



## **Cost Action 920**

### **Foodborne Zoonosis: a Co-ordinated Food Chain Approach**

**WG2/WG4 Joint Meeting**

**"NEW TECHNOLOGIES IN THE  
FOOD CHAIN AND EMERGING  
ZOOONOTIC AGENTS "**

**Bertinoro - 3-5 ottobre 2004**

**Dipartimento di Scienze degli Alimenti  
Alma Mater Studiorum - Università di Bologna**

## SESSION 1

# **Influence of new technologies along the food chain on the emergence or selection of new pathogens**

ORAL PRESENTATIONS

# FOOD SAFETY ASPECTS OF THE MODERN LOW HEAT FOOD PROCESSING

**S. Knochel**

(Institute of Food Science, Royal Veterinary and Agricultural University, Denmark)

Heating is the most universal process in food preparation and preservation. Although some new technologies are being used most processes are still done with conventional heating technologies. While emphasis was previously on canning and long-term preservation focus has now shifted to mild heat treatments and subsequent chill storage and it is getting increasingly important to know where the safety limit is in terms of microbiological food safety. Both processors and public health authorities need to know which hazards are relevant, which factors influence safe thermal treatment, and how we may begin to predict inactivation or potential for subsequent growth. Examples of heat resistance differences between and within species will be given and the influence of matrix-bacterial interaction before, during and after heat treatment adaptation will be described. The potential for growth during heat treatment, cooling and storage will be discussed with emphasis on *C. perfringens*. While differential resistance due to the intrinsic properties of the microorganisms is of utmost importance, the variations in processing conditions may have an even greater impact on the assessment of process safety which will be illustrated by examples. Finally, some of the more recent technologies employing new combinations or principles will be mentioned.

## MICROBIAL BIOFILM IN FOOD INDUSTRY

**B. Carpentier**

(AFSSA, Maisons-Alfort, France)

“Biofilms are cells immobilized on a substratum and often embedded in a matrix of microbially produced organic polymers” (Characklis, 1989). According to this definition, individual cells adhering to surfaces can be classified as biofilm. Biofilm cells are phenotypically distinct from their planktonic counterparts. A major feature of biofilms is the high proportion of persister cells remaining after an antimicrobial treatment. The biofilm matrix contains water, extracellular polymers (mainly exopolysaccharides and exoproteins), nucleic acids and entrapped substances. Exopolysaccharides allow microcolony formation and resistance to desiccation. Exoproteins play a role in adhesion and proteinaceous extracellular appendages can be involved in the formation of thick biofilm. In the food industry, biofilms frequently do not form a continuous film but rather distant microcolonies. Gram<sup>+</sup> bacteria usually have a smaller potential for forming biofilm than Gram<sup>-</sup> bacteria. *L. monocytogenes* adheres to substratum as single cells, but its adhesion and its susceptibility to disinfectant can be modified when associated with another bacterial strain. This is one of the possible reasons why some *L. monocytogenes* strains are persistent in some premises. All biofilm cells do not show the same attachment strength. The proportion of cells easily detached depends on the bacterial strain, the substratum, the age of the biofilm and also on the cleaning or disinfecting product that had come in contact with the biofilm. Any contact with a biofilm leads to detachment but it is very difficult to detach all cells. Therefore the effectiveness of cleaning is limited as is the assessment of the microbial load of a substratum. To decrease biofilm settlement it is necessary to choose good construction materials, to comply with hygienic design rules and to limit, as far as possible, the presence of water on surfaces. Indeed, water is necessary to bacterial growth but may also be a source of undesirable micro-organisms.

## THE USE OF LACTIC ACID BACTERIA TO CONTROL ZONOTIC PATHOGENS

**M. Chemaly**

(AFSSA Ploufragan, France)

LAB consisted of a heterogeneous group of Gram positive bacteria producing lactic acid as the key metabolite. LAB are involved in large number of spontaneous food fermentations and are closely associated with animal and human environment. Their history of safe use combined with a variety of interesting metabolites have led to wide industrial applications. One of the projects involving LAB in our laboratory aimed to evaluate their effect on the decrease of *Salmonella* carriage in young turkeys. Animals reared in isolators and inoculated at the first day by *S. Typhimurium* received by drinking water a selection of LAB and other “natural” compounds. Animals are followed until twelve days after the compound administration and then sacrificed in order to control *Salmonella* level in the caeca. Comparisons between the control batches (animals inoculated by *Salmonella* but not treated) and treated ones allowed the identification of the most interesting compounds. The addition of LAB in drinking water led to a decrease of 2.5 log<sub>10</sub> in *Salmonella* level comparing to the control group. LAB showed the most significant effect amongst other compounds and are selected to be evaluated in a field trial.

# FURANONES AS POTENTIAL INHIBITORS OF PATHOGEN GROWTH

**M.E. Guerzoni**

(DIPROVAL, Alma Mater Studiorum- University of Bologna, Italy)

Furanones are naturally occurring or formed during food processing compounds which participate in a variety of disparate biological phenomena including signalling mechanisms between individual organisms. The high specificity and sensitivity of responses that they generate are consistent with a basic function in living cells (Slaughter, 1999). They can be hydro or liposoluble or volatile depending on the substituents on the central ring. The later feature is a prerequisite for air-mediated transmission. Winzer *et al.* (2002) reported the extracellular production of 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF), 4-hydroxy-5-methyl-3(2H)-furanone (MHF) and a furanosyl borate diester (AI-2) in a boron rich medium by *Vibrio harveyi*.

In a previous research a strain of *Lactobacillus helveticus* producing a furanone under stress conditions, and endowed with the ability to produce autolysins as well as morphological changes in different microbial species, has been identified.

The aim of this preliminary work was to evaluate whether this molecule, presumably associated with quorum sensing phenomenon, can be used as a non-conventional antimicrobial compound. The results obtained confirmed its antimicrobial activity against *Salmonella* Enteritidis also when used at levels < 0.5 ppm.

## References

Slaughter J. C. (1999). The naturally occurring furanones: formation and function from pheromone and food. *Biological Reviews*, 74 (3), 259-276.

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## METALLOPROTEINS MODULATING SALMONELLA-HOST INTERACTION: SUPEROXIDE DISMUTASES AND OVOTRANSFERRIN

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The ability of bacterial pathogens to survive and multiply in the infected host is critically dependent on their ability to recruit adequate amounts of transition metals. Metals are essential cofactors in several proteins, including the *sodC*-encoded periplasmic Cu,Zn superoxide dismutase, which protects bacteria from phagocyte-mediated oxidative killing. Most *Salmonella* strains possess two *sodC* genes, *sodC1* and *sodC2*, located on a prophage and on the chromosome, respectively. Our studies indicate that both genes contribute to *Salmonella* virulence, although the relative importance of *sodC1* and *sodC2* depends on the specific serotype.

To limit bacterial proliferation, the host produces proteins able to sequester transition metals. Some of these proteins, which exert antimicrobial activity, are located in the egg white. Among these, ovotransferrin (Otr) is a glycoprotein belonging to the transferrin family synthesised by the liver and also present in the oviduct. The antibacterial activity of Otr depends on its capacity to bind with extraordinary affinity two Fe<sup>3+</sup> ions per molecule, thus sequestering iron necessary for bacterial survival and growth. This action is largely bacteriostatic as it is reversed by the addition of ferric ions. However, Otr also shows a strong bactericidal activity, which depends on its binding to the bacterial surface.

Here we report the inhibition of *S.choleraesuis* growth due to the iron binding ability of Otr. Moreover, we show that Otr has an antibacterial activity, unrelated to Otr iron-withholding, which is associated to its binding to the bacterial surface. Both actions of Otr suggest that this glycoprotein could play a role in nonimmune antibacterial defence in birds.

**RISK FACTORS FOR MORTALITY IN INVASIVE HUMAN LISTERIOSIS - DENMARK  
1994-2003**

**P. Gerner-Smidt, S. Ethelberg, P. Schiellerup, B.G. Bruun, J.J. Christensen, J. Engberg, V. Fussing, A. Jensen, C. Jensen, E.M. Nielsen, A.M. Petersen and M. Weischer**  
(Statens Serum Institut, Denmark)

Background: Listeriosis is a notifiable disease in Denmark. Invasive listeriosis carries a high case-fatality rate (CFR). The current study was undertaken to identify important risk factors for adverse outcome of invasive listeriosis excluding materno-foetal cases.

Methods: Data on the cohort of notified cases with invasive listeriosis for the 10-year period 1994-2003 were reviewed

Results: 299 invasive cases of listeriosis were reported. 64 (21%) died of their disease. The yearly incidence of disease ranged from 0.42 to 0.73 per 100.000 inhabitants. Septicaemia and meningitis caused the same mortality, but no mortality was observed in patients with focal infections at normally sterile body sites (bones, joints and peritoneum). Increasing age, being infected with strains of serogroup 4 as compared to serogroup 1/2, or being infected with strains of particular ribotypes were associated with increased CFR in the univariate analysis. In the multivariate analysis, the serogroup remained as a risk factor for death (OR 2.1, 95%CI: 1.1-3.9, serogroup 4 ~ 1/2) but the ribotype of the infecting strain disappeared as a risk factor when the serogroup of the infected strain was taken into account. Underlying conditions in young patients < 70 years of age were strongly related to death (OR 10.7, 95% CI: 1.4- 84) whereas underlying conditions were of no importance for mortality in the old age group = 70 years (OR 0.97, 95% CI: 0.4- 2.3). The underlying conditions most strongly associated with death in the young age group were non-haematological cancer and alcoholism.

## SESSION 1

# **Influence of new technologies along the food chain on the emergence or selection of new pathogens**

POSTER PRESENTATIONS

# WHOLE GENOME COMPARISON OF ENTEROHEMORRAGIC *E. COLI*: DEFINITION OF THE MINIMUM GENOMIC CORE FOR PATHOGENICITY

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*E. coli* O157 and other enterohaemorrhagic *E. coli* (EHEC) are important emerging foodborne pathogens. The main virulence features of EHEC include the presence of a pathogenicity island (PAI) termed Locus of Enterocyte Effacement (LEE) and the capability to produce Shiga toxins. Sequencing of *E. coli* O157 EDL 933 strain has recently shown the presence of numerous putative Pathogenicity Islands (PAIs) in the chromosome of this organism some of which might be involved in virulence.

We adopted a whole genome approach aimed at the identification of the complete genomic core needed for virulence of EHEC based on the comparison of low pathogenic *E. coli* strains isolated from pigeon, containing the LEE and producing an host adapted shiga toxin, with the genome of EHEC O157 spotted onto commercial microarray slides. Cy-3 and Cy-5 genomic DNA from two O45 strains isolated from pigeon has been used in hybridisation experiments. Scanning of the microarray slides allowed us to identify eleven O157-specific ORFs belonging to PAIs referred to as O#52, O#57, O#93 and O#166 in the EDL 933 strain. PAI O#93 is a virulence determinant of EHEC O157, corresponding to the phage carrying the Shiga toxin 1 coding genes, while the PAI O#166 encodes the pathway for degradation of small organic acid compounds and a transport system from *Klebsiella pneumoniae* whose involvement in the EHEC virulence is unclear.

PAIs O#52, and O#57 are prophage-related (CP-933-X, and CP-933-O respectively) and contain genes possibly involved in the virulence. PAI O#52 encodes an endodeoxyribonuclease and other determinants conferring the capability to grow in presence of methyl-viologen and its presence has been demonstrated also in other Shiga toxin-producing *E. coli* strains belonging to O91 serogroup isolated from humans. PAI O#57 harbours genes coding for a putative intestinal colonization factor and a putative cell killing protection factor that may be involved in the colonisation of the host gut. Studying the distribution of these PAIs among different EHEC pathogroups is in progress to assess if they can be considered as part of the minimum genomic core for EHEC pathogenicity.

SESSION 2

**SWINE AS A SOURCE OF EMERGING  
ZONOSIS**

ORAL PRESENTATIONS

## **HEPATITIS E VIRUS INFECTIONS: AN EMERGING ZOOONOSIS**

**M. Martin**

(Veterinary School, University of Barcelona, Spain)

In developing countries hepatitis E is an endemic disease caused by a virus (HEV) transmitted via the fecal-oral route. The clinical course of hepatitis E is similar to that of hepatitis A, although high fatality rates, up to 25%, have been reported in pregnant women, mostly in the third trimester of pregnancy, that develop an acute fulminant hepatitis of yet unknown origin. In industrialized areas, HEV is considered an emerging disease and recent studies indicated that most probably is a zoonosis. Of the different animal species that could act as reservoirs of the infection, pig is of the greater epidemiological importance. Several studies carried out mainly in the United States, Europe and Japan, showed that HEV strains of swine origin are closely related to human isolates of HEV of the same geographical areas. Besides, swine farmers and veterinarians had a higher seroprevalence against HEV than the general population. No effective treatment is known for this infection, and the only available prevention is to avoid contact with contaminated environments. Recently, human cases of hepatitis E have been reported in which the source of infection was the consumption of uncooked deer or wild boar meat. In several countries health authorities are beginning to be aware of the zoonotic risk of this infection. Identification of sources of infection and risk populations are some of the recommendations made by the WHO to control the disease.

# EMERGING CLONES OF SALMONELLA ENTERICA SEROTYPE TYPHIMURIUM AND 4,5,12:I OF SWINE ORIGIN

**A. Ricci**

(Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua, Italy)

The laboratory surveillance of Salmonella in Italy has a high level of integration among the different involved institutions, and it is represented by the Enter-net system which concerns notifications from human cases, food items and environmental sampling, and the Enter-vet network, which collects information on isolations from animals and from food of animal origin. An accurate analysis of the two databases allowed us to detect an increase, in 2002 and 2003, in the prevalence of *S. Typhimurium* (ST) NT (not typable) and of an atypical monophasic strain, defined as 4,5,12:i-, both in human cases and in veterinary samples. ST NT accounted for the 25% of the ST isolated in humans during this period, and for the 20% of the ST isolated from samples of swine origin. Among these strains, we found a high percentage attributable to a unique PFGE pattern (XB79), which does not appear to be frequent in other animal species. Also the resistance profile seems to be typical of human and swine strains.

*Salmonella* 4,5,12:i- accounted for the 2,5% of veterinary isolates in 2002, and for the 4,2% in 2003, being the third most common strain in pigs and pig products (6,8% and 10,4% respectively). This strain appears to be emerging also in human cases, even if its prevalence can be underestimated due to its strong phenotypic similarity with *S. Typhimurium*. This problem can be easily overcome submitting suspect monophasic strains to a multiplex PCR, obtaining the confirmation of them being H 1,2 negative.

## **HEPATITIS E VIRUS IN PIGS IN THE NETHERLANDS**

**Martijn Bouwknecht \***, **Froukje Verschoor\***, **Paul Roholl\*\*** and **Wim H.M. van der Poel \***.

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Hepatitis E virus (HEV), is a non-enveloped RNA (7.5 kb) virus, classified separately in the family of Hepatitis E like viruses. Hepatitis E virus (HEV) is endemic in much of the developing world, where it is a major cause of viral hepatitis. Clinical illness resembles other forms of acute viral hepatitis. In the industrialised countries of Europe, seroprevalence is rather low (1-3%) but in recent years there has been an increasing number of diagnoses of this infection in people who have not been abroad. The source of these infections is unclear. In the Netherlands over the last five years about twenty cases of HEV infections in humans have been confirmed by RT-PCR at RIVM. At least 15 of these patients had not been abroad and must have been infected with an endemic HEV strain. Sequencing of the PCR products revealed that for of these patients the closest sequence was a swine sequence also detected in the Netherlands. To gain more insight in the prevalence of infectious HEV in swine in the Netherlands we tested 60 pooled swine farm fecal samples and 36 individual swine fecal samples by RT-PCR and found a farm prevalence of HEV of 37% and an individual animal prevalence of 30%. Electron Microscopy (EM) study of the samples revealed HEV-like virus structures of expected size in just a few of the samples. HEVs from swine in the Netherlands were clustered in at least two groups, together with European and American isolates from swine as well as humans. The close relationship of swine HEVs with human HEV sequences, indicates that animals may be reservoir hosts of HEVs and could be a source of HEV infections in humans. It is unclear if swine excrete infectious HEV and how HEV may be transmitted from animals to humans, but it should be elucidated if foodborne and waterborne transmission is possible.

# THE STYRIAN *SALMONELLA* SURVEILLANCE PROGRAMME FOR PORK PRODUCTION

**P. Wagner and J. Köfer**

(Department of Veterinary Administration, Styrian Government, Austria)

In the Austrian Province of Styria the implementation of Directive 92/117/EEC, which requires measures to be taken for the control of "food-borne diseases", resulted in the setting-up of a *Salmonella* surveillance programme for pork production. After implementing a baseline study a serological monitoring programme based on meat juice samples and a bacteriological monitoring programme based on swabbing of carcasses and retail pork cuts in meat cutting plants were established. Following a representative sampling plan a total of 34,170 muscle samples from pigs originating from 3,417 finishing farms were serologically tested for the presence of *Salmonella* antibodies in the period from 1999 to 2003. More than 95 % of the samples investigated were below the negative cut off < 20 % based on the 5-year average. By using a geographical information system regional differences in the mean extinction values were detected. The prevalence of *Salmonella* contamination in pork was determined by testing a total of 11,330 wipe samples from wholesale pork cuts. *Salmonella* spp. was detected in only 15 cases (0.13 %) of a total of 11,330 bacteriologically tested wipe samples from meat processing plants. The proportion  $\hat{p}$  of *Salmonella* contaminated pork in the total population estimated from the annual sample decreased from 0.36 % (CI  $0.23 \leq p \leq 0.85$ ) in 1999 to 0.14 % (CI  $0.07 \leq p \leq 0.24$ ) in 2003. The contamination of Styrian pork with *Salmonella* is extremely low and thus poses a negligible risk of infection to consumers.

## **A FAMILY CLUSTER OF HAEMORRHAGIC COLITIS CAUSED BY CONSUMPTION OF ESCHERICHIA COLI O157 CONTAMINATED SALAME IN ITALY**

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Most foodborne *E. coli* O157 cases have been traced to foods of bovine origin and cattle are considered the main reservoirs of the organism, nevertheless non-bovine food vehicles have also been identified. This report describes a family cluster of *E. coli* O157 cases caused by an uncommon food vehicle. In a family living in North Eastern Italy, the daughter had mild diarrhoeic symptoms, while the wife (aged 56) and the husband (aged 64) were admitted to the hospital with bloody diarrhoea, nausea and severe abdominal pain. In both of them, the colonic mucosa appeared oedematous and hyperaemic at endoscopy and stool cultures tested positive for sorbitol non-fermenting *E. coli* O157.

The epidemiological survey showed that they lived in an urban area, did not have animals and the only food eaten in common was a dry fermented traditional salame produced in a local meat plant. It was purchased at retail level and consumed on day 6 and day 4 before hospitalisation, at about 50 days of seasoning. The remaining part of the salame was collected by the local health authority at the family house and submitted to the laboratory. *E. coli* O157 was isolated from a 50 g in depth aseptically collected sample, analysed by a sensitive procedure based on immunomagnetic separation (ISO 16654:2001), while 3 further 25g samples tested negative. The product  $A_w$  was 0.90 and the agar-immunodiffusion test demonstrated the presence of only pork meat, thus excluding beef, ovine and chicken meat. All the *E. coli* O157 strains isolated from wife, husband and food were identical: they carried *vt2* and *eae* genes and shared the same PFGE pattern. This finding confirmed the role of a food vehicle normally considered uncommon for the animal origin of the meat and its processing, including fermentation and drying steps.

# **FIRST ISOLATION OF NOROVIRUSES FROM CLINICAL SAMPLES IN POLAND**

**B. Mizak , J. Król, I. Kozyra**

(National Veterinary Research Institute, Poland)

The importance of foodborne viral infections is increasingly recognized. Food handlers can transmit infection during preparation or serving. Fruit and vegetables may be contaminated by fecally contaminated water used for growing or washing. In Poland the cause of most nonbacterial gastroenteritis outbreaks remained unknown. The aim of presented studies was to estimate the etiological agents of foodborne human gastroenteritis cases in Poland.

We examined faecal samples collected from 7 symptomatic individuals with nonbacterial acute gastroenteritis. All stool samples were examined by RT-PCR and hybridization methods for the presence of genotype I and II of Noroviruses, rotaviruses, enteroviruses and astroviruses. Nucleic acids were isolated with modified chloroform-butanol method described by Le Guyader et. al. (Int. J. Food Microbiol. 87, 2003).

In three out of seven examined samples Norovirus genotype I (samples No 2, 5 and 7) was detected. Samples No 2 and 5 were also positive for Norovirus genotype II. In stool samples No 5 and 7 we also detected rotavirus. None of the examined samples were positive for enteroviruses and astroviruses.

The results of presented studies are the first description of Norovirus isolation in Poland.

## **YERSINIA PSEUDOTUBERCULOSIS INFECTIONS TRACED TO FRESH CARROTS**

**J. Takkinen**

(National Public Health Institute, Finland)

In March 2004, the number of *Yersinia pseudotuberculosis* notifications increased suddenly in Finland compared to previous months. *Y. pseudotuberculosis* causes acute gastroenteritis characterized by fever and abdominal pain. Post infectious complications, like reactive arthritis and/or erythema nodosum may occur.

Overall, 125 cases were notified to the National Infectious Diseases Register (NIDR) by the end of July, 2004. Of the notified cases, 57 were women and 68 men. The incidence was highest (4,6/100 000) in young children and descended steadily to 0,5/100 000 in adults over 60 years of age. The epidemic curve showed two peaks: between weeks 12 and 17, and 23 and 30. An outbreak with 48 cases was detected among schoolchildren in one municipality during the first peak. Only 4 (3%) cases from the school outbreak were reported to the NIDR. Strains (n=48) sent to the Laboratory of Enteric Bacteria were of the serotype O:1.

Preliminary interviews implicated that grated carrot was a possible vehicle of infection. Case-control study showed that cabbage – grated carrot meal served in March was a probable source of outbreak (crude OR 3.5, 95%CI 1.0 – 16.1).

The tracing of carrot producers led to two farms. *Y. pseudotuberculosis* was isolated from fluid of spoiled carrots and from mouldy carrots in one farm. The farm located in the region where most of the cases were reported. Rodent samples from the farm field yielded to positive findings in shrews. The isolates from the positive farm samples and majority of human samples showed the same PFGE pattern.

## **BOVINE NON-EHEC O157:H7 STRAINS AS A SOURCE OF EMERGING CONCERN?**

**Tóth, Z Lancz and B.Nagy**

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

We examined colon (n=428), and faeces (n=114) of healthy slaughter cattle originating from three slaughter houses, and 100 faeces and 114 milk from a dairy farm, for E.coli O157 using specific immuno magnetic separation (IMS) and “VTEC-screen” kit. Part of the samples was also examined with O26- and O111 specific IMS. Positive isolates were PCR tested for relevant virulence marker genes.

A total of 7,4 % (32/428) colon samples, 7 % (8/114) of milk and 2,8 % (6/214) of stool samples contained E. coli O157 strains. Only 6 % of these samples were suspect to harbor VT-producing bacteria, out of which we were able to identify 22 VTEC (9x O157, 1x O26 and 12x OX) strains.

The isolated O157 E. coli strains (46) proved to be overwhelmingly of O157:H7 EPEC pathotypes (69.6 % eae-positive). A further 17.4 % (8/46) was typical EHEC (stx<sup>+</sup>, eae<sup>+</sup>), and 2,2% (1/46) were VTEC, and 10,8 % (5/46) did not have either stx or eae gene. The EHEC O157 strains of colon-, and stool origin had stx1, stx2 genes, the one EHEC O157 strain with milk origin was stx2<sup>+</sup> only. Most of the O157:H7 strains had enthly, and paa, espD, and the entero-aggregative heat stable toxin (east1) gene. None of the strains were cnf<sup>+</sup>. Altogether 8 strains had cdtB gene. Typed cdt genes genes (5) were all cdt-III. All E. coli O157 strains were motile, and produced H7 flagella, did not ferment sorbitol, did not produce colicin, and – interestingly - none of the strains were multiresistant.

Our data suggest that the frequent EPEC O157:H7 strains could be of future concern as potential reservoirs for EHEC.

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**ANTIMICROBIAL RESISTANT *SALMONELLA* IN IMPORTED AND DOMESTICALLY  
PRODUCED FRESH MEAT AS A SOURCE OF HUMAN INFECTIONS – THE DANISH  
EXPERIENCE**

**S. Aabo**

(Danish Institute for Food and Veterinary Research, Denmark)

The occurrence of antimicrobial resistance among *Salmonella enterica* has increased in recent years. As consumers are exposed to *Salmonella* from domestically produced as well as imported meat, resistance in both sources are important. In this study, 6,321 *Salmonella* isolates were obtained from humans and Danish produced and imported meat between July 1998 and July 2002. The isolates were serotyped, phage typed and tested for susceptibility to antimicrobial agents. A statistically significant higher proportion of resistance, multi-resistance, and quinolone resistance was observed among *Salmonella* isolates obtained from imported meat (58%, 28% and 26%, respectively) compared to Danish produced meat (26%, 4% and 4%, respectively). Resistance was found in a number of different sero- and phage types. Furthermore, comparison with the resistance levels in serovars from humans suggested imported meat as a major source of infections with antimicrobial resistant *Salmonella* in humans. These results demonstrated that programs for controlling resistant *Salmonella* isolates in meat cannot be limited to the national level but should be of worldwide concern, and that multi-resistance is only for a small part related to *S. Typhimurium* DT104.

## **DISTRIBUTION OF TOXB, AN ESCHERICHIA COLI O157 VIRULENCE GENE, AMONG ATTACHING AND EFFACING E. COLI (AEEC)**

**R. Tozzoli, S. Morabito, A. Caprioli**

(Istituto Superiore di Sanità, Department of Food Safety and Veterinary Public Health)

*Escherichia coli* O157 and other Attaching and Effacing *E. coli* (AEEC) are pathogenic bacteria responsible for enteric diseases in humans and animals. AEEC pathogenicity is mainly due to the presence of the Locus of Enterocyte Effacement (LEE) and of the Shiga toxin-converting phages. Besides these well-established virulence determinants, additional factors harboured by Mobile Genetic Elements (MGE) are likely involved in AEEC pathogenesis. In particular, a large plasmid (pO157), consistently present in EHEC O157 strains, carries several putative virulence genes, such as those governing the production of enterohaemolysin (ehxA), a katalase-peroxidase (katP), a serine protease (espP) and a very large gene, *tox*B, which is long about 10 Kb. This gene encodes a factor able to inhibit the host lymphocyte activation and to confer a marked increase in the capability of adhesion *in vitro*.

So far, the complete sequence of *tox*B gene has been detected only in the two *E. coli* O157 strains whose genomic sequence has been completely determined. Recently the presence of a 600 bp sequence corresponding to the 5' terminus of *tox*B has been identified by PCR in AEEC strains belonging to serogroups O121, O26, O103, and O145. However, the analysis involved only a small portion of such a large gene, therefore, we decided to investigate on the presence of the entire *tox*B sequence. We deployed molecular tools based on three PCR amplifications targeting different regions of *tox*B to investigate on the distribution of this gene in a collection of AEEC strains belonging to different serogroups.

A total of 99 AEEC isolates were examined, they included 60 Verocytotoxin-producing strains (23 O157 and 37 *E. coli* non-O157). As expected, all the *E. coli* O157 isolates were positive in all reactions, giving amplification products of the expected size. Among the non-O157 AEEC strains investigated, ten out of 21 *E. coli* O26 isolates investigated were positive with all the three primer pairs, while other six isolates were positive with at least one of the primer pairs used. In addition, a few strains belonging to serogroups O111, O86, O118, O127, O121, O123 and O145 resulted positive to PCR with at least one of them.

The results obtained in this study showed that *toxB* gene is present in all the *E. coli* O157 strains assayed. Moreover, this new virulence determinant seems not to be restricted to this serogroup, being highly associated also with strains belonging to O26 serogroup.

**PREDOMINANT ROLE OF ACTIVE EFFLUX IN THE ACQUISITION OF RESISTANCE CHARACTER IN *IN VITRO*-SELECTED ENROFLOXACIN, CIPROFLOXACIN AND NALIDIXIC ACID RESISTANT MUTANTS OF *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM**

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The role of AcrAB-like efflux pumps and of *gyrA* mutations in the acquisition of resistance character were studied in *Salmonella enterica* serovar Typhimurium mutants that were selected with enrofloxacin, ciprofloxacin or nalidixic acid from two field strains (ST8, ST15) and one reference strain (ST ATCC 14028). When the antibiotic susceptibility of the selected resistant mutants were tested in the presence of the efflux pump inhibitor (EPI) Phe-Arg-*b*-naphthylamide (PA $\beta$ N) (also termed MC207,110), heterogeneous MIC values reductions from 2-64 fold for enrofloxacin and nalidixic acid and 2-8 fold for ciprofloxacin were detected. In particular any differences were observed among enrofloxacin mutants of all three strains when PA $\beta$ N was used. With regard to nalidixic acid mutants, the lowest PA $\beta$ N concentration tested was sufficient to revert the acquired resistant character of ST ATCC 14028 nalidixic acid mutants whereas the highest PA $\beta$ N concentration showed the highest reduction of MIC values on ST 15 and ST 8 nalidixic acid mutants.

For the ciprofloxacin resistant mutants, the lowest PA $\beta$ N concentration was sufficient to revert the acquired resistant character for all but three mutants (S.T 8 mutants 11,12 and 13) for which the higher PA $\beta$ N concentration was required.

Single or none mutations in *gyrA* gene were found in all selected resistant mutants without any correlation to their MIC values.

These results strongly suggests that the acquisition of enrofloxacin, ciprofloxacin and nalidixic acid resistance character in the *in vitro*-selected resistant mutants of *S. Typhimurium*, is due predominantly to an AcrAB-like system other than *gyrA* gene mutations. Moreover the heterogeneous effect of the EPI PA $\beta$ N with regard to the different antibiotics tested leads to suppose that the AcrAB-like system is binding site specific or that other mechanisms (i.e. reduced permeability; other target gene mutations) may be involved.

SESSION 2

**SWINE AS A SOURCE OF EMERGING  
ZONOSIS**

POSTER PRESENTATIONS

**MOLECULAR EPIDEMIOLOGY OF THE EMERGENT DRUG-MULTIRRESISTANT  
TYPE OF *SALMONELLA* SEROTYPE TYPHIMURIUM CARRYING THE HYBRID  
VIRULENCE-RESISTANCE PLASMID PUO-STVR2**

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The aim of the present work was to trace the epidemiological surveillance in Asturias, Spain, of an emergent type of *Salmonella* serotype Typhimurium carrying a selftransferable virulence-resistance plasmid (pUO-StVR2 of ca. 140 kb). This plasmid is a derivative of the serotype specific *spv*-plasmid (pSLT90 of 94 kb) that has gained a R-region containing a class 1 integron with the *oxa1-aadA* gene cassette configuration, in addition to other resistance determinants (Antimicrobial Agents Chemother. 46:2977-2981). Forty-six Typhimurium clinical isolates recorded in the LSP during 2001-2002, together with 22 isolates implicated in a nursery outbreak occurred in 2004 and 4 food isolates collected during 1998-2003 were *oxa1*-positive, generated amplicons of 2000 bp associated to the variable region of class 1 integrons, and contained plasmids of ca. 140 kb on which the *spv* and *oxa1* probes were located. These three features support the presence of pUO-StVR2 in the isolates, which could be then ascribed to the emergent type under surveillance. The clinical isolates recovered over 2001-2002 were discriminated into 5 *XbaI*-macrorrestriction profiles (similarity  $\geq 70\%$ ). The most frequent profile, already represented in previous years (1993-2000), was collected from meat and caused the nursery-outbreak. The pandemic Typhimurium DT104 (*pseI*+), the emergent monophasic [4,5,12,1:-]-U302 (*temI*+), and the emergent type carrying pUO-StVR2 (*oxa1*+) respectively represented 38.9, 11.1 and 19.7 % of the Typhimurium clinical isolates recorded in Asturias during 2001-2002. These data support the important role that the latter has nowadays in human salmonellosis, and suggest the horizontal transmission of pUO-StVR2 between strains differentiable by *XbaI*-PFGE.

**PHAGE TYPING AND ANTIMICROBIAL RESISTANCE IN S.TYPHIMURIUM AND S. ENTERITIDIS FROM HUMAN AND ANIMALS IN ITALY IN 2003 (ENTER-NET AND ENTER-VET SURVEILLANCE)**

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Salmonelle are widely distributed in nature. *S. Typhimurium* (STM) and *S. Enteritidis* (SE) are the most common causes of salmonellosis man. In this study we report data on phage typing and antimicrobial resistance of STM and SE isolated from human and animals during 2003 in Italy.

815 isolates of STM and SE were phage typed and tested for antimicrobial susceptibility.

In human, DT104 and DTNT (not typeable) were 28.7% and 22.1% respectively. In animals DTNT was 15.5% (82% from swine) and DT104 was 12.0% (44% from swine). Resistance to ampicillin (Am), streptomycin (S), sulfamethoxazole (Su) and tetracycline (Te) in human and animal isolates were from 50% to 100%, both in DT104 and NT, while the resistance to chloramphenicol (C) was about 100% in DT104 and from 0% to 40% (poultry) in NT. The other phage types of STM showed similar rates. SE phage types in humans and animals were PT4, PT2, PT1 and PT14b. In human, the higher resistance rates were to Su (20.6%) nalidixic acid (Na, 9.3%) and Am (6.1%). In swine resistance to Te, Na and Su were 50%, 41.7% 25.0%, respectively; in poultry resistance to Su was 36.7%, to Na 16.3%, to S 6.1% and to Te 2.0%.

In general, STM showed higher rates of resistance and multiresistance than SE. In STM, the resistance to C, associated with AmCSSuTe R-type was associated with DT 104, while the same multiresistance without C was mainly observed in DT NT. In SE, association between particular resistance and phage types were not clearly observed.

## EXPOSURE ASSESSMENT OF SALMONELLA IN PORK IN BELGIUM

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*Salmonella* is the leading cause of foodborne disease worldwide. The prevalence, the contamination level, the serotype and phage-type distribution are essential for an efficient risk assessment study. Since 1997, the Belgian zoonosis surveillance program surveys the *Salmonella* contamination in cattle, pig and poultry. Between 100 and 300 samples have been sampled each year for each matrix. Detection of *Salmonella* was carried out with the official method SP-VG-M002, consisting of a pre-enrichment into buffered peptone water followed by culture onto Diasalm. A loopful was streaked onto xylose lysine desoxycholate agar. Characteristic colonies were confirmed by biochemical and serological tests. Typhimurium isolates were phage-typed. For each pork carcass, four sites were swabbed, constituting one sample of 600cm<sup>2</sup>.

A significant and continuous lowering of *Salmonella* prevalence is observed since 2000 (17-32% in 25g in 2000). In 2003, the prevalence in pork varied from 6% (minced and cutting meat) to 15% (carcasses). Most of the isolated strains belonged to the 3 following serotypes: Typhimurium (43%), Derby (21%) and Brandenburg (11%). Between 17 and 22 % of Typhimurium isolates were characterized DT104 or closely related.

It is assumed that the rate and the level, and thus the risk, is lower for pork than for poultry.

These results should be used to take preventive measures to decrease the *Salmonella* contamination rate and to prevent cross-contamination at the retail and consumption stages. A special attention should be drawn to control DT104 Typhimurium strains, which may be associated with multi-resistance to antimicrobials used in human and veterinary medicine.

**PREVALENCE OF ANTIBODIES TO HEPATITIS E VIRUS IN VETERINARIANS  
WORKING WITH SWINE AND IN SWINE WORKERS IN ITALY.**

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Hepatitis E virus (HEV) is the causative pathogen of enterically transmitted non-A, non-B hepatitis. HEV-like viruses have been isolated in many animal species especially in pigs and the disease is thought to be a zoonosis. Reports have been published of elevated anti-HEV antibodies levels among swine veterinarians and swine workers in USA, Moldova, Taiwan and Greece. The aim of this work was to estimate the prevalence of anti-HEV antibodies in veterinarians and swine workers in Italy. 84 sera samples have been collected during the 30<sup>th</sup> meeting of Soc. Ital. Patol. e Allevamento dei Suini (SIPAS, 2004), and tested with a commercial ELISA kit for anti-HEV IgG. None of the sera tested was positive to HEV antibodies. The HEV seroprevalence in the Italian normal population is around 1-5% while, nowadays, there are no data available about the virus circulation in pigs. Because previous studies in other countries have reported of a higher anti-HEV prevalence in people working in contact with swine compared to the normal population, our different result could be explained in different ways: 1) the HEV prevalence in the Italian pig population is much lower than the prevalence in other countries so, in Italy, swine workers don't have a higher risk to get infected; 2) the HEV pig strains circulating in Italy are only scarcely infectious to human beings or, 3) the ELISA test used in this study was not enough sensitive.

## RE-EMERGENCE OF *SALMONELLA* ENTERITIDIS IN SICILY, 2002-2003

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After an upward trend paralleling that occurring in most European countries, since October 2002 serotype Enteritidis has again gained the first position among human isolates in Sicily. The aim of this study was to assess epidemiological features of *S. Enteritidis* in Sicily during this re-emergence period, by applying a multiple typing approach on a sample of fifty isolates. Fourteen isolates were recovered from eggs and layer hens; five human isolates were from epidemiologically defined outbreaks and the remaining 31 were from apparently sporadic isolates.

Phage typing differentiated the 50 isolates into nine types. The most common were PT6 and PT4 (17 and 15 isolates, respectively). PT8 and PT6a were found in 4 and 3 human isolates.

*BlnI* PFGE analysis identified six pulsotypes: the predominant B2 was found in 31 isolates, whilst type B3 was detected in six isolates and the remaining types only in three or less isolates.

Seven different plasmid profiles were found: 31 isolates harbored a single plasmid of approximately 38 MDal. Plasmids of 4.2 and 4.0 MDal were present only in PT6 strains, while plasmids of 33 and 70 MDal were identified in PT6a strains.

Results of the three typing techniques subdivided the isolates under study into 17 combination profiles.

The presence of low molecular size plasmids has proved to be the only specific marker of PT6 strains in our geographical area. Emergence of PT6 strains phenotypically distinct, but genetically closely related to PT4 strains, seems the key attribute of the resurgence of *S. Enteritidis* in Sicily.

## SESSION 3

# **The role of the environment in the transmission of food-borne zoonotic pathogens inside and outside the farm**

ORAL PRESENTATIONS

# THE FATE OF FOOD-BORNE PATHOGENS IN ANIMAL WASTES

**G. Duffy**

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Animal wastes and effluents from farming operations including manures and slurries, are frequently applied as fertilizer to land used for crop or silage production and for animal grazing. It is well documented that many potentially harmful pathogens are shed in animal faeces and there is growing concern about the number of sporadic and outbreak cases of illness linked to zoonotic pathogen as a result of direct contact with faecal material. Infection may occur either as a result of handling contaminated mud in fields or from ingestion of produce grown in contaminated manures or slurries. A range of pathogens including Verocytotoxigenic *E. coli*, *Campylobacter*, *Salmonella* and *Cryptosporidium parvum* have been detected in the faeces of ruminant and non ruminant farmed animals, wild animals, domestic pets and birds. Many of these pathogens are well adapted to survive in animal faeces and can persist for extended periods ranging from several weeks to many months. This persistence makes animal waste important as a potential vehicle for transmission within herds, farms, the fresh food chain, and the wider environment. Appropriate handling of animal waste is necessary to control the spread of pathogen and to limit the significant risks of human infection. It may be necessary to hold manure/slurry for extended periods prior to spreading on farmland, or for use in the production of food crops, particularly foods that are to be consumed in the raw or minimally processed state. Alternatively, it may be necessary to apply processes such as composting, heat drying or digestion which can expedite the decline of pathogens in animal wastes. However there is also a need for research work to develop economical and practical systems for treatment of manures and slurries. The risk from direct contact with faecal material at farms and petting zoos is also recognised and many public health authorities have put forward measures for strict practices to limit the risk of infection particularly for young children visiting these environments.

# EMERGING CAMPYLOBACTERIACEAE IN THE FOOD AND WATER CHAIN: THE CAMPYCHECK PROJECT

**B. Keevil**

(School of Biological Sciences, University of Southampton, UK)

*Campylobacter jejuni* is the major cause of human gastro-enteritis worldwide, responsible for 400 to 500 million cases of diarrhoea each year. *C. jejuni* is excreted in the faeces of infected animals and humans. It can therefore be transmitted from animal to person, through ingestion of faecally contaminated water or food, or by direct contact with contaminated environmental surfaces. Recent research in the UK, USA and elsewhere suggests true infection rates of Campylobacteriosis are probably at least 10 times higher than currently reported, questioning whether *C. jejuni* alone is responsible and whether current detection methods are adequate.

Although the presence of new *Campylobacteraceae* was demonstrated over 10 years ago, very few substantial studies have been published to determine the true prevalence of these new species in the clinical environment. Until formal studies are established using methods capable of isolating and identifying these bacteria the extent of their clinical relevance will remain an unknown. Even when the clinical picture has been addressed there is hardly any information on the environmental or animal reservoirs that harbour these new *Campylobacteraceae*. Indeed the approved isolation procedures across Europe and elsewhere are designed to primarily isolate thermo-tolerant *Campylobacter spp.* such as *C. jejuni* and *C. coli*, and are known not to isolate these emerging *Campylobacteraceae*. The aim of this CAMPYCHECK research project is to address the limitations of current isolation and identification methods and establish the prevalence of these microorganisms in patient and animal faeces and the food and water chain in three different continents.

## **SALMONELLA SURVIVAL IN AMOEBA FROM CATTLE SLURRY**

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Denmark)

*Salmonella* often encounter an extra animal stage during transmission from one animal to another. During this stage, e.g. in dung or slurry, the bacterium may be taken up by protozoa present in the environment. In this study we have examined the capability of strains of *S. Typhimurium* (Stm) and *S. Dublin* (Sdu) to survive inside amoeba of *Naegleria* spp. isolated from cattle slurry. Both strains readily infected the amoeba. After 4 hours, Stm dropped by almost 3 log<sub>10</sub> units but then increased by approx. 2 log<sub>10</sub> units after 24 hours, showing that Stm can multiply inside the amoeba. The Sdu strains only dropped 2 log<sub>10</sub> units after 4 hours and remained at this level for 48 hours with out signs of growth. Since motility genes have been shown to be important for survival of *L. pneumonia* inside protozoa, we constructed motility and chemotaxis mutants of Stm and Sdu. Mutation of flagella genes and chemotaxis genes *cheA* and *cheR* resulted in decreased invasion (4 h) in Stm. After 24 hours the *cheA* mutant had grown to wild type level, while *cheR* and *fljB* mutants showed decreased levels after 48 hours. All chemotaxis and motility mutants of Sdu showed reduced invasion and survival compared to the wild type strain.

## **PREVALENCE OF *CLOSTRIDIUM BOTULINUM* IN FOODS AND ENVIRONMENTAL SAMPLES**

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*C. botulinum* surveys in fish have been performed in Nordic countries, but few data are available on its prevalence in environmental and food samples from other European countries. Information on the prevalence of *C. botulinum* in the environment and food is of critical importance for an assessment of botulism hazards. In a coastal area of the Northern France, near the Canche river estuary, a severe outbreak of wild avian botulism related to type E occurred in 1996, suggesting a potentially high local prevalence of *C. botulinum* and fish products as a possible source of contamination. The objective of our study was to investigate the prevalence of *C. botulinum* in this area and to perform a survey on food raw materials used in refrigerated processed foods of extended durability (REPFEDs) manufactured in France which are becoming increasingly popular in Europe, showing for instance a 7% increase in production in France between 2000 and 2001.

*C. botulinum* types A, B, E and F investigations in fish and environmental samples from Northern France and food raw materials used in REPFEDs were carried out with a new PCR-Enzyme-linked Immunosorbent assay (PCR-ELISA) and compared with the standard method of mouse bioassay. This PCR technique based on identification of the most conserved region of *bont* genes permits the simultaneous detection of *C. botulinum* A, B, E and F.

During a first survey, the prevalence of *C. botulinum* types A, B, E, F was determined in 214 fresh fish and environmental samples collected in Northern France. The prevalence of *C. botulinum* in seawater fish and sediment was respectively 16.6% and 4%, corresponding to respectively 3.5-7 *C. botulinum* MPN/kg and 1-2 *C. botulinum* MPN/kg and is in the low range of *C. botulinum* contamination reported elsewhere. The toxin type identification of the 31 naturally contaminated samples was 71% type B, 22.5% type A and 9.6% type E. Type F was not detected. The high prevalence of *C. botulinum* type B in fish samples is relatively exceptional, compared with the high prevalence of *C. botulinum* type E reported in many worldwide and North Europe surveys.

However, fish processing and fish preparation in France have not been identified as a significant hazard for human type B botulism.

The second survey was performed with food raw materials (meat, fish and other ingredients) used in REPFEDs manufactured in France. Portions of 25 g to 50 g of food were analysed. Eight out of the 102 samples of fish and shellfish, 12 out of the 143 samples of meat and poultry , 1 out of the 62 samples of aroma, sauce and gravy , 4 out of the 25 samples of thickening agents , 3 out of the 26 samples of dehydrated dairy ingredients, and none of the 65 samples of spices, herbs and dehydrated mushroom were positive for *C. botulinum* in PCR-ELISA, i.e., 6.6 % of all the samples tested. The 28 positive samples comprised 10 type A, 10 type B, 4 with both types A and B, and 4 undetermined by PCR-typing. No sample positive for type E was detected. Of the 28 samples positive in PCR-ELISA, 15 were also positive in the mouse bioassay. The MPN count was between 1 to 3 *C. botulinum*/kg of food, which is similar to or in the lower range of values reported in the literature. Consequently, despite possible contamination, the risk of *C. botulinum* poisoning in REPFEDs is probably low if the products are produced, distributed, retailed and stored under a strict control of refrigeration temperature for one month, the shelf-life commonly displayed for REPFEDs in France.

# IS IT POSSIBLE TO ESTIMATE PREVALENCE OF VTEC O157 BY SAMPLING MANURE ?

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Verotoxin producing *Escherichia coli* O157:H7 can persist for prolonged periods in cattle manure and cattle manure slurry. This constitutes a major problem, as the spread of manure may act as a mean of pathogen dissemination. On the other hand, this persistence could be used to screen for presence of VTEC O157 on cattle farms. The aim of the study presented here was to elucidate whether cattle manure slurry could be used to determine prevalence of VTEC O157 on herd level.

In 1999, 125 dairy cattle farms, randomly selected from all over Sweden, were sampled by rectal fecal sampling and by one manure slurry sample each. 16 of the dairy cattle herds were found positive for VTEC O157 after rectal fecal samples, and 9 of these also by the manure samples. One farm was found positive by the manure sample only. This study indicated that half of the farms would be found positive with this more simple way of sampling. All isolates were examined by PFGE, and it was shown that strains from rectal fecal samples and strains from manure slurry from the same cattle farm were identical or very similar.

In 2001, manure slurry samples were collected from dairy cattle farms in a limited part of Sweden. In total 585 farms were sampled, of which 13(2,2%) were found positive for VTEC O157. The diverging results of the two studies will be discussed.

**STAPHYLOCOCCUS AUREUS ISOLATES IN POSSESSION OF PYROGENIC TOXIN SUPERANTIGENS AND RECOVERED FROM HUMAN NASAL SAMPLES AND MANUALLY HANDLED FOODS FELL INTO THE SAME GENOMIC GROUPINGS**

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The genetic relationships between *Staphylococcus aureus* recovered from nasal samples of healthy people (42 human-isolates) and manually handled foods (34 food-isolates) over 1996-2002 in Asturias, Spain, all of them in possession of some classical pyrogenic toxin superantigen (enterotoxins SEA to SED and/or toxic shock syndrome TSST-1) were established.

Human- and food-isolates were respectively discriminated into nine toxin-genotypes (six - *sea*, *sea-tst*, *tst*, *seb*, *sec*, and *sed*- common to both groups); 36 *Sma*I-genomic profiles (3 common), and 15 *Eco*RI-plasmid profiles (two common as well as plasmid-free). *Sma*I-genomic profiles with a Dice's similarity coefficient  $\geq 0.7$  were included into a genomic-grouping or lineage. Eight lineages were differentiated, six of them included both human- and food-isolates, and two of these included outbreak-implicated isolates. A strong relationship between toxin-genotypes with both *Sma*I-lineages and *Eco*RI-plasmid profiles was revealed. Two lineages, one related with *tst* or *tst-sea* and the other with *sec* or *sec-sed* genotypes, were the most frequent and could be considered as endemic, but only the second was outbreak-related. When *Sma*I-genomic and *Eco*RI-plasmid profiles were hybridized with toxin-probes it was observed that each probe mapped only on distinctive *Sma*I-fragments a part of *sed* that also mapped on three plasmid. When *sej*- and *ser*-probes were included, they mapped together with *sed* on the chromosome (*Sma*I-fragment of ca. 320 kb) and on plasmids of three sizes (33, 36 and 53.5 kb). The plasmid (ca. 53.5 kb) carrying *sed-sej* and *ser*-like and the chromosomal location of *sed-sej-ser* are findings of new description.

## SESSION 3

# **The role of the environment in the transmission of food-borne zoonotic pathogens inside and outside the farm**

POSTER PRESENTATIONS

# HORIZONTAL TRANSMISSION OF *ESCHERICHIA COLI* O157:H7 DURING CATTLE HOUSING

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*E. coli* O157:H7 is an important zoonotic pathogen as it can cause hemorrhagic colitis and hemolytic uremic syndrome in susceptible humans. Ruminant livestock, in particular cattle, are considered the primary reservoir for this organism. This study examined the transmission of *E. coli* O157:H7 within groups of cattle during winter housing. Holstein Friesian steers were grouped in 6 pens of 5 animals. A diet of ad-lib grass silage was fed to 3 pens and ad-lib concentrates to 3 pens. An animal inoculated with and known to be shedding a marked strain of *E. coli* O157:H7 was introduced into each pen at Time 0. Faecal, hide and environmental samples were taken regularly over a 23-day period. CCTV cameras were installed for a 24-hour period. Within 48 hrs *E. coli* O157:H7 was cultured from the hides of 20 of 30 cohort animals. The faeces of cohort animals, pen and water samples were positive within 3 days. Positive hide and faecal samples were obtained up to day 23. There was no difference between groups on different diets. Camera footage from each pen showed on average 13 instances of cattle grooming per hour. The study suggests that transmission of *E. coli* O157:H7 between animals may occur following ingestion of the pathogen at low levels and that the hide may be an important source of transmission.

## SESSION 4

# **Poultry as a source of emerging zoonosis**

## ORAL PRESENTATIONS

# AVIAN RESERVOIRS OF EPSILOBACTERIA (ARCOBACTER, CAMPYLOBACTER, HELICOBACTER): A PUBLIC HEALTH PERSPECTIVE

**S.L. On**

(Danish Institute for Food and Veterinary Research, Copenhagen, Denmark)

The class Epsilonobacteria represents a distinct phylogenetic lineage of Gram-negative bacteria that includes the genera *Campylobacter*, *Arcobacter*, and *Helicobacter*, among others. There are more than 70 taxa within the group and approximately 50 of these are known or suspected pathogens, causing a wide range of diseases in humans, animals, or both. Certain zoonotic species are known to be frequent causes of human gastroenteritis and the clinical and economic significance of the bacteria should not be underestimated.

Although Epsilonobacteria as a whole may be found in almost every animal species as normal flora, surprisingly few taxa seem to occur in birds. Whether or not this is scientifically factual, or simply a consequence of the problems in recovering these fastidious organisms from samples, is debatable. However, the importance of avian species as sources of human Epsilonobacterial infection is significantly illustrated by *C. jejuni*, the most commonly reported human gastrointestinal pathogen worldwide. Both poultry and wild birds appear to be sources of human disease, although it is highly likely that not all strains of *C. jejuni* pose the same level of risk to human health. The contribution of other Epsilonobacteria such as *A. butzleri*, *H. canadensis* and *H. pullorum* to human disease is largely unknown, a consequence of inadequate isolation and identification procedures. Nonetheless, specific studies clearly suggest these taxa are frequently carried by poultry or wild birds and thus may represent at least as great a risk to human health as *C. jejuni*.

In this talk, topical aspects of the taxonomy, detection, identification, prevalence, epidemiology and public health significance of the avian Epsilonobacterial species will be discussed.

## AVIAN BOTULISM IN FRANCE

**G. Salvat**

(AFSSA, Ploufragan, France)

Botulism in mammals and birds is associated with one of the antigenic type (A to G) of the most powerful toxin known, botulinic toxin. Botulism among human is a rare foodborne disease in France and its prevalence is constant in human representing c.a. less than 20 cases/year, most of them associated with type B toxin. The main sources of botulism among humans are home-made vegetables canned food and raw cured sausages and ham. These traditional sources are decreasing, while sea food associated cases (typeE toxin) are increasing.

Clinical botulism among birds (mainly broilers and turkeys) is most of the time due to type C and D and regular cases (c. a. 20 cases/year) were noticed for 15 years in western part of France. The first case of type E botulism in avian species appeared in 1997. Whatever the type of toxin, mortality rate ranges from 4% to 100% but is high most of the time. The main sources of poultry contamination are dead or healthy carrier animals (birds, rodents, insects...), feces, environment feed and water. Clinical symptoms associated with avian Botulism is flaccid paralysis. Death occurred after a long agony and is associated with the paralysis of respiratory and cardiac muscles. The risk of human botulism associated with the consumption of poultry meat is weak in France. Despite that, food-borne cases of botulism associated with poultry meat cooked sausages were described in Morocco and in France (2003). The origin of *C.botulinum* type B that were responsible for the French case was not completely elucidated. Heavily contaminated raw poultry meat and spices combined with low cooking temperature and lack of refrigeration during the hot 2003 summer were certainly responsible for the 3 human cases reported in this outbreak.

Reference : AFSSA, 2002. Rapport sur le Botulisme d'origine aviaire et bovine. 82 pages.  
[www.afssa.fr](http://www.afssa.fr)

## ***HELICOBACTER PULLORUM*, AN EMERGING ZOOONOTIC PATHOGEN?**

**L. Ceelen**

(Department of Pathology, Bacteriology and Avian Diseases, Faculty of Medicine Science, Ghent University, Belgium)

*Helicobacter pullorum* has been detected in the caecum and on the carcass of broiler chickens, in the liver and the intestine of laying hens and in the faeces and biliary tree of humans. This species has been associated with avian hepatitis and enteritis and with diarrhoea, gastroenteritis and liver diseases in human beings. Broilers are speculated to be the source of infection for humans, due to carcass contamination with intestinal contents in the abattoir. To date, hardly any data are available on the actual prevalence of this organism in poultry and humans. In addition, very few information about the antibiotic susceptibility and potential virulence markers of *H. pullorum* has been published so far.

First, a study on the prevalence of *H. pullorum* was performed, involving intestinal and liver samples of 110 broilers from 11 different flocks, and 531 faecal samples of human gastrointestinal patients. PCR revealed 34% of the broilers and 4.3% of the gastrointestinal patients were found positive for *H. pullorum*.

Secondly, the *in vitro* susceptibility of *H. pullorum* isolates to different antimicrobial agents was examined. Acquired resistance was only detected to spectinomycin. The other tested antibiotics showed a monomodal distribution of minimum inhibitory concentrations reflecting normal susceptibility levels of this species.

Finally, the production of cytolethal distending toxin as possible virulence trait and the presence of the *cdtB* gene encoding the active subunit of this protein were studied. All 14 tested isolates harboured the *cdtB* gene, but biological activity typical for CDT was only demonstrated in one *H. pullorum* strain under the circumstances adopted in this study.

## ARCOBACTER, AN IGNORED FOODBORNE PATHOGEN

**K. Houf**

(Department Veterinary Public Health, Ghent University, Belgium)

The first reports of *Arcobacter* isolation go back to 1977, in which aerotolerant campylobacters were associated with bovine and porcine abortion. At present, the genus *Arcobacter* comprises four species and is classified as a second genus within the family *Campylobacteracea*. Arcobacters differ from the closely related *Campylobacter* species by their ability to grow at lower temperatures and in air. During the past decade, improvements in isolation (4) and identification techniques (6) have led to the discovery of *Arcobacter* species as both human and animal pathogens. Two species, *A. butzleri* and, more rarely, *A. cryaerophilus*, have been associated with enteritis and occasionally bacteremia in humans (9). Patients with *A. butzleri* infections report diarrhoea associated with abdominal pain, nausea and vomiting or fever also occur (9). A third species, *A. skirrowii*, has recently been isolated from a person with chronic diarrhoea (10). During an 8-year study, arcobacters were isolated from 77 patients. In 82.1% of the patients, *Arcobacter butzleri* was the only pathogen isolated in the stool samples (9).

The three human associated species have also been incriminated with animal illness such as reproduction disorders, mastitis and gastric ulcers in farm animals, but have also been isolated from healthy livestock (7).

Arcobacters are common on food of animal origin, and food is therefore considered as one of the main sources of *Arcobacter* to humans. Food contamination is assumed to occur by faecal contamination during slaughter (2,4). Especially poultry products are commonly contaminated with arcobacters on the surfaces (1,2,3). Contamination levels as high as several thousands cells per gram skin or meat are detected (2). In contrast, in a recent study, no arcobacters were recovered from 120 cloacal swabs in contrast to 100% contamination of neck skin samples of the same flock (data not published). These findings suggest that arcobacters may not belong to the natural poultry flora, and that the chicken carcass contamination must have another yet unknown source.

Arcobacters have also been isolated from pork and beef samples, but in contrast to the chicken, *Arcobacter* has been frequently isolated from porcine and bovine faeces (7,8). In a recent study, 55 of 294 examined pigs had a bacterial load of  $10^2$  to  $10^4$  cfu/g faeces and another 66 animals excreted less than  $10^2$  cfu/g faeces. *A. butzleri* was the most frequently occurring species, but co-colonisation was not uncommon (7). Of 276 cows examined, 4 had a bacterial load of more

than  $10^2$  cfu/g faeces and low levels were detected in 26 animals using enrichment. *A. cryaerophilus* was the most dominant species in cows (data not published). The prevalence and colonisation levels in bovine faeces turned out to be far below those in porcine faecal samples (7,8).

Diarrhoea caused by members of the *Campylobacteraceae*, and *Arcobacter* in particular, is presumed to be a self-limiting disease, though severity or duration of symptoms may necessitate antibacterial therapy. When an antibiotic is recommended for treatment, the most commonly prescribed drugs are erythromycin or a fluoroquinolone such as ciprofloxacin (5). Tetracycline, doxycycline, and gentamicin are sometimes listed as alternative drugs for treatment.

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## **CAMPYLOBACTER IN NORVEGIAN POULTRY A DISAPPEARING PROBLEM?**

**M. Hofshagen**

(Norwegian Zoonosis Centre, National Veterinary Institute, Norway)

Campylobacteriosis is the most commonly reported bacterial gastroenteritis in humans in Norway, and consumption of poultry meat purchased raw has been identified as a significant risk factor. The action plan against *Campylobacter* spp. in Norwegian broilers was implemented in May 2001, with the objective to reduce human exposure to *Campylobacter* spp. through Norwegian broiler meat products. The action plan is a joint effort involving Governmental agencies, academia, and the poultry industry, and is coordinated by the Norwegian Zoonosis Centre.

The action plan consists of three parts; a surveillance program including all Norwegian broiler flocks slaughtered before 50 days of age, a survey of broiler meat products, and a follow-up advisory service on farms with flocks positive for *Campylobacter* spp. In the surveillance, pre-slaughter sampling of the flock is performed eight to four days before slaughter. Positive flocks are slaughtered at the end of the day, and the carcasses from these flocks are either heat treated or frozen before being marketed. All flocks are retested at the slaughter plant.

Results from the start of the action plan in 2001 and up to October 2004 will be presented. There has been a significant reduction in the proportion of positive flocks from 2002 onwards. This reduction can partly be attributed to the advisory service and general improvement of hygienic practices in the Norwegian poultry industry. There are also indications of a positive public health effect.

# **ZOONOSIS CONTROL WITHIN THE SCOPE OF THE AUSTRIAN POULTRY HEALTH SERVICE (GGD)**

**H. Schliessnig, S. Weber, J. Köfer**  
(Austrian Poultry Health Service, Austria)

## **Introduction and objectives:**

### **Reduction of Salmonella contamination in poultry production:**

The heart of the poultry data pool (GDV) is an internet application based on an Oracle database. Salmonella outbreaks in poultry farms can thus be isolated quickly and efficiently. The GDV automatically sends all electronically signed test results to veterinarians, veterinary officers and farms by fax or e-mail. The Poultry Health Service (GGD) was thus able to quickly detect an outbreak of *S. enteritidis* in broiler flocks in 2003 and to eliminate the source of phage type 21 (foreign hatching eggs).

### **Risk management and assessment in human medicine:**

Most human Salmonella infections in Austria are caused by table eggs. The GGD thus uses the database in cooperation with the Salmonella Centre in Graz in order to trace the cause of such infections. These efforts are further supported by egg labelling regulations and the official laying hen register. If sampling procedures in suspect farms produce positive results, a suspension of deliveries is ordered by the competent authorities.

### **SUPPLY CHAIN DOCUMENTATION FOR TABLE EGGS**

The traceability for egg packing facilities (Regulation (EC) No 178/2002) is implemented in the GDV by a separate supply chain documentation program (Egg-Web). This allows individual batches to be recalled in the case of contaminated eggs. The GGD thus covers all areas from farm to fork.

### **DATABASE EVALUATIONS FOR AUTHORITIES BY THE GGD**

The GDV provides the basis for a wide range of evaluations.

The zoonosis report as well as drug and vaccine administration are documented and can be evaluated.

# CONTAMINATION LEVEL OF CAMPYLOBACTER IN ITALIAN POULTRY CARCASSES

**V. Bondioli, A. De Cesare, G. Manfreda**

(Department of Food Science, Alma Mater Studiorum-University of Bologna, Italy)

It is generally assumed that Campylobacter contaminates poultry meat during processing, surviving throughout the food chain supply to constitute a risk for human health.

The aims of this study were (1) to use a direct plating method to quantify Campylobacter in fully processed broiler carcasses of different weight, sampled after the air-cooling operation; (2) to evaluate if there is a seasonal variation in the prevalence of Campylobacter in Italian poultry flocks; (3) to estimate the contamination level trends in three farms tested along one year.

More than two hundred broiler carcass rinses were obtained from one Italian slaughterhouse. During each sampling date, ten carcasses from the same flock with a mean weight of 3.5 kg and ten carcasses from a different flock with a mean weight of 2.5 kg were placed into sterile plastic bags and rinsed with sterile water then spread plated on Campy-Cefex agar plates. The 3.5 kg carcass flocks were always reared in three different farms tested four times along a one year period. The 2.5 carcass flocks were reared in farms randomly selected.

Any statistically significant difference ( $P < 0.05$ ) was observed between the Campylobacter contamination levels detected in carcasses with mean weight of 3.5 and 2.5 kg (5.06 *vs* 5.25 Log CFU/carcass). Independently from the carcass weight, the Campylobacter loads were statistically significant lower in winter and spring than in autumn (5.03 and 5.06 Log CFU/carcass *vs* 5.39 Log CFU/carcass) whereas any statistically significant difference was observed with the loads registered during the summer period. In relation to the Campylobacter contamination in the 3.5 kg carcasses, statistically significant differences were observed between the Campylobacter loads in the carcasses reared in the three farms tested and between the loads detected in the same farm along the year.

## SESSION 4

# **Poultry as a source of emerging zoonosis**

## POSTER PRESENTATIONS

# TYPING OF CAMPYLOBACTER JEJUNI STRAINS OF POULTRY AND HUMAN ORIGIN

**I. Steinhauserova, M. Nebola, M. Mikulicova**

(University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic)

Campylobacter jejuni and C. coli are among the commonest causes of acute bacterial enteritic diseases in humans. Campylobacter sp. findings are very frequent in the environment of chickens slaughters and chicken farms. The aim of study has been typing of strains of Campylobacter jejuni isolated from slaughtered poultry from various chicken farms by using PFGE method.

PFGE analysis. Lysis of harvested and washed bacteria was performed in 1% agarose blocks with 1mg/ml proteinase K in ESP buffer. The lysed agarose blocks were equilibrated in restriction enzyme reaction buffer, and the consequent DNA digestion with SmaI was performed for 5 h at 30°C. For PFGE a Bio-Rad CHEF-DR III apparatus was used.

Results and discussion. The PCR assay was based on primers specific for 23S rRNA to differentiate thermophilic Campylobacter spp. C. jejuni clones were further subtyped by flagellin PCR/RFLP. AfaI, MboI and HaeIII restriction length polymorphisms showed 19 subclone genotypes. In human isolates from patients with gastroenteritis 26 subtypes were also found. Four subclones (1, 4, 8, 15,) with the highest frequency both in human and poultry samples were further characterized by PFGE analyses with the restriction endonuclease SmaI. The findings of poultry isolates was that PFGE genotypes within some flock with the same PCR subtype were identical and considerably different between distinct PCR subtypes. In no case identical PFGE but different flagellin PCR subtypes were found. None of these poultry genotypes were identical with human SmaI-defined subtypes.

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