants of Stx2 have been described in literature and nomenclature is confusing. Different investigators examined the structure-function relationship of the Shiga toxins, e.g. through site-directed mutagenesis, via the generation of hybrid toxins or occasionally of naturally occurring Stx2 variants. The present study compares the cytotoxicity of 14 naturally occurring Stx2 variants for Vero cells. Toxin sequences were identified using PCR-RFLP (De Baets *et al.* 2004), were cloned in an inducible expression vector and were subsequently transformed in an *E. coli* host. The toxin yield was estimated in immunoblot and toxicity for Vero cells was assessed in a quantitative manner. Stx2 toxin variants were identified that differed in their cytotoxicity for Vero cells by a factor 1000.

**TETRACYCLINE RESISTANCE GENES OF *SALMONELLA* AND  
*E. COLI* : POSSIBLE LINKS OF *tetB* TO VIRULENCE AND PREHARVEST  
SURVIVAL**

**P. Zs. Fekete, N. Nógrády, F. Olasz, G. Blum-Oehler, B. Nagy**

(Veterinary Medical Research Institute of the Hungarian Academy of Sciences,  
Hungary)

The tetracycline resistance genes of animal *Salmonella* and animal pathogenic *E. coli* bacteria were identified and typed by phenotypic methods and by PCR. *Salmonella* Hadar and *Salmonella* Typhimurium isolates originated from Hungarian poultry samples. We also determined the *tet* gene type of Hungarian and Austrian enterotoxigenic (ETEC) and/or verotoxigenic (VTEC) *E. coli* strains of porcine, as well as extraintestinal and commensal *E. coli* strains of poultry origin. Additionally, porcine ETEC/VTEC strains from the USA were also investigated.

The *tetA* and *tetB* genes were found in the central-european *E. coli* strains (without reference to the pathotype) and in the *Salmonella* Hadar isolates. Interestingly, almost all of the porcine *E. coli* strains from the USA were characterized by the *tetB* gene. In a few instances *tetA/tetB*, *tetA/tetC*, or *tetA/tetD* genes also occurred.

The *tetG* gene was characteristic of the Hungarian *Salmonella* Typhimurium isolates.

To reveal the co-occurrence of the antibiotic resistance and virulence genes, the tetracycline resistance plasmid (pTC) of a *tetB* positive porcine ETEC strain (EC2173) (O147:F18, STa, STb) was characterized as a model system. Sequencing studies revealed that the *tetB* gene was located on the pTC plasmid as part of a Tn10 transposon. STa and STb enterotoxin genes were also located on this pTC plasmid as part of Toxin Specific Locus (TSL) (Fekete et al, I. J. Med. Microbiol, 2003). Such pTC like plasmids were detected with high frequency (62%) in F18 fimbriated ETEC strains, but were present in only 33% of the K88 (F4)<sup>+</sup> ETEC strains, indicating an association of fimbrial (F18) and enterotoxigenic (*sta*, *stb*) virulence genes with the *tetB* gene.

Due to the fact that tetracyclines have been widely used in veterinary medicine during several decades, it is postulated that some tetracycline resistance genes (like *tetB* on pTC), could have played a role in preharvest survival and spread of some pathogenic strains of *E. coli* and of *Salmonella*.

Acknowledgement:

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## GLYCOPEPTIDE AND STREPTOGRAMIN RESISTANCE IN ENTEROCOCCI ISOLATED FROM AUSTRIAN ANIMAL HUSBANDRY

A. Eisner

(Institute of Hygiene, Medical University of Graz, Austria)

Nosocomial infections with enterococci increasingly cause great concern because of their ability to acquire high-level resistance to antimicrobial agents which limits medical treatment options. The glycopeptides vancomycin and teicoplanin as well as the semisynthetic streptogramin combination quinupristin/dalfopristin (Synercid) are alternatives for treatment of infections due to multiresistant enterococci. In addition to selection in hospitals, another potential source of acquiring resistance is the therapeutic use of antibiotics in veterinary medicine or the application as growth promoters in animal fattening.

We investigated the prevalence of enterococci with resistance to glycopeptides and streptogramins in Austrian animal husbandry. 619 fecal specimens (pig, cattle, poultry) were screened for the presence of glycopeptide-resistance and 300 specimens for the presence of streptogramin-resistance in enterococci. Determination of resistance genes was done by PCR.

A total of 286 glycopeptide-resistant strains were isolated from the animal fecal samples. *E. faecium* with *vanA*-mediated resistance was frequent in broiler chickens (42%) but rare in cattle and pig samples. Streptogramin resistance was only found in enterococci originating from broiler specimens with a resistance rate of 15% for the *E. faecium* isolates.

Our results document an existing reservoir for glycopeptide and streptogramin resistance in Austrian poultry. Further studies are necessary to elucidate the significance of this resistance reservoir for human infections.

## **POSTER SESSION**

## **SALMONELLA ENTERICA SEROVAR TYPHIMURIUM, SDIA AND STRESS RECOVERY**

**M. Crhanova, D. Gregorova, P. Adams, P. Barrow, I. Rychlik**

(Veterinary Research Institute, Hudcova 70, Brno, Czech Republic)

SdiA is a member of the LuxR family of transcriptional regulators function of which in *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) is relatively poorly understood. Therefore conditions under which *sdiA* is expressed in *S. Typhimurium* were studied. Strains containing transcriptional promotor fusion *sdiA-luxCDABE* were inoculated into LB medium with pH ranging between 4.0 and 7.5 and incubated in microplates at 37 °C. *sdiA* was maximally induced when inoculated to LB pH 4.3. Acid pH of the medium increased during cultivation and the maximal *sdiA* expression was observed, when the medium just reached neutral pH in the wild type strain. This phenomenon was not observed in *sdiA* mutant. When both the strains were shifted to LB pH 3 in time of the peak expression of *sdiA*, the mutant strain was more resistant to acid stress than was the wild type. This fact led us to a hypothesis, that *sdiA* is involved in switching off the stress defense mechanism.

Microarray analysis was used to confirm this hypothesis. As expected, genes upregulated in *sdiA* mutant were related to the stress defense. Genes suppressed in the *sdiA* mutant were related to the virulence. When genes induced in *sdiA* mutant were compared with microarray analysis of gene expression in stationary phase *S. Typhimurium* in minimal medium and in LB medium supplemented with polymyxin, a considerable overlap in upregulated genes was observed. Interestingly, most of these genes belonged to the RpoS regulon. Exact relationship between *sdiA* and *rpoS* is further studied.

## USE OF DIAGNOSTIC MICROARRAYS FOR TYPING OF ESCHERICHIA COLI

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Numerous phenotypic and genotypic methods have been employed to detect, classify and type pathogenic *E. coli* strains for clinical diagnosis, epidemiological investigations, or routine surveillance. However, all methods are based on the detection or identification of one or only a few specific markers. Microarrays allow the simultaneous detection of a range of genetic markers, resulting in a comprehensive picture of an isolate's pathogenic potential. We present a diagnostic microarray, containing a total of 32 PCR-based gene probes specific for extraintestinal as well as intestinal *E. coli*. The technique was established using reference strains for each *E. coli* pathogroup. For diagnostic evaluation 40 *E. coli* strains isolated mainly from neonatal meningitis (NMEC) cases were screened for the presence of virulence genes. Furthermore, the prototype of a oligo-based microarray for genetic serotyping is presented. Serotyping of *E. coli* strains is tedious, requires a large set of antisera, and is sometimes not fully reliable, e.g. some strains turn out as non-typeable (NT) or as non-motile (NM). In order to overcome these drawbacks we are aiming at replacing serotyping by a genetic alternative. A system, that mimics the serotype most optimally will have to focus on the same properties. This is why we chose the key genes responsible for expression of the antigens exploited by serotyping. These genes are *wzx* (flippase) and *wzy* (repeat unit polymerase) which encode key enzymes in the bio-synthesis of the O-specific side chain, and *fliC*, which codes for the flagellin monomer. A DNA microarray is currently under development. It will contain spots with oligo DNA probes representing around 90% of the *fliC*- and around 20% of the *wzx/wzy* genes of *E. coli*. Pathogenic serovars will be over-represented. In our experience DNA microarrays have the potential to replace or complement laborious classical techniques.

## **OSMOTIC STRESS INFLUENCES BIOFILM FORMATION IN *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM**

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*Salmonella enterica* serovar Typhimurium (STM) is an important gram-negative pathogen causing gastroenteritis in humans and animals. Its pathogenicity increases with the ability to form the biofilm, a population of microbial cells growing on a solid surface. The aim of our study was to investigate the efficiency of the biofilm formation in STM F98 strain in the presence of various stress factors (sodium nitrite, polymyxin, paraquat, methylmethane sulphonate, trimethylamine-N-oxide (TMAO), dimethylsulphoxide, mitomycin, sucrose and sodium chloride) added into LB culture. Bacteria were incubated aerobically for 48 h at 37°C in a 96 polystyrene microplate well. The biofilm formation was investigated by the electron microscopy (negative staining, cationized-ferritin labeling) and by visualization using 0.1% safranin. For the quantitative evaluation of the rate of attached bacteria, the dye bound to the adherent cell was resolubilized with acetic acid and the absorbance of solution from each well was measured at  $\lambda = 518$  nm. Results showed that TMAO, sucrose and sodium chloride were the main factor affecting the biofilm formation. The highest value of the biofilm formation was in accordance with electron - microscopy observation. Characteristic multicellular aggregates enclosed by the extracellular matrix were observed densely labeled with the cationized ferritin. Bacteria growing in LB medium only did not form the biofilm. The data suggest a critical role of ionic and non-ionic osmolytes in STM F98 strain biofilm regulation under environmental osmotic stress conditions.

## **IDENTIFICATION OF GI O#57, A PUTATIVE PATHOGENICITY ISLAND OF EHEC O157, ASSOCIATED TO ATTACHING AND EFFACING *ESCHERICHIA COLI***

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Attaching and Effacing *E. coli* (AEEC) are characterized by the presence of a pathogenicity island (PAI), the Locus of Enterocyte Effacement (LEE), conferring the capability to form the attaching and effacing lesion. They include enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC).

Sequencing of EHEC O157 strain EDL 933 has shown that about 20% of its genome is made up by horizontally acquired genomic islands (GIs) and many of these can be considered as putative PAIs.

In the attempt to define a minimum genomic core needed for AEEC virulence, we compared DNA from low pathogenic *E. coli* strains isolated from pigeon with the genome of EHEC O157 spotted onto commercial microarray slides. The pigeon isolates are LEE positive, produce the *stx2f* variant but have never been reported in human cases with severe EHEC disease. We identified two GIs, O#52 and O#57, that were absent from both the pigeon isolates. GI O#52 encodes an endodeoxyribonuclease and other determinants conferring resistance to methylviologen. GI O#57 harbours genes encoding a putative intestinal colonization factor and a putative cell killing factor possibly involved in the colonisation of the host.

The presence of these two GIs has been investigated by PCR in a panel of AEEC strains, including 23 EHEC O157, 39 EHEC non-O157 and 42 EPEC strains from different sources. Other 25 strains belonging to Enteroaggregative, Enterotoxigenic, and Necrotoxigenic *E. coli* were included for comparison. Both GIs were present in all the EHEC O157 assayed. GI O#52 was very common among all the *E. coli* pathogroups investigated. Conversely, PAI O#57 was strictly associated with AEEC, being present only in the most of EHEC and EPEC strains.

## DETECTION OF GENOMIC VARIABILITY AMONG ISOLATES OF *CAMPYLOBACTER JEJUNI* FROM CHICKEN AND HUMAN

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*Campylobacter jejuni* and *C. coli* are among the commonest causes of acute bacterial enteritic diseases in humans. During examination of the slaughtered poultry, there were detected very different findings of *Campylobacter sp.* In some farms incidence was repeatedly found 80 - 100%, while in other farms about 30 - 40%. The question of whether isolates *C. jejuni* from poultry flocks represented different clones or had a common clonal ancestry was solved by subtyping by *fla*-PCR/RFLP and by pulsed-field gel electrophoresis (PFGE) after *Sma*I digestion. Among 96 poultry strains, 11 *fla*-RFLP and 11 PFGE subtypes were found, while 110 human strains could be divided into 23 *fla*-RFLP and 32 PFGE subtypes. Altogether, 31 *fla*-RFLP and 32 PFGE subtypes were found. Analyses showed that some flocks were colonized with clones demonstrating identical profiles both after *fla*-RFLP typing and PFGE analysis. Two flocks bred simultaneously in the same farm showed identical *fla*-RFLP profiles but altered PFGE profiles. Both flocks might be infected with the same clone but during the colonization in the second flock the infection with the clone mutant at the restriction site for *Sma*I prevailed. After eight months in the same farm a new flock was colonized with other clone differing both in the *flaA* gene and in the restriction sites for *Sma*I. In other flocks various PCR/RFLP profiles were found with the same PFGE profile. But detailed analysis showed the possible transition from one *fla*-RFLP subtype to another after mutation of the recognition site referring to only one restrictive enzyme. Only in six cases (5.5%) *fla*-RFLP and PFGE subtypes combinations found in poultry and humans were identical.

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## **ANALYSIS OF ENVIRONMENTAL SIGNALS CONTROLLING *SALMONELLA* ENTERICA SEROVAR TYPHIMURIUM VIRULENCE GENE EXPRESSION**

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Using promoter fusions we analyzed responses of 25 genes associated with *Salmonella* virulence. Factors selected were those modeling the environments which *Salmonella* experiences when colonising a host beginning with acidification, followed by responses to bile salts and butyrate, and intracellular survival. In promoters originating from SPI3, SPI4 and virulence plasmids genes we never observed any activity *in vitro*. Expression profiles of gene promoters related to the invasion of the epithelial cell (*hilA*, *hilC*, *hilD*, *invF*, *sicA*, *sopA*, *sopB* and *sopE2*) were nearly identical. These promoters were active in LB pH 7.4 and LB pH 7.4 with butyrate but were suppressed by acidification, bile salts, and in minimal medium. Genes related to *Salmonella* intracellular survival (*phoP*, *ssrA*, *ssaB*, *ssaG*, *pipB*, *sifA*, and *sifB*) were characterised by their expression in LB pH 7.4 and M9 minimal medium, although their expression profiles were not as uniform as those of invasion-related genes. Increased expression of intracellular survival related genes in stationary phase culture in minimal medium culture was confirmed by microarray analysis which revealed high level of expression of SPI2 genes in 2-day-old stationary phase culture in minimal medium.

## IDENTIFICATION AND TYPING OF *STAPHYLOCOCCUS AUREUS* ISOLATED ALONG THE MILKCHAIN, IN TANZANIA, AFRICA

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*Staphylococcus aureus* (*Staph. aureus*) is regarded as an important mastitis pathogen in the bovine in Tanzania and is the most frequent pathogen isolated from mastitic milk. Infected wounds, lesions, and boils of food and milk handlers may also be sources of contamination, as well as coughing and sneezing by individuals with respiratory infections. Yet there are no data available about the prevalence of *Staph. aureus* and other major foodborne pathogens in milk in Tanzania. The aim of the study was (i) to evaluate the prevalence of *Staph. aureus* in milk (ii) to characterize their toxic potential, (iii) their sensitivity to antibiotics and (iv) to determine their clonal identity.

The dairy economy of Tanzania is based on a traditional extensive matter of production. The traditional smallholder sector (Maasai pastoralists and urban farmers) contributes about 85% of the total output of milk.

Maasai are above all cattle people. Milk is their everyday food. Animals in the pastoral farming system are short-horn Zebu (*Bos indicus*) cattle kept in a free range grazing system and using communal grazing grounds and watering points. Animals are not supplemented and receive no veterinary attention.

Urban farmers keep mostly crossbreeds or European pure breeds. Animals are zero-grazed with owners practising a cut and carry type of feeding. On average urban farmers keep 4-5 cattle. Their knowledge about keeping of animals, milking and processing of milk is very poor. In the rural regions milk is collected by private milk traders. They in turn receive milk either directly from the Maasai pastoralists or from milk vendors who collect milk from the cattle bomas and deliver it at the many makeshift milk collection points along the main road.

Only 5% of milk produced in Tanzania is processed in licensed dairy plants. Most of the milk is sold directly. 200 milk samples were collected along the milk-chain. 23 small-scale producers, 64 milk vendors, 4 milk collection centres and 16 milk kiosks were included in this study. The study was undertaken in Dar Es Salaam and in the north part of Tanzania, in the region of Victoria Lake.

The prevalence of *Staph. aureus* in milk was found to be 18,5 %.

37 strains of *Staphylococcus aureus* have been characterized by five different methods: (i) by their production of lecithinase, lipase, pigment and sheep haemolysin, (ii) by multiplex PCR toxin typing assay (iii) by their sensitivity to antibiotics, (iv) by pulsed field gel electrophoresis (PFGE) and (v) phage typing using the international set and supplementary phages.

**APPLICATION OF MOLECULARBIOLOGICAL FINGERPRINTING METHODS  
FOR THE RISK ASSESSMENT OF PERSISTENT INFECTIONS CONSIDERING  
*YERSINIA ENTEROCOLITICA* AS EXAMPLE**

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*Yersinia (Y.) enterocolitica* is considered to be a foodborne pathogen. Many fingerprinting methods had been described before such as PFGE, RAPD and MLST. But one of the auspicious procedures is seen in the upcoming Amplified fragment length Polymorphism (AFLP) technique. It is by far easy to use and provides a high reproducibility. This study is embedded in a research project focussing the risk of pathogenic *Y. enterocolitica* in broilers. During a 36-days period possible *Yersinia* spp. colonies were isolated from faeces and different tissues of infected broilers in order to investigate the practicable recovery of the used *Yersinia enterocolitica* DSM 13030. Before the usage of the above fingerprinting methods all strains were first determined by PCR (species, genus, pathogenicity) and so far by biochemical testings. The aim of this study was to evaluate two different fingerprinting methods, PFGE and AFLP, regarding to their reproducibility, simplicity and time-consumption. PFGE was done by using restriction enzymes like *XbaI*, *EcoRI*, *MseI* and *NotI*, the AFLP procedure was carried out with the enzymes *EcoRI/MseI* and *HindIII/HhaI*. Many optimisation steps had to be negotiated for both methods to receive analysable results displayed in fragment length figures.

## **SENSITIVITY AND SPECIFICITY OF TESTS USED FOR DETECTING VEROTOXIGENIC ESCHERICHIA COLI AND PREVALENCE ASSESSMENT OF ANIMAL CARRIERS AT SLAUGHTERHOUSES**

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The prevalence of pathogenic *Escherichia coli* carriers among slaughtered cattle is a key factor for microbial risk assessment. Probability of faecal contamination exists even in production units with good hygienic standards and risk prevention strategies, based on meat chilling and cooking before consumption, should be accompanied by efforts to maintain the prevalence of carriers at low level. The probability of detecting the different strains of verotoxigenic *E. coli* at low level in cattle faeces depend on the analytical methodology and because many diagnostic tests are currently used the risk assessment should encompass it. Results of epidemiological studies carried out at slaughterhouse or at farm level in Italy show that strains detected in cattle only partially overlap with those isolated by human cases, but often the presence of non-O157 verotoxigenic *E. coli* has not been investigated in animals because the diagnostic procedures were complex and time consuming and the low number of isolates selected for phenotypic/genotypic profiling could have hampered the detection of some strain. Among cattle showing an high prevalence of O157:H7 *E. coli* carriers, we have observed a striking high prevalence of isolates showing the *eaeA* gene but lacking the STx genes. In other animals from the same farm we have isolated verotoxigenic strains. These results have been confirmed in two laboratories in Italy and Spain. Phage typing and PFGE profiles gave evidence that mixed *E. coli* populations with different virulence profile coexisted in the same group of calves and up to three different pathogenic strains was isolate the same calve.

## TRANSFER OF $\beta$ -LACTAMASES GENES FROM *S. TYPHIMURIUM* DT104 TO *S. AGONA* AND *E. COLI* K12 IN BROTH, MILK AND MINCE BEEF

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Multi-drug resistant *Salmonella* Typhimurium DT104 has emerged as a global health problem over the last decade. Its typical ACSSuT resistance profile has been reported in other bacterial strains, suggesting the dissemination of resistant genes. A donor and recipient *E. coli* K12 strain were co-inoculated into broth, milk and minced beef and incubated at 4, 15, 35 and 37°C for 18 h and 36 h. *S. Typhimurium* DT104 was found to transfer ampicillin resistance to a strain of *E. coli* K12 strain. The  $\beta$ -lactamase genes *carb* and *tem* were confirmed by PCR to have transferred from the donor strain (*S. Typhimurium* DT104) to the recipient (*E. coli* K12) strain. Disc diffusion testing showed the *E. coli* K12 transconjugant to have the ACSSuT resistance profile, suggesting the transfer of the chromosomal antibiotic resistance gene cluster from *S. Typhimurium* DT104. Ampicillin resistance transfer from a strain of *S. Typhimurium* DT104 to a strain of *S. Agona* was also attempted, but the ampicillin resistant transconjugant was not stable and reverted to being ampicillin susceptible. Transfer rates were found to be similar in all transfer media at 25 and 37°C ( $10^{-4}$  to  $10^{-7}$  transconjugants per recipient), however transfer was found to occur in minced beef at 15°C ( $10^{-6}$ , transconjugants/ per recipients cells), but not in broth or milk. No transfer was found to occur at 4°C.