5th World Congress
Foodborne Infections and Intoxications

- Abstracts -

7 - 11 June 2004
Berlin, Germany

Federal Institute for Risk Assessment

FAO/WHO Collaborating Centre for Research and Training in Food Hygiene and Zoonoses

5th World Congress
Foodborne Infections and Intoxications

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Under the auspices of

Renate Künast

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5. Weltkongress
Lebensmittelinfektionen und -intoxikationen

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Unter der Schirmherrschaft von

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F-01

Securing food safety - the role of risk assessment in an expanding free trade zone

Podger, G.
European Food Safety Authority (EFSA), Brussels, Belgium

F-02

"Securing food safety - adjusting the means to match the requirements of the developed and the abilities of the developing countries " (requested)

Schlundt, J.
World Health Organization, Genf

Keynotes

K-A01

Monitoring and control of zoonoses – the food chain approach

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Many foodborne zoonoses are prevented and controlled through the application of HACCP or HACCP-like strategies in the post-harvest stage of food production, whereas little, or nothing at all, is done to control zoonoses in the pre-harvest stage (on the farm). For some foodborne zoonoses this post-harvest approach may suffice, whereas for others it is not sufficient to provide adequate and sustainable public health protection.

The added benefit of including pre-harvest monitoring and control is well documented for some foodborne zoonoses. *Salmonella* can be effectively controlled in primary slaughter poultry production as well as in laying hens. The objective of pre-harvest control may vary from elimination to reduction, and the means of control will have to be chosen accordingly. *Salmonella* can be effectively reduced and/or kept at a low rate in primary pig production, through the combination of monitoring and specific interventions to prevent propagation and spread.

The global incidence of Campylobacteriosis has been increasing in the nineties, probably due to the increased marketing of fresh poultry products. There is growing evidence from a number of Nordic countries that the introduction of *Campylobacter* into poultry houses can be prevented, under some circumstances, which permits the production of *Campylobacter*-free flocks of chicken, and consequently *Campylobacter*-free poultry products.

Preventing the emergence and spread of antimicrobial resistant foodborne pathogens, such as quinolone-resistant *Campylobacter* and *Salmonella*, requires intervention on the farm. Avoiding misuse and overuse of antibiotics, and choosing older narrow spectrum classes of antibiotics over modern extended spectrum antimicrobials in food animal production are important elements in prevention and control of antimicrobial resistant pathogens in the food supply.

There is a need to develop and implement quality assurance systems, equivalent to the HACCP systems in food industry, that can be applied in primary food production. There is furthermore a need for the veterinarians to begin actively promoting food safety on the farm, and to expand the traditional “fire brigade-like” role of the veterinarian responding to outbreaks of clinical disease.

It is important to further develop and implement testing and sorting systems, where information about the hygienic quality of animals delivered for slaughter can be used by food processors to optimise food safety. Testing and certification systems in primary production will motivate producers to accept their role in food safety assurance.

In general, food safety has to be considered in an integrated food chain-approach, which involves testing and quality assurance in all stages of production. In addition to implementation of modern diagnostic tools for monitoring and modern principles of production hygiene and quality assurance, improved emphasis should be given to the “human factor”. It is well documented that it is not always a lack of knowledge, but also lack of compliance with known and accepted procedures (e.g. hand hygiene), that compromises food safety. Behavioural science is an important element in food safety research.

K-A02

Food-borne viruses in Europe

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Noroviruses are among the most common causes of community-acquired gastro-enteritis in persons of all age groups, and a common cause of outbreaks of vomiting and diarrhoea in people in close confinement, seen in hospitals, nursing homes, schools, and cruise ships. Noroviruses belong to a separate genus within the family *Caliciviridae*. The noroviruses are non-enveloped RNA viruses, that are resistant to various adverse conditions outside the host, and are extremely infectious. Virus is transmitted from person to person via fecal material or vomit, which may contaminate food and water and the environment. Noroviruses have been found in food animals (cattle and pigs) and veterinarians have increased prevalence of antibodies to these animal viruses, suggesting that zooonotic infections occur.

The increasing use of molecular detection and typing methods is providing better insight in the epidemiology of noroviruses. Noroviruses now are known to be extremely diverse, with several distinct lineages – called genotypes- co-circulating in the community. The use of molecular epidemiology to trace noroviruses has been used in a European collaborative research project aimed at elucidating the importance of food-borne transmission in the epidemiology of noroviruses. The lessons learnt from this project will be presented, including unpublished data. The results and their implications for other (emerging) food-borne viruses will be discussed.

K-A03

Modern tools for detecting and investigating outbreaks of foodborne infections

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Public health investigation of foodborne outbreaks is a common and important activity. Public health officials investigate outbreaks to control an immediate hazard, to identify new threats as they emerge, and to learn how they can be prevented. Research agendas stimulated by the findings of outbreak investigations have been a major impetus to improve food safety. The nature of the outbreaks that are detected, and the speed with which they are investigated and controlled, depends very much on the methods that are used. The combination of molecular subtyping and field epidemiology is transforming the detection and investigation of many outbreaks.

Beginning in 1996, we have developed and implemented a new strategy of public health surveillance for bacterial foodborne infections in the United States, PulseNet. Our network of public health laboratories conducts routine subtyping of clinical isolates using pulsed field gel electrophoresis (PFGE). Via PulseNet, the standardized PFGE patterns of a growing list of pathogens can be compared via the internet with the national database, and with the current observations in other States. With rapid typing and comparison, PulseNet identifies clusters of infections with the same PFGE pattern.

When such a cluster is detected, public health epidemiologists and microbiologists in all 50 states and Canada are alerted to look for more cases of the same pattern. Epidemiologists interview the cases to identify food exposures that may be similar, and may launch a rapid case-control investigation may occur. If a specific food exposure associated with illness is identified, its source is traced, and the combination of epidemiologic and laboratory information can be powerful enough that regulatory actions are taken rapidly even if the organism has not been recovered from the implicated food. The pattern is shared with food regulatory agencies, who use the same method in their laboratories to characterize isolates from animals and foods. Thus, PulseNet improves both the detection and the investigation of outbreaks. In 2003, 18,208 patterns from *Salmonella*, 4,488 patterns from *E. coli* O157:H7, and 1,535 patterns from *Listeria* were characterized in PulseNet; a total of 104 clusters were further investigated.

The classic foodborne outbreak affects many people in a single location, and may be the result of a local foodhandling error. The Anew paradigm@ outbreaks revealed by subtype-based surveillance are often widely dispersed, with few cases in any one location, and would have been missed entirely without subtyping. The outbreak may be the result of contamination early in food production and preparation, well before final food handling. Identifying them and investigating them involves collaboration across jurisdictions. The result of thorough multidisciplinary investigation may identify problems that affect the entire industry, and correcting them can make the entire food supply safer.

Recent investigations of clusters of *E. coli* O157:H7 infection, *Salmonella* infections and of *Listeria* infections in the United States have identified food industry challenges that require more better prevention strategies. In the future, application of comparable methods in Asia, Latin America and Europe will make it easier to identify clusters with international scope, and collaborative international epidemiological investigations can lead to better food safety for all.

K-A04

Network and epidemiological surveillance of foodborne diseases

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Foodborne disease (FBD) surveillance is essential to characterize the epidemiological dynamics and directing the planning of control and prevention strategies and policies. Also, it is an important tool for assessing the impact of the food safety programs and identifying the areas requiring urgent investigation, particularly at the local level. Furthermore, FBD surveillance is the basis to conceive national strategies to reduce the risk related to the consumption of contaminated food. Therefore, the current scarcity of reliable data on FBD outbreaks (less than 11,000 reported to the Regional Information System on FBD - SIRVETA, in the last 10 years) in the countries of the Americas (WHO/AMRO Region) is the biggest obstacle to carry out preventative interventions based on information. The main cause of the lack of information is that, almost all counties are collecting and reporting syndromic data (e.g., diarrhea or food poisoning), and in almost all cases, there is no formal laboratory-based surveillance.

PAHO/WHO is promoting the use of an integrated food-chain surveillance (IFCS) which consists on the collection, analysis, and interpretation of data from animals, food, and humans. IFCS allows the attribution of burden of illness to specific food categories through the use of detailed information from monitoring food and animals. One of the strategic action lines used is to promote IFCS by networking. There are three important networks in which PAHO/WHO is promoting and supporting countries: the Inter-American Network of Food Analysis Laboratories - INFAL, presently integrated by 54 laboratories from 28 countries, with the general objectives of (a) achieving the methodological equivalence of the food analysis laboratories and (b) promoting the implementation of equivalent systems of quality management. INFAL is promoting integration of the laboratories into the national programs of food safety and epidemiological surveillance. The second network is the WHO-GLOBAL SALM SURV (all countries of the Region), which has the objective of strengthening the capacities of the participating countries in the surveillance and response systems and to contribute to the global effort of containment of antimicrobial resistance of foodborne pathogens. PAHO/WHO supports the regional reference centers and also participates in the training activities. Finally, PAHO/WHO has established, in a joint effort with the U.S. Centers for Disease Control and Prevention and the National Institute of Infectious Diseases from Argentina the PulseNet for Latin-America. The aim of the network is to strengthen the surveillance of FBD in Latin America based on biological molecular techniques.

It is clear to PAHO/WHO that networking actively improves our efforts to strengthen the capacity in countries for surveillance and laboratory confirmation of FBD through provision of training, guidelines and standards, reagents, external quality assurance, advice and follow-up.

K-A05

The impact of feeds on the safety of foods

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Introduction

Food security and food safety are key elements of health and well being of mankind. Food security means to have sufficient food; food safety means freedom or minimal content of undesirable substances in food coming from natural or anthropogenic origin. Animal nutritionists consider all the contaminants along the food chain (soil-plant-feed-animal-food of animal origin). The paper deals with the influence of feeds on the safety of foods.

Grouping of contaminants/undesirable substances

Recently SCAN (2003) distinguished four categories of undesirable substances:

- Ions and elements (incl. NO_3, heavy metals etc.)
- Mycotoxins and products of microorganisms
- Organic contaminants (like PCB's, dioxins)
- Botanic impurities (plants with toxic substances)

D'MELLO (2003) distinguished in biotoxins (plant toxins, bacterial pathogens und toxins, shellfish toxins, mycotoxins) and anthropogenic contaminants (pesticides, PCB, dioxins, heavy metals, N-compounds, veterinary drugs, prions, radionuclides etc.). Apart from real contaminations food safety is in the public also associated with the introduction of novel feeds incl. feeds from genetically modified organisms in the food chain (EFSA 2004) and with so-called scandals and further uncertainties. Furthermore mistakes in animal feeding may also influence safety.

Importance of substances

Significance of various substances for food safety depends on:

- Occurrence of contaminants in feeds
- Intake of contaminated feed by animals
- Absorption of contaminants by animals in dependence on various factors
- Excretion, metabolisation and carry over of absorbed contaminants in animal tissues (e.g. meat, fat) or products (milk, eggs)

Some substances influence animal health and have a very low carry over factor (e.g. some mycotoxins like deoxynivalenol), other contaminants do hardly influence animal health, but they could be accumulated in food of animal origin (e.g. some heavy metals, halogenated substances) and there are also substances, which influence animal health and can be transferred into animal tissues as well (e.g. some microorganisms).

Possibilities to avoid contaminations or to detoxify

Feed producers and animal nutritionists have to use all the opportunities to avoid or decrease contamination of feeds with undesirable substances along the food chain (detect and block up of sources). The so-called "Positive list of feedingstuffs" may contribute to avoid contaminations in feeds and to increase feed and food safety. Among the decontamination procedures there are different reports on the efficiency of substances added to the diet to detoxify undesirable substances like mycotoxins. More experiments to study the mode of action of such substances to get reproducible results seem to be necessary.

Conclusions

One important objective of animal nutritionists is to detect sources of contaminants along the food chain and to find out ways of their elimination.

During the last decades research in the field of feed and food safety contributed to a considerable decrease of many undesirable substances in foods of animal origin.

K-B01

Problems with the identification of new hazards for men - *M. paratuberculosis*

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K-B02

Antimicrobially resistant microorganisms in food

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K-B03

Campylobacter – 25 years old and still an emerging disease?

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In 1977 Martin Skirrow published the selective culture technique that enabled the identification of campylobacter as the most common cause of human intestinal infectious disease in many industrialized countries. Early studies established that this disease was primarily due to *Campylobacter jejuni* and *C.coli*. These infections were rapidly shown to be usually sporadic and foodborne. Despite more than 25 years of investigation, campylobacteriosis is still considered an emerging disease. The reason for this seems to reflect our paucity of knowledge about the epidemiology, pathogenicity, population structure and ecology of these organisms.
The epidemiology of campylobacter infections is complex. In the UK the reported incidence of campylobacteriosis continued to rise until only recently and in many other countries this incidence is still increasing. Until all potential sources, and their relative risks, are identified such changes in incidence will remain unexplained. Campylobacters are ubiquitous in the environment, and can be recovered from the faeces of most domestic and wild animals. Nevertheless, in the early 1980s, the handling and consumption of contaminated poultry meat was quickly implicated as the major source. The initial strength of this observation has tended to constrain subsequent source investigations and, consequently, control strategies in the food chain. Most recent epidemiological studies indicate that other sources, especially water, may be more important than originally thought. Another epidemiological anomaly is the seasonality observed in human infections, which also remains unexplained. Other questions still unanswered include “what are the mechanisms of pathogenicity by which campylobacters cause disease” and “can such virulence properties vary between strains”? Perhaps most intriguingly is the question “why is colonisation in birds and most mammals asymptomatic but doses as low as 500 cfu can cause severe enteritis in susceptible humans”? The absence of a suitable animal model of campylobacteriosis is a major hindrance to such studies. Properties such as invasiveness and cytolethal toxin (CDT) production in vitro are well established, but the roles of these in human disease are unknown. Recent evidence indicates that the immune experience of any potential host plays a significant role but it is also clear that potential virulence properties vary between strains and this may account for differences in disease presentation. Phenotypic diversity within campylobacter strains has been recognised for some time. Nevertheless, the extreme weak clonality of the species has only recently been recognised with the development of genotyping techniques. This population structure, which appears to be a consequence of genetic instability, considerably hampers our understanding of sources of infection and virulence potential, confounding accurate risk assessment and the identification of changing trends. However, such properties may explain how these organisms survive the ecological bottlenecks of hostile environments during food processing and host-to-host transmission. Such genetic instability, which provides excellent mechanisms for adaptation, may prevent control strategies such as treatments with probiotics or bacteriophages from being sustainably effective. The adaptability of this bacterial species is clearly demonstrable by its rapidly increasing antimicrobial resistance.
Overall campylobacters are remarkably successful organisms, which have exploited novel ecological niches generated by humans during the intensification of food production. Controlling this will provide interesting challenges for the next 25 years.

K-B04

TSE and food

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vCJD is a fatal human neurodegenerative disease linked to the alimentary transmission of the cattle disease bovine spongiform encephalopathy (BSE). There is no treatment available for the disease and the number of affected individuals is unknown due to numerous uncertainties such as the minimal infectious dose by oral route for humans, the incubation period and the possibility of a secondary interhuman transmission which would amplify the epidemic.
The BSE agent is a TSE (transmissible spongiform encephalopathy) agent also named a prion. It harbours particular features when compared to other TSE agents like that inducing scrapie in sheep, and has accidentally crossed several species barriers. The major biological properties of prions and the public health problems raised by BSE and the contamination of humans will be discussed.

We established a model for the study of vCJD by transmission of BSE to the nonhuman primate species cynomolgus macaque. Cynomolgus BSE recapitulates the strain specific characteristics of vCJD with respect to neuropathology, biochemical properties and tissue distribution of the disease-associated prion protein. Data will be shown on how this model can be used to study the risk of iatrogenic primate-to-primate transmission of the disease through subclinical vCJD carriers. A special attention is given to determine the efficiency of the intravenous route of infection compared to other routes, and the infectivity of different blood components at the preclinical and clinical stage of the disease. A second focus of these studies is to determine the minimal infectious dose of BSE for primates and the pathogenesis of the oral route of infection. This is part of a collaborative European study enabling to perform end-point titrations of cattle BSE brain by the oral and intracerebral routes. This study will further provide informations on the time-course of the infection by the oral route, and the relative efficiency of this route when compared to the intracerebral/intravenous routes.

K-C01

Risk assessment of foodborne pathogens

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In just ten years time, quantitative microbiological risk assessment has become a standard tool for food safety professionals. This rapid development has been promoted by both political and scientific factors. From a political point of view, the Sanitary and Phytosanitary Agreement of the World Trade Organization has been a major driving force. This has led to adoption of risk analysis principles by Codex Alimentarius, and to a number of expert consultations and workshops organized jointly by FAO and WHO. These activities will lead to a series of guidelines for risk assessment, of which the recently published “Guidelines for Hazard Characterization of Pathogens in Water and Food” are the first in print. Also, international experts have produced a number of risk assessment documents, describing specific hazards and commodities. From a scientific point of view, risk assessment has become an indispensable tool to oversee the dynamics of pathogens in complex food chains and to analyze the effects of possible interventions in a systematic manner. Non-linear relationships and the effects of peak exposures can be accounted for using risk assessment models. It has also become highly visible that not only prevalence but also numbers of micro-organisms must be known to estimate risk.
By combining risk assessment with epidemiological, economic and social analyses, powerful decision support tools can be generated. This will be illustrated by the CARMA project, a multidisciplinary project in the Netherlands to assess methods aimed at reducing the incidence of campylobacteriosis. Two central questions are to be evaluated: what are, from a quantitative point of view, the most important routes of exposure of the Dutch population, and what is the efficiency and efficacy of possible intervention measures. A central part of the project is the development of risk models that quantify the dynamics of Campylobacter (currently focussed on the chicken meat chain) and that compare exposure from various sources. The baseline risk model is completed with an economic model that describes the disease burden and cost of illness in the current situation. In consultation with risk managers and stakeholders, a series of interventions has been defined at the farm, during broiler processing and directed towards consumers. These will be compared with respect to their expected effects on public health, their costs and their acceptance by stakeholders. More information on the project can be found at www.rivm.nl/carma.

K-C02

Interactions between risk assessment and risk management

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Published frameworks for the conduct of risk assessments consistently include a principle that articulates the need for “functional separation” of risk assessment from risk management. Experiences gained as a result of conducting quantitative microbiological risk assessments at both the national and international levels have reinforced that this is one of the most misunderstood risk assessment / risk management principles. The underlying reason for separating the two activities is to ensure that the risk assessment is not biased by the risk managers. However, this has too often been interpreted as a need to completely separate the two activities. Experience during the past decade has proven that the controlled interaction between risk assessors and risk managers is critical to the development of effective risk assessments. Without this interaction, there is a strong potential for risk assessment teams to produce unneeded, unfocussed, or incomplete risk assessments; an outcome that will result in a diminution of the usefulness of this powerful tool in the minds of the risk managers. There are several critical phases in the development of a risk assessment that require effective interaction, including the defining of the risk management questions that the risk assessment will address, identifying and agreeing upon the underlying assumptions that will be employed in developing the risk assessment model, establishing the criteria for including or excluding data sets, and interpreting the risk assessment findings. An additional reality that must be considered is often the risk managers are the key subject matter experts whose involvement is critical to the development of an effective risk assessment. A number of strategies for the timely, controlled interaction between risk assessors and risk managers have been recommended by several international consultations and workshops. Furthermore, a number of countries have been developing tools and processes that can be used to help identify and avoid both intentional and unintentional biases.

K-C03

"HACCP and microbiological risk assessment – commonalities and differences"

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In recent years the control over the safety of food production has become tighter and tighter. Food safety management systems such as Hazard Analysis Critical Control Points (HACCP) and the pre-requisite systems Good Manufacturing Practice (GMP) and Good Hygiene Practice (GHP) have provided the professional players with excellent tools to assure product safety. Specific concepts have been developed, i.e. microbiological criteria, control measures, product and process criteria, which support proper co-ordination of operational processes. Stakeholders in food safety management such as governments, trade or sector organisations have developed guidelines, best practice advice, regulations and food safety standards further aiding assurance of safe food production.
Considering that in any country many different food chains exist, with an enormous variety in structures, logistics and chain participants, and that they will undoubtedly change rapidly, scale-up and diversify continuously, food safety management at any scale (regional, national, local, factory) is a challenge. Ideally (1) each food production chain is managed integrally, across all steps or links in the chain; (2) there is explicit knowledge about the success of this management, i.e. whether the food safety management systems in place work to the extent projected; (3) the success of food safety management is reflected in the health status of the population concerned.
At the governmental level, food safety control by necessity covers the range of different food chains relevant to a certain product or product group, including all relevant producers, manufacturing sites and food service establishments within the country as well as those importing into the country. FAO and WHO have called upon countries to apply modern food safety systems and associated standards to protect consumer health. Appreciating the complexity of the current food safety supply within and across countries, both organisations advocated using Risk Analysis as the single framework for building food safety control programs. Partly through the activities of Codex Alimentarius and *ad hoc* expert consultations, FAO and WHO have developed the various phases in Risk Analysis, namely Risk Management, Risk Assessment and Risk Communication. With respect to food-borne pathogens, Risk Analysis is about to be generally accepted by governments as the framework to estimate the impact of a particular hazard on public health and to define an appropriate level of public health protection against that hazard. Public health protection is paramount, but the facilitation of fair trade is a second important area of application.
The introduction of the Risk Analysis framework and the conduct of a series of risk assessment studies by governments have provided a novel basis to make decisions on the policies for public health protection. New concepts have been introduced in the process: Food Safety Objective (FSO), Performance Objective (PO) and Performance Criterion (PC). FSO and in where appropriate PO will be used by governments to communicate their public health protection policy to particular food chains, i.e. guiding them in what level of safety performance is expected. The new concepts may sound completely new, but are rather more like a new language, introducing some new words for already existing activities. To provide FSO, PO and PC, still, control measures, product, process or microbiological criteria will be used. The established and rather successful food safety management systems we have today (i.e. HACCP, GHP, GMP) will continue to be used in order to meet the expected food safety at consumption. This paper explains how risk analysis and risk assessment, including the new concepts, relate to our current food safety management systems. Its key message is that they will not become obsolete but remain a necessary, integral part of the future food safety management.

K-C04

Risk communication: Balancing science, policy making and public perception

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Health and environmental scientists, professional risk managers and the general publi strongly disagree about the seriousness of many risks. Most members of the public ar concerned about long-term effects of risks, equity and fairness issues, lack of personal contro and the pace of technological diffusion into their cultural environment, whereas professiona toxicologists and risk managers focus on the task to minimize the probability of adverse effect caused by a potentially hazardous agent or activity. To bridge the gap between th professional mandate and the public perception of risk, two-way-communication has to b initiated between scientists, risk managers, interest groups, and representatives of the affecte public. This dialogue should serve three major functions:

- to facilitate understanding of different risk perspectives among scientists regulators and stakeholders as well as groups of the public;
- to enlighten all these constituencies about different rationales for dealing wit risks of infections and intoxication;
- to develop appropriate procedures for conflict resolution.

A prerequisite for a successful communication is the willingness of each group to respect th perspective of all the other participating groups and to include their concerns into the decisio making process.

The conference paper reviews the literature on the three main functions of risk communicatio message recognition, mutual understanding and respect as a prerequisite for trust building an resolution of risk-related conflicts. The paper discusses the structure of the communicatio process from a descriptive and a normative point of view and draws on empirical studies abou risk perception and communication. The argument will be made that risk cannot be understoo as a monolithic concept that penetrates different research disciplines and risk managemen camps. Risk should rather be seen as a mental instrument that allows prediction of futur hazards and facilitates risk reduction measures. Due to the inherent ambiguity and uncertaint of conceptualizing risk, different concepts of risk compete with each other and rely on differen rationales. The main goal of risk communication is therefore integration of different concepts o risks, in particular with respect to setting priorities in risk reduction and mitigation. The autho will introduce a recent initiative by the OECD Chemical Risk Group to accomplish this goal.

Social and economical aspects of foodborne infections and intoxications in developed and developing countries

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Foodborne diseases (FBD) represent an important public health problem that significantly affects people's health with serious socio-economic implications.

It is recognized that FBD produce losses of billions of U.S. dollars yearly in developing as well as developed countries; however, the real magnitude of the problem is still unknown in spite of important efforts to estimate it, especially in countries where the surveillance and reporting systems are inadequate. For instance, it has been estimated that each year FBD causes approximately 76 million illnesses, 325,000 hospitalisations and 5,000 deaths in the USA and that the annual costs produced by some of the principal foodborne pathogens (*Campylobacter jejuni*, *Clostridium perfringens*, *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Salmonella spp*, *Staphylococcus aureus* and *Toxoplasma gondii*) varies from U.S $6.5-13.3 to U.S $19.7-34.9 billion depending on the parameters used. Lately, a preliminary study was carried out for European Union countries, taking into account only four of the major pathogenic bacteria (*Salmonella spp*, *Campylobacter spp*, *Listeria monocytogenes* and *Escherichia coli* 0157:H7), and the minimum losses were calculated at 3.2 to 4.4 billion Euro per year.

The estimation of annual costs of FBD varies significantly from country to country and from one study to another, depending on the quantification of some parameters such as the value of human life. It should also be pointed out that in most instances, the losses have been calculated on the basis of reported cases and therefore the real numbers and costs figures may be several times those presented by the authors.

Ongoing changes in the food supply, the identification of new foodborne diseases and the availability of new surveillance data are factors that influence the cost figures published and therefore, they should be viewed with caution.

The problem of FBD and its associated costs is multi factorial and its prevention and control requires a multidisciplinary and intersectorial approach through the participation and collaboration of multiple partners at the level of decision makers and experts.

K-E

Modern harvesting, processing and packaging technologies and foodborne diseases

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A high number of foodborne diseases is registered worldwide. Particularly in poor regions a dramatic situation concerning morbidity and mortality is given. But also in the developed industrial countries with advanced level in food hygiene and modern technologies of slaughtering, processing and packaging a not acceptable number of food infections is observed.

Best technologies alone are not able to prevent foodborne diseases. In the case of the zoonotic pathogens coming into the food chain by animals with latent infections measures in the pre-harvest area are needed to improve the situation. Even the most progressive slaughtering process cannot eliminate the introduced pathogens completely. The aim during slaughtering is to reduce the contamination (cross contamination) and to stop the multiplication of the microbes. There is some hygienic progress, e. g. the chilling process in poultry, but something is still waiting for innovations, e. g. the scalding. Many reports on the decontamination of the surfaces of carcasses after slaughtering by using different chemicals or hot steam are published. An interesting way to avoid cross contamination during the portion process seems to be the Laserjet cutting. An effective method of decontamination even in the depth of the carcasses is the irradiation, but the majority of consumers refuses it.

In the field of processing there are a lot of successful “traditional” technologies focussed on killing or prevention of enrichment of pathogenic microorganisms, e. g. heating, chilling, drying, curing, acidification etc. Today many data on the physiology of pathogenous microorganisms are available. The food safety can be improved strongly by using the extensive knowledge on the conditions for multiplication, survival and inactivation of the pathogens for the innovation of processes in food technology (predictive microbiology, hurdle effect acc. LEISTNER, 1999).

More and more alternative technologies have been developed considering the consumers demands for minimally processed, “natural” food. Especially the combination of “mild” preservation methods (“non thermal processing”) operates successfully, e. g. high hydrostatic pressure together with low temperature pasteurization or utilisation of pulsed electric fields.

The trend to prefer fresh, not preserved foods demands new methods in packaging too, e. g. the clean room technology using a localized air delivery system, the modified atmosphere packaging or the “active packaging”. A good example is the case-ready fresh meat packaging as a very successful product of the self-service retail sector today. “Active packaging” is an innovative food packaging concept. This packaging technique is concerned with substances that absorb antimicrobial and other agents. These agents can be incorporated in or coated onto food packaging materials.

Modern technologies offer some benefits for improving food safety provided that the people are using them properly. The practice shows, that incorrect handling (neglect of simple hygienic principles) still is the most common cause for foodborne infections.
In conclusion only by the combination of pre-harvest measures, modern technologies in harvesting, processing and packaging and well qualified staff the health risks for the consumer can be reduced sufficiently.

K-F01

Travel associated foodborne infections

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Foodborne infections are the leading cause of health problems during and after travel to areas of low hygienic standards. The majority of cases presents as traveller's diarrhea (TD). However, some foodborne infections can cause severe or chronic enteritis (e.g., shigellosis, typhoid fever, amebic dysentery, helminth infections), or diseases of other organ systems (e.g., hepatitis A and E, poliomyelitis, tissue parasitoses).
Recent studies in short term travellers have shown TD incidence rates between 14 and more than 60%, depending on the destination and various risk factors. Mean duration of untreated TD is 3 to 5 days. However, in 8-15% a prolonged course occurs and in 1-3% chronic diarrhea (> 4 weeks) will develop. 50% of patients with TD will be incapacitated for at least 1 day, 20% are confined to bed for 1-2 days and 5-15% will seek professional help.
The etiology of TD is heterogeneous. Most common are bacteria, notably enterotoxigenic *Escherichia coli* (ETEC), enteroaggregative *E. coli* (EaggEC), diffusely adherent *E. coli* (DAEC), campylobacter, salmonella, shigella, and viral pathogens. Various virulence factors of pathogenic *E. coli* and genetic host factors have been determined, that are associated with symptomatic infection. Giardia and other parasitic pathogens (i.e., cyclospora, cryptosporidia) are less frequent causes of TD. However, in persisting gastrointestinal disorders of returning travellers they play a more important role.
Newer diagnostic methods (PCR, coproantigen-ELISAs) increase the diagnostic sensitivity and spectrum considerably. Microbiological diagnosis is indicated in severe or chronic enteritis, and in the immunocompromized. However, to apply the complete spectrum of available methods is economically and medically not justified, and the diagnostic work-up has to be done in a step-wise manner.
Several antibiotics are effective in the treatment and prevention of TD. However, the empiric use of antibiotics like quinolones or azithromycin should be restricted to the treatment of dysenteric and febrile cases of enteritis. New antisecretory drugs, nonabsorbable antibiotics, and new vaccines against cholera, ETEC and rotavirus are under clinical development.

K-F02

Foodborne diseases in communal catering

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Foodborne diseases in communal catering are of special interest based upon the numerous consequences.
Due to the centralisation of catering to a small number of food producing factories and a few local producers it is possible that food produced in a modern technology e.g. cook & chill is distributed to various parts of the country and served at the same time.
As a result an increasing number of people are supplied by a few firms. Logically, danger to health to a larger circle of people increases.
That means that there is a high risk of deficits with respect to the population's health and economy of the country and may lead to financial risks for the producers of food.

Based upon the experience of the German Bundeswehr - one of the largest producers of communal catering in Germany - I will try to give evidence in respect of registering and epidemiological evaluation of foodborne diseases and of analysing the effects of HACCP implementation.
The "alert system" of the Bundeswehr has shown positive results in discovering epidemiological sources of foodborne diseases. The analysis of the last 20 years statistics shows microbiological results and points out new questions for the future.
It could be proved, that there are connections between the introduction of HACCP system, the choice of disinfectant and the detected spectrum of microbiological agents in cases of food- borne diseases. For example the number of infections caused by Staphylococcus aureus decreased, whereas the number of Salmonella infections remained constant. It is considered that the incidence of Salmonella-infections can only be influenced by reducing the contamination rate of meat, poultry, eggs and their products.

The illness caused by Campylobacter agents play also an important role in the communal catering; this has been indicated by faecal tests. But until now there is no proof of epidemiological relation because these agents have not been detected in cooled 48 hour food retain samples.

The high number of illnesses caused by Bacillus cereus is of extreme interest.

Up to the year 2000 illnesses of viral origin in the Bundeswehr correlated with those in the local civilian population. The epidemiological (presumptive) diagnosis "viral enteritis" respected the fact that faecal tests were incompletely done and the results of the microbiological food testing didn't give adequate results.
In the year 2002, in some parts of Europe and Germany there was an increase of gastro-enteritis illnesses caused by "Norovirus". Additionally in January 2002 a new type of Norovirus was detected and suspected as causal agent.

Is this new type of "Norovirus" as highly pathogenic as supposed?
What kind of food can be a vehicle in the chain of infection?
Where is the point of contamination – food preparation and/or food service?
These and other questions connected with the detection of the virus in food will be discussed.

K-G01

Global trade and food safety

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The formation of the World Trade Organisation (WTO) in the year 1994 has led to a significant increase in the international trade in foods. The volume of the world food trade is estimated between 300 and 400 billion US Dollar. The Uruguay Round Agreements represent a milestone in the multilateral trade system as for the first time, agriculture and food were incorporated under effective rules and disciplines. In 1995 the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), included in the General Agreement on Traffics and Trade (GATT) entered into force.

The SPS Agreement acknowledges the right of governments to take sanitary and phytosanitary measures necessary for the protection of human health, but only in as far as human, animal or plant life or health are at risk, not to discriminate between Member Governments. The measures should be based on scientific principles and not be maintained without scientific evidence. Only the exporting countries are in a position to guarantee compliance with the requirements of the importing countries because of their access to information on the animal health situation and on the food manufacturers. Only the food control authorities of the exporting country would know whether a food manufacturer has an effective HACCP system in place. Inspections by importing members (e.g. the Food and Veterinary Office of the EU) checking compliance will the rules on a sampling basis may help to establish trust (or distrust) in those guarantees.

Statistics on foodborne infections and intoxications are not available world-wide. The globalisation of food trade and increasing problems with emerging and re-emerging foodborne diseases have increased the risk of cross-border transmission of infectious agents. Because of the global nature of food production, manufacturing and marketing infectious agents can be disseminated from the original point of production and packaging to locations thousands of miles away. Reports on outbreaks due to Typhoid Fever associated with imported fruit in Florida in 1999, Cyclosporiasis associated with imported raspberries in Philadelphia in 2000, *Shigella sonnei* associated with imported oysters in Japan in 2001 or with norovirus and hepatitis A virus associated with clams in Washington in 2001 show the impact of the international trade on food safety aspects.

The Codex Alimentarius Commission has identified and elected standards, guidelines and recommendations for food additives, veterinary drugs, pesticide residues, contaminants, methods for analysis and sampling, and codes and guidelines of hygienic practice. Codex has also developed standards for food products to help prevent fraud. Hereby, the Commission complies with the approach on harmonisation in Article 3.1 of the SPS Agreement: "To harmonise sanitary and phytosanitary measures on as wide a basis as possible. Member states shall base their sanitary and phytosanitary measures on international standards, guidelines or recommendations..."

WTO Member States may introduce or maintain measures resulting in a higher level of sanitary protection than would be achieved by measures based on international standards. However, the Members are bound to ensure that their measures are based on an assessment, as appropriate to the circumstances of the risks to human, animal or plant life or health taking into account the risk assessment techniques developed by Codex Alimentarius or other relevant international organisations.

K-G02

Bioterrorism – an emerging threat to food safety

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In the aftermath of the Anthrax attacks in the US in late 2001, preparedness for bioterrorist attacks was geared up worldwide. The US Centers for Disease Control and Prevention (CDC) have published a list of agents which potentially could be used for deliberate release, also for the contamination of food. Besides biological agents, various toxins and chemicals are potential candidates. Risk assessments have already been carried out for some of these agents, for others they are underway or planned.
Agents best suited to poison the food supply include those that are easily disseminated, cause high morbidity and lethality, cause social disruption and need special precautions with respect to public health preparedness. As one example of this category, Clostridium botulinum toxins could be used as aerosol or as contaminant for drinking water. Food borne anthrax as another example is occurring naturally in regions where raw meat is consumed and an absence of food safety programmes results in the slaughter and consumption of animals infected with anthrax. The second most important category of biological agents requiring public health attention consists of organisms that are easy to disseminate, cause moderate morbidity and low lethality and require specific enhancement of diagnostic and surveillance capacities. Among those are Ricin and Staphylococcus Enterotoxin B (SEB), both toxins, as well as Salmonella, Shigella dysenteriae, E. coli O157:H7 and Vibrio cholerae. There are additional pathogens that could be employed for criminal acts, among them hepatitis A or Cryptosporidium. However, due to the difficulties generating larger aerosols and the uncertainties of dilution effects when water systems are contaminated, the poisoning of food supplies with potential biological warfare agents may well be an attractive alternative for terrorists.
Contamination of food for a great number of persons would require contamination at the source (=production) level. Here prevention is only possible by rigorous control strategies established by the food producers. On the other hand, even the intentional contamination of only a small number of products can have a destabilising effect in the population and absorb resources in particular within the public health service.
Most likely, an outbreak due to an intentional contamination of food would be handled in the beginning like an unintentional food borne outbreak. Thus, the adequacy of response will depend on the public health capacity to respond to food borne outbreaks in general. The preparedness for intentional contamination of the food supply requires an interdisciplinary approach:
Capacity for rapid microbiological diagnosis including unusual agents and the possibility of rapidly establishing whether strains are identical (DNA fingerprints, sequencing), thus suggesting exposure to a common source (microbiologists).
Capacity for early recognition and treatment of human cases (epidemiologists and clinicians). This requires detection by alert clinicians or laboratory workers who rapidly report clusters or even single cases of unusual illness. With the surveillance system established under the new Infection Protection Act in Germany, rapid data transmission together with comprehensive case information is implemented. The development of outbreak detection algorithms is still a challenge. Also necessary are adequate infrastructure and stocks of medical supplies to support the care of patients with a rare disease.
Capacity for quick response aiming particularly at the prevention of secondary spread (public health service).
Capacity for timely trace back (veterinarians, food safety specialists).
Capacity for cooperation and communication of all disciplines on all administrative levels.
Assessing vulnerability: effectiveness of the food safety infrastructure, magnitude of the threat associated with a particular agent and mode of delivery and the capacity for an emergency response.

Abstracts

S-A01

Preparation and realization of a monitoring project for Campylobacter in broiler chickens in Germany

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Campylobacters are generally regarded as one of the most common bacterial cause of diarrhoea in humans. Recently, an increasingly high incidence of campylobacteriosis in humans has been reported. The reasons for the increase of human campylobacter infections during the last years are unknown. In Germany 2003 campylobacteriosis has become already second in number (46.282) after salmonellosis (61.578).
Campylobacter infections are predominantly believed to be foodborne, via raw or contaminated milk, contaminated water and ice, but above all due to undercooked poultry meat and foods contaminated through raw poultry meat. In poultry *Campylobacter* spp. occur in the gastro-intestinal tract and do not cause disease.
Symptoms of human campylobacteriosis typically start two to five days after infection and include abdominal pain, diarrhoea (often with bloody faeces), fever and headache. They usually last three to six days. A fatal outcome is rare but complications (e.g. bacteriemia or Guillain-Barré syndrome, a neurological disorder) are possible.
The Zoonoses Directive 2003/99/EC requires (Art.4) that „EU Member States collect relevant and comparable data in order to identify and characterize hazards, to assess exposures and to characterize risks related to zoonoses and zoonotic agents“. It mentions campylobacteriosis and its agents (App. I).
Against this background the Federal Institute for Risk Assessment (Bundesinstitut für Risiko-bewertung, BfR) prepares for a monitoring project for the occurrence of thermophil *Campylobacter* spp. (*C. jejuni*, *C. coli* and *C. lari*) in broiler chickens at slaughter in Germany. For that purpose at least 80% of the national broiler flocks should be investigated. Only slaughterhouses that receive batches of more than 2000 birds are considered for inclusion in the sampling frame. All farms delivering their animals to these slaughterhouses will be sampled once during the summer period (May until October) and once during the winter period (November until April) to include the seasonal variation of Campylobacter prevalence.
10 caecal samples per batch per farm will be collected at the point of evisceration. These samples will be sent as intact caeca to the laboratory where the caecal contents will be aseptically removed and pooled. The processing and isolation of *Campylobacter* spp. takes place in veterinary public health laboratories and follows ISO 10272.
For further investigation (determination of species and resistance behaviour) isolates will be sent to the Federal Institute for Risk Assessment. All data will be collected and statistically evaluated by this Institute.

S-A02

Persistence of *Salmonella* Montevideo on a large broiler farm over a 30 month period: Observations on sampling and control

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Salmonella enterica serovar Montevideo is a group C *Salmonella* (6,7,14 : g,m,[p],s : [1,2,7]) which is relatively infrequent in UK chickens (56/869 [6.4%] incidents in 2002) and in humans but which may become persistent in the environment of poultry farms, feed mills and hatcheries.

The study site was a large modern farm comprising 8 x 33,000 bird houses linked in pairs and operated on an all in/all out basis. Infection with *S.*Montevideo is thought to have originated from a persistently contaminated feed mill and had been identified intermittently on the farm prior to this study but was thought to be currently absent. Screening samples comprising eight pooled litter and four pooled dust samples were taken from each house and cultured by a sensitive Buffered Peptone Water, Diasalm, Rambach Agar technique. Despite all routine company monitoring samples being negative, *S.*Montevideo was found in a high proportion of samples from all houses, with dust being the most sensitive sample (dust 57/64 [89.1%], litter 31/64 [48.4%]). This sample type sensitivity trial was maintained throughout the study and, as the level of infection fell, litter became a much poorer sample although the company's own monitoring results did improve. Introduction of boot swabs further improved the company monitoring and these also yielded more positive results than litter samples during the study, but not as many as dust.

Sampling carried out in three of the houses after cleaning and disinfection using a glutaraldehyde based product demonstrated 14.4%, 18.4% and 38.8% of surface samples still containing *S.*Montevideo. In particular, feeding systems, water systems and flooring remained significantly contaminated. Repeat sampling following a change in cleaning contractors demonstrated some improvement but similar problems were still present at a lower level. Flock infection rates did reduce following these improvements but at least one house remained infected at each sampling round, with infection apparently cleared from some houses and 'moving' to others on several occasions. Further improvements were made to disinfection by introducing a phenolic and formaldehyde based programme. This produced good results with little residual contamination found. Samples taken from birds at slaughter from one positive flock found 1/100 caeca and 4/100 neckflaps containing *S.*Montevideo. Following the upgrade of disinfection, *S.*Montevideo continued at a low level but sampling of houses and equipment immediately before entry of new chicks identified significant contamination in feed pipes, drinkers, weighers and partitions, as well as in rainwater washing down contaminated dust from roof mounted exhaust baffles, in some of the houses.

It was clear that the empty period between flocks was too short (maximum seven days) to adequately clean and disinfect all these very large houses and to maintain effective biosecurity between houses that were already cleaned and others still in the process of cleaning. The most significant problem was application of disinfectants and fogging agents to wet surfaces on the same day as washing. This pattern of residual contamination appears to be an increasing problem on large, modern broiler farms but the significance of infection with environmentally persistent serotypes in relation to human health risk is uncertain.

S-A03

Safety and quality practices in closed-house poultry production in Thailand: Lessons from avian influenza outbreak in 2004

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Food safety and quality that are demanded by domestic and international trades are the major concerns in food production chains. Safeguarding these concerns presents challenging tasks across the entire farm-to-fork continuum. This progression is comprised of various segments that include farm levels, industries, transportations, markets, consumers and governmental departments. The devolution of these tasks among the stakeholders and the heterogeneous nature of safety measures at each stage require coherent good management practices. And, successful achievements of these need continuous efforts in identifying practical and cost-effective measures for reducing or eliminating the food-borne risks to consumers, for example, the avian H5N1 influenza.

The recent outbreak of the avian influenza (bird flu) in Asia, including Thailand, has heightened public awareness on requirements of good production practices in poultry and poultry products for both local and international needs. In addition, accurate and timely dissemination of information regarding diseases like this bird flu are important issues that affect public perception and implementation of preventive measures.

Lessons of avian influenza outbreak in terms of building cost-effective monitoring and surveillance systems can be good basis for this disease and other poultry-borne zoonoses. Apart from these systems, research should be conducted with aims of finding out the reason(s) that led to this outbreak. The laid down regulations of “good” agricultural practices in each stage in the whole poultry chain production in Thailand will be examined. This would assist in identifying the critical points that might have led to this outbreak. The knowledge gained would be used in formulating preventive measures at these points.

S-A04

A laboratory perspective on the *Vibrio parahaemolyticus* monitoring program in Pacific oysters (*Crassostrea gigas*) in British Columbia, Canada from 1997 to 2003

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In 1997 an unprecedented outbreak of raw oyster - related *Vibrio parahaemolyticus* (Vp) gastroenteritis occurred throughout the West Coast of Canada. Since July 1997, the CFIA Burnaby Laboratory (Boundary) has been conducting environmental *Vp* monitoring of oysters from 7 sites which represent the bulk of commercial oyster harvest in B.C. The lab uses the standard 3 tube MPN method and pursued the validation of direct plating methods using DIG-labelled probes and multiplex PCR assays that targeted the tlh, tdh and trh genes, and the R72H DNA sequence in Vp. Special projects were also initiated every summer to confirm Vp profiles in the harvest and distribution chain and data gathered were used to adjust harvest/handling practices for shellfish. In 2003, the multiplex PCR assay for tdh, trh and R72H (taxonomic identifier) was validated and will be used in an MPN format to enhance the monitoring program. The use of laboratory data from monitoring and special projects as part of an overall risk reduction strategy for this organism reduced the confirmed illnesses from over 100 in 1997 down to single digits in the last few years.

S-A05

Surveillance of zoonotic bacteria in farm animals in The Netherlands, 1998-2002

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The EU Zoonoses Directive obliges the Member States to collect data on the occurrence of zoonotic agents in animal populations. For this purpose, a national programme for surveillance of zoonotic bacteria in farm animals was implemented in April 1997 by the RIVM in order of and in collaboration with the Inspectorate for Health Protection and Veterinary Public Health (VWA/KvW).
The prevalence of *Salmonella* spp. in layer flocks has significantly decreased in the period 1998-2002. The estimated salmonella prevalence in 2002 was 13%, with *S*. Enteritidis accounting for one third of the positive flocks. Prevalence estimates for *Salmonella* spp. in broiler flocks, in 1998-2002, did not yet yield a decreasing trend. The estimated salmonella prevalence in 2002 was 11%, with *S*. Paratyphi B var. Java accounting for one third of the salmonella positive flocks. The prevalence of *Salmonella* spp. in finishing pigs showed a decreasing trend between 2000 and 2002, with an estimate of 30% in 2002. *S.* Typhimurium accounted for half of the salmonella positive herds in the last two years, with multiresistant phagetype DT104 accounting for approximately 20% of the positive herds. The prevalence of *Salmonella* spp. in dairy cattle and veal calves remained at a relatively low level during the study period, *S.* Typhimurium being the most frequently isolated serotype.
The prevalence of *Campylobacter* spp. in broiler flocks did not increase nor decrease continuously between 1998 and 2002. In 1999-2002, the estimated flock prevalence roughly averaged around 20%, with *C. jejuni* being the predominant species.
The crude prevalence estimates for *E. coli* O157 in dairy herds increased in subsequent years, resulting in a prevalence estimate of 14% in 2002. Also, the prevalence of *E. coli* O157 in veal herds increased in the period 2000-2002, yielding an estimate of 24% in 2002. This increasing trend can largely be attributed to an increase in the fraction of pink veal herds in the sample during the study period combined with an increase of *E. coli* O157 in this type of veal herds.

S-A06

Prevalence of Shiga toxin-producing *Escherichia coli* (STEC) shedders in meat processing companies

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Shiga toxin-producing *Escherichia coli* (STEC) belong to a group of gut-pathogenic microorganisms, which were made responsible for food-poisoning first in 1982 (CDC, 1982). Ever since there has been a number of outbreaks and sporadic illnesses world wide. Among food, meat products are of great importance as a zoonotic spread of EHEC.
This work was to investigate the role of staff as a possible source of entry for Shiga toxin-producing *E. coli* in meat processing companies. For that purpose regular stool samples of staff members were sampled during two studies and assayed for STEC with genotype-oriented (PCR) methods.
A total of 1566 faecal samples were examined from 244 employees of five meat processing companies in the years of 1997 to 1998 and 2001 to 2002, respectively. The prevalence of STEC in stool samples of employees in the production line was 9% in plant I, 3% in plant II, 10% in plant III and IV and 1,1% in plant V.
The isolated STEC were further differentiated with following results: In plant I Shiga toxin-producing colonies cultivated from one of the two human shedders possessed only Shiga toxin-gene and belonged to serotype O40:H8. Isolated colonies from the other shedder have the virulence marker Shiga toxin-gene and enterohaemolysin and the serotype O91:H-/H14/H21. Furthermore, in this plant an intermittent shedding of STEC over a period of 10 months could be documented. In plant II Shiga toxin producing isolates (serotype O23:H-) were obtained without eaeA-gene. Two of the Shiga toxin-positive staff members shed isolates with the complete virulence markers (Shiga toxin-gene+, eae-gene+, EHEC-hyl+) and serotypes O103:H2 respectively O26:H-. Two asymptomatic shedders were detected in plants III and IV. The isolates belonged to the serogroups O76 and O91. They possessed the shigatoxin gene and enterhemolysin gene and only the shigatoxin gene, respectively.
These results document the importance of asymptomatic carriers as a possible source of entry for EHEC/STEC in meat producing companies. Therefore regular examinations of staff are necessary to guarantee product safety. The source of infection for staff members could not be ruled out in this studies.

S-A07

Noro-/calicivirus food- and waterborne outbreaks in Sweden 1999-2003

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Norovirus is at present the most common pathogenic agent causing foodborne outbreaks in Sweden. Between 1999 and 2003 there were on average 13 reported foodborne outbreaks per year (7-16 per year) with approximately 600 cases (331-1086) per year. In total 64 outbreaks with 3174 cases. Compared with foodborne *Salmonella* which has had 49 outbreaks and 1165 cases during the same period.
The outbreaks are evenly distributed throughout the year, with a small increase during March and a decrease during the month of November when there has only been one reported outbreak in 2002. In approximately 55 % of the outbreaks a suspected food vehicle was incriminated. As there are very few food items where it is possible to demonstrate calicivirus (e.g. oysters, mussels and perhaps raspberries), the epidemiological investigations are even more important than in other foodborne outbreaks.
The most implicated food in Sweden in norovirus outbreaks is raspberries. After New Year one or several outbreaks from oysters are reported and before Christmas always at least one outbreak from Christmas buffet. Indirect contamination of food via an infected food handler is more likely in food that requires extensive handling such as salad, tarts and cold cuts.
Outbreaks with norovirus normally give rise to a greater number of cases (approximately 50 cases per outbreak) than other foodborne pathogens. 33 % of the outbreaks originate from restaurants and canteens, only 6 % of the outbreaks have been from households, but that may be an underestimation. 44 % of the outbreaks have a contributing factor most commonly associated with kitchen personnel who are ill.
It is possible that there may be more norovirus outbreaks identified in the future as the methods to reveal outbreaks increase and improve. More information and strict food hygiene rules for food handlers are needed.

S-A08

Epidemiology of norovirus in Switzerland: Key findings from recent studies

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Recent international studies have shown that viral infections, and especially noroviruses (NV) (former name *"Norwalk-like viruses"*), are the most frequent cause of acute gastroenteritis in the community regarding the endemic and the epidemic situation. In the past ten years, NV outbreaks were increasingly recognised in Switzerland. However, solid epidemiological data were missing due to the fact that NV are not routinely searched for in diagnostic laboratories and that there is no legal obligation to report known cases. For this reason, the Swiss Federal Office of Public Health launched a series of studies to assess the epidemiological situation of NV in Switzerland. The key findings from these studies will be presented hereby.
NV are omnipresent in Switzerland. They were found in surface waters and NV sequences were detected in several brands of bottled mineral water and in imported oysters. Phylogenetic comparisons showed that the isolated NV sequences from water samples were closely related to human stool isolates of NV from the same period. Furthermore, a two-year screening of 699 bacteriologically negative patient stool samples (at least shown negative for Campylobacter, Shigella and Salmonella) revealed that 125 specimens (17.9%) were found to be NV-positive by RT-PCR. Between January 2003 and December 2003 73 NV outbreaks were registered mainly in the German speaking part of Switzerland due to the original study design. Therefore the real number must be at least doubled. 25 outbreaks (34%) took place in nursing homes and asylums for disabled and 18 (25%) in hospitals and health resorts. The mean number of cases per outbreak was found to be 60 (median: 35, range: 3-650) and attack rates ranged between 30-90%. The transmission pathways could be identified in 54 of the 73 outbreaks and forty-four times (81%), person-to-person transmission was the primary infection route. Two outbreaks (6%) were possibly waterborne and in 7 (13%), a foodborne transmission may have been the cause. The results of a case-control study confirmed the low importance of foodstuffs as NV fomites in comparison to the predominant person-to-person transmission. The same study also concluded that approximately 36% of NV cases, considered to be sporadic, were in fact part of a probable NV infection chain.
It can be summarised that NV infections are occurring in Switzerland mainly in closed and semi-closed settings (such as hospitals and nursing homes) and that the person-to-person transmission is the major infection route. Foodborne outbreaks may occur, but play a minor role in the overall epidemiology of the NV in Switzerland. Finally, all waterborne outbreaks happened due to deficiencies in infrastructure or failures in the water treatment process.

S-A09

Concentration and detection of norovirus in various food matrices by RT-nested-PCR

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Noroviruses are a leading cause of gastroenteritis and are the most common cause of outbreaks in semi-closed environments such as hospitals, nursing homes and cruise ships. They are transmitted by person-to-person contact and fecally contaminated food. Nevertheless food is rarely routinely tested for viral contamination, and if so, testing is limited to only a few selected foodstuffs like shellfish or water. Furthermore, the methods commonly used and described often include numerous, complex and time consuming steps for virus concentration and isolation.
To improve the investigation of foodborne outbreaks of gastroenteritis, we developed a sensitive and convenient method for routine diagnostics to detect Noroviruses from various food matrices. For seeding experiments, 25 g of food were artificially inoculated with 10fold dilutions of a stool sample containing Norovirus genotype II. The food samples were extracted for virus concentration by addition of 5 to 30 ml phosphate-buffer followed by homogenisation in a stomacher filter bag. The total liquid phase was further processed by centrifugation and prefiltration of the supernatant through a 0,45-µm-pore-size filter. The final volume was reduced by ultrafiltration of the filtrate in an Amicon 100 kD microconcentrator to a final virus-containing concentrate of max. 1 ml. RNA was extracted from the complete ultrafiltration concentrate to a small volume of 50 µl by a guanidium thiocyanate-silica method. Thus, by using the total volume of virus concentrate in every step, a maximum yield of virus RNA from the original 25 g of food was achieved.
We used a two-step RT-nested-PCR protocol based on the ORF-1-gene region for virus detection. The sensitivity of the PCR-assay was tested using serial dilutions of a stool sample and compared with virus detection in various food matrices that were artificially contaminated. Norovirus could be detected in 10^5-fold dilutions of a stool sample. Sensitivity with various seeded foods (e.g. lettuce, alfalfa, noodles, raw minced meat, carrot salad, melon, soy sprouts, cooked zucchini with spring onion, and meat tomato sauce) was found to be decreased only by factor 10.
In the state of Baden-Wuerttemberg, the method is currently being applied to food samples associated with Norovirus outbreaks of gastroenteritis. So far 96 food samples from 11 outbreaks have been examined. Food matrices tested ranged from vegatable soups, cooked vegetables, desserts, delicatessen salads, meat products and sauces. In one case, Norovirus was detected in potato salad. The two-step method that we have developed is simpler and more efficient than commonly described methods to test for Norovirus and thus has the potential to reduce the time required to isolate sources of contamination and become a standard in Norovirus testing.

S-A10

Novel aspects in the pathogenesis of rotavirus infections: possible consequences for food safety

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Group A rotaviruses are among the most important etiological agents of severe diarrhoeal illness of infants and young children world-wide. Furthermore, rotaviruses are the most significant cause of viral gastroenteritis in young animals, including mammalian (simian, porcine, bovine, ovine, caprine, equine, canine, feline, lapine, and murine) and avian (chicken, turkey, and pigeon) species. Generally rotaviruses are transmitted by the fecal-oral route, and infection is thought to be confined to the intestine. There, rotaviruses replicate in the upper half of the intestinal villi of the jejunum and ileum. The destruction of the nondividing, absorptive enterocytes results in a generalized reduction of the net digestive capacity of the small intestine, a functional deficit known as malabsorption. When the undigested material enters the large intestine, the absorptive capacity may be exceeded, resulting in diarrhoea. Young individuals may die as a result of dehydration or secondary bacterial infection, but most recover within 3 to 4 days.

Until recently, extraintestinal rotavirus infections have been thought to play an insignificant role in disease because they were rarely observed and often were transitory. Reports of rotavirus RNA in the cerebral spinal fluid and serum of children infected with rotavirus suggested that rotavirus escapes the intestine into the circulatory system. Indeed, recent data demonstrate that rotavirus can escape the gastrointestinal tract, resulting in antigenaemia and viraemia: (i) Rotavirus antigen was detected by an enzymatic immunoassay (EIA) in the sera of experimentally infected suckling and adult mice, suckling rats, adult rabbits, and newborn calves. (ii) Gnotobiotic piglets inoculated with virulent human rotavirus developed diarrhoea, shed virus nasally and rectally, and had viraemia lasting at least for 5 days. (iii) In a rotavirus rat model, rotavirus-specific antigens were detected by ELISA in serum, the small intestine, stomach, liver, and spleen; rotavirus was cultured from the liver and serum indicating that these samples contained infectious virus.

These findings are important for the understanding of the pathogenesis, immunology and clinical manifestations of rotavirus infections. An extraintestinal persistence of rotaviruses, following viraemia, may also have considerable impact on the epidemiology, and, due to the zoonotic characteristics of these viruses, also to a possible risk of foodborne infection. Speculations on the role of animals as a source of rotavirus infections in humans have been intensified by the following observations: (i) certain animals share a neutralizing antigen with human rotaviruses; (ii) certain naturally occurring animal rotavirus strains may infect humans.

We have developed a competitive reverse transcription –polymerase chain reaction (RT-PCR) to detect and quantify the RNA of group A rotaviruses. The efficiency of the protocol was confirmed by the reproducible amplification of 10 RNA molecules in clinical samples obtained from various animal species (pigs, calves, rabbits, and horse) and man. The application of this method will allow to get more insight into the significance of the new aspects described above, particularly with regard to (i) rotavirus infections of slaughtering animals, (ii) interspezies transmission, and (iii) food safety.

S-A11

Raw milk associated Campylobacter infections in Germany: Investigations in outbreaks by modern methods

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Campylobacter infections are one of the most important foodborne diseases in many countries, and this is also true in Germany (second frequent foodborne disease after salmonellosis). During the last years most reports on the sources of Campylobacter infections were concerned with poultry, especially in sporadic cases and the results were mostly obtained by epidemiological studies.

This report describes investigations of 15 outbreaks of Campylobacter infections in Germany, most of them associated with raw milk, and all involving more than 1000 patients. In some of the outbreaks it was possible to find distinct dose-response relationships between the amount of consumed raw milk and the illness of patients. These relationships confirm also the very low infective dose of thermophilic Campylobacter species in human infections.

With regard to the methodology to investigate outbreaks the results confirm that a combination of epidemiological examinations with microbiological and molecular investigations in isolated strains are a useful, modern and reliable method to verify the sources of outbreaks and the routes of transmission. The results of the investigations show furthermore the possibility to identify outbreak related strains over a period of some weeks by molecular methods in spite of the relative weak clonality, relative genetic instability and great variability of Campylobacter populations. The PFGE is a recommended method for this aim

Regarding the prevention of foodborne infections it can be concluded that the consumption of raw milk poses an underestimated risk of acquiring campylobacteriosis and perhaps other infections. For this reason only the consumption of pasteurized milk and milk products is advisable.

S-A12

Detection of *Salmonella* in food using real-time PCR

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A real-time PCR based method for the detection of *Salmonella* in food was developed and validated. The assay is based on the amplification and detection of the highly conserved *Salmonella* genes *ttrC* and *ttrA* which are part of the *ttrRSBCA* operon located in the pathogenicity island 2. These genes are involved in the tetrathionate respiration of *Salmonella*. Specific primers and a TaqMan fluorescent probe were designed. For detection of possible PCR inhibitors, which might derive from the sample DNA, an internal amplification control (IAC) was constructed. The IAC is co-amplified with the target primers and detected by a specific probe. The real-time PCR assay detected all 110 reference *Salmonella* strains correctly when a wide range of *Salmonella* serotypes representing strains of *S. enterica* subspecies I-VII and *S. bongori* were tested. All 75 non-*Salmonella* strains tested were negative. Using a 10^4 cfu/ml cell suspension as template in the real-time PCR (50 cells per reaction) the detection probability of the PCR assay was 100% and for a 10^3 cfu/ml cell suspension it was 96% in the presence of 150 copies internal control DNA. The accuracy of the PCR assay was validated on naturally and artificially contaminated samples from pig and poultry. Pre-PCR treatment was based on an initial pre-enrichment step followed by an easy closed-tube resin based DNA extraction protocol. The results gave high agreement in comparison to the traditional culture method. It was also shown that the assay has the potential to quantify *Salmonella* DNA correctly, which plays an important role in risk assessment studies. This assay addresses the increasing demand of quality assurance laboratories for rapid and safe diagnostic detection methods. It has the great potential to become the validated open-formula method for the detection of *Salmonella* from food and environmental samples.

S-A13

Detection of polymorphism of bacterial interspersed mosaic elements (BIMEs) using AFLP-PCR assay: a novel marker for epidemiology studies of *Escherichia coli*

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The simple, robust and reproducible method for molecular typing of *Escherichia coli* strains was developed using the polymorphisms in chromosomal sequences of bacterial interspersed mosaic elements (BIMEs) detected by a combined AFLP (amplification fragment length polymorphism)-multiplex PCR (polymerase chain reaction) assay. The method was based on detection of patterns obtained by the combinations of three different amplification products of variable sequences localized between the genes mtlA and mtlD, lamB and malM, araA and araD. The lenghts of these amplicons were variable due to strain-specific arrangements of noncoding intergenic sequences BIMEs and boxCs, which are located between conserved genes. The applicability of the method as an epidemiological tool was tested on the group of 870 *E. coli* strains isolated from veterinary and human sources. The observed fragment lengths varied from 170 to 554 bp and were combined producing 18-20 genotypes. Significant differences in the frequencies of these genotypes were found between isolates of human and veterinary origins, source of certain specimens (urine, feces, milk, beef meat etc.) or geographical localization. Our results indicate that polymorphism in BIMEs sequences could serve as a novel marker for epidemiology studies of *Escherichia coli*.

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S-A14

Multi-centre ring trials for validation of *E. coli* O157 PCR detection system.

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Enterohemorrhagic *Escherichia coli* O157 is an important food-borne pathogen and a causative agent of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). There have been several published PCR assays for detection of *E. coli*, but not all published PCR could be successfully adopted in different laboratories. Therefore as part of a major international project for the validation and standardization of PCR for detection of five major foodborne pathogens, three *E. coli* PCR systems have been selected and evaluated through national and international ring trials. The present study describes the development of a PCR system for the detection of *E. coli* O157 including also an internal amplification control. This was based on *rfbE* O157 gene sequence. With these oligonucleotide primers designed a 500 bp PCR product could be amplified. This PCR system was optimised varying the PCR parameters. According to the results of the ring trials, This PCR appeared to be highly specific and sensitive.

S-A15

Staphylococcus aureus isolated from cows, goats, sheep, rabbits and chickens: Genotypic variation as revealed using molecular typing techniques

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Staphylococcus aureus causes a variety of infections in animal hosts such as mastitis in cows, goats and sheep, staphylococcosis in rabbits and osteomyelitis in chickens. While it is generally accepted that *S. aureus* strains are host specific, the relatedness of animal-associated clones to human clones has not been assessed by sequence typing methods.

Molecular typing methods were used to investigate the evolutionary relatedness of 120 isolates of *S. aureus* from animal hosts (49 from cows, 34 from goats, 14 from chickens, 12 from sheep, 10 from rabbits and 1 from a cat) from a number of geographic locations. These techniques included MLST (Multi-locus Sequence Typing), spa typing (Protein A typing) and agr typing (accessory gene regulator typing). Of the 120 isolates 48 were also typed using the nucleotide sequences of several predicted surface-associated proteins (*sas* Typing).

MLST revealed that a limited number of clones are responsible for many cases of animal-associated staphylococcal infection with certain clones predominating among different animal hosts. The commonest MLST types were ST133 (22), ST5 (8), ST71 (10), ST97 (7), ST126 (9) and ST151 (6). ST133 also had the greatest number of genotypic variants and appears to be an animal specific genotype. *spa* and *sas* typing revealed that animal-associated clones show significant variation in terms of the nucleotide sequences of genes encoding surface proteins and that animal-associated strains have many novel *sas* alleles. The majority of isolates were *agr* type 1 and 2. No correlation between *agr* type and host or type of infection was observed

A small number of sequence types are responsible for staphylococcal infection in different animal hosts in different geographic locations, with most sequence types capable of infecting both humans and animals. Several of the sequence types found do not appear to be represented in the human *S. aureus* population. These may be subpopulations that have evolved to specifically infect animals. This is evidenced by the presence of unique *sas* alleles in these animal-associated strains that are not found in the human database of *sas* genes.

S-A16

A multi county outbreak of *Salmonella* Hadar in Sweden involving several different authorities

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In Sweden the reported number of domestically acquired human cases of *Salmonella* Hadar is low. Normally in the order of ten cases per year. During the summer and autumn of 2003, an increased incidence of infection caused by this serotype was observed in Sweden. At the end of the year, 53 cases had been notified in three different clusters. The median age was 28 and 26 of the cases were women.

This is an example of a complex outbreak which over a period of six months involved at least ten environmental health protection boards, twelve county medical offices and numerous county veterinarians, government authorities and food establishments.

In the first cluster, mainly in the southern and western parts of the country, 25 cases fell ill between 21 July and 17 August. It was established, early on that several of the cases had eaten chicken salad purchased from the same salad producer, who purchased their chicken for the salads, grilled and sliced, from producer A.

Salmonella Hadar was recovered from processed chicken. Analysis by pulsed-field gel electrophoresis documented that isolates from the chicken belonged to the same clone as the outbreak strain.

In the second cluster, in the Stockholm area and the middle part of Sweden, 24 cases fell ill between 28 August and 21 October. A case-control study was launched to identify the source of infection. Twenty-three cases were compared with 39 controls. Statistical analysis showed ready-made sandwiches to be the main risk factor with an odds ratio (OR) of 13.33 (95 % CI 3.68-48.30). *Salmonella* Hadar was isolated from a ready prepared chicken mixture. Several of the sandwiches contained prepared sliced chicken delivered from producer A.

At the end of the year an additional four cases of *Salmonella* Hadar, distributed throughout the country, were reported. This time chicken salad was the suspected source.

No other human *Salmonella* Hadar outbreak had been reported from any other European country.

The investigation of this outbreak involved a great number of people from several authorities and agencies, involved in communicable disease control, consuming many man hours. Despite very strong epidemiological data indicating producer A to be the source of the outbreak neither producer A nor the responsible authority have shown any particular interest in the problem and the production has continued uninterrupted ever since the problems started.

This outbreak demonstrates how an investigation may be delayed in reaching a successful conclusion because of the involvement of multiple agencies, the wide distribution of cases in time and space and the lack of cases in the area which produced the product containing the source of infection.

S-A17

An explosion of Salmonella infections in 2003 in the Netherlands: hot summer or side effect of the avian influenza outbreak?

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In June 2003, the Dutch National Salmonella Centre reported a significant excess Salmonella isolation rate compared to former years in most regional public health laboratories. This solely concerned *Salmonella* Enteritidis (SE), not *S.* Typhimurium, nor other *S.* serotypes or *Campylobacter* spp.. At the end of 2003, this amounted to an extra 540 laboratory confirmed cases for the whole of the Netherlands, which would mean an estimated 7500 extra cases of gastro-enteritis caused by SE in the general population, which is an increase of 50%. At first the excess was attributed to the exceptional hot weather up to August, with temperatures far above normal. In the WHO/CCASH-project, time series analysis of salmonellosis in 10 European countries clearly demonstrated an added effect of temperature on the risk for food poisoning apart from a general effect of season itself. In the Netherlands (data covering 1984-2001) this effect was exceptionally strong for SE (a linear 12,6% increase per ^{o}C). However, on inquiry among the members of the ENTERNET surveillance network it appeared that most European countries had not experienced an excess of Salmonella infections in the same time-period, except Belgium and England & Wales. This made a large role of the hot summer unlikely which was confirmed after estimation of the number of extra cases on the basis of temperature alone. In Holland surveillance programs show that the Salmonella control program in poultry has been successful in reducing SE in broilers almost to exclusion, whereas in commercial layers still up to 7-9% of the flocks are SE-positive. This makes raw shell eggs the sole suspect food vehicle for causing the 2003 excess of SE-infections in humans. However, phagetyping of SE combined with antimicrobial resistance testing showed remarkable differences between humans and poultry, pointing to a source from outside the Netherlands. In 2003, twice as much phagetype 1 (Pt 1) was found among SE isolates from Dutch patients (14,5%) as between 1998-2002, 54% of them being resistant to nalidixic acid (Na) and decreased susceptible to ciprofloxacin. In Dutch poultry, between 1998-2003, Pt 1 accounted for about 5% of all isolates of SE (almost all from layers), but never nalidixic acid resistant. Human infection with Pt 1(Na) in the Netherlands appeared to be 3 times more often travel-related as other SE-phagetypes, more than 50% related to Spain and Portugal. A series of outbreaks with SE in the UK in 2002 led to the investigation of raw shell eggs and amongst other phagetypes found Pt 1(Na) associated with Spanish eggs. Salmonella was found in 0,3% of the eggs produced in the UK and in 5,1% of eggs imported from Spain. Salmonella is found in only 0-0,03% of eggs produced in Holland, i.e. 10 respectively 160 times lower than those in the UK and Spain. Nonetheless it is estimated that each year about 35% of the Dutch cases of human salmonellosis originates from eggs. After the outbreak of avian influenza in Dutch poultry in the spring of 2003, shortage of eggs was replenished with imports mainly from Germany, Poland, Italy and Spain (8-fold increase). The lesson is that with the low level of contamination of Dutch eggs even small but relatively strong contaminated imports may have a huge impact on the incidence of human salmonellosis. Continuous surveillance, especially of imported eggs, is therefore strongly recommended. To trace back the source of cases of salmonellosis, sero- and phagetyping of positive findings, next to testing for antimicrobial resistance, is essential in decision making and should be a basis for intervention.

S-A18

A nation-wide outbreak of *Salmonella* Agona in infants due to aniseed in herbal tea, Germany, October 2002 – July 2003

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Background: In February 2003, a three-fold increase of notified *Salmonella* Agona infections was observed in Germany. Two-thirds of the cases were infants younger than one year of age. An investigation was started to determine the outbreak's extent, risk factors and source.

Methods: Cases were identified through the notification system and reference laboratories. Parents of cases were interviewed by a standardised questionnaire (eg, on food exposures). A case-control study was conducted, defining cases as children aged <14 months with acute *S*. Agona infection from October 2002 to July 2003. Four age-matched controls per case were randomly selected from population registries. Food control authorities and laboratories were contacted to identify food contaminated with *S.* Agona. All human and food isolates were subtyped by phagetyping and pulsed-field gel electrophoresis.

Results: Overall, 40 geographically dispersed cases were identified; 31 cases and 130 controls were included in the case-control study. Of the cases, 68% had consumed tea products containing aniseed compared to 7% of the controls (OR 28, 95% CI 10-78). In logistic regression, this risk factor was confirmed (adjusted OR 31, 95% CI 10-95), whereas breast-feeding was protective (OR 0.2, 95% CI 0.1-0.7). In 33% of the cases (controls 15%) tea had not always been prepared with boiling water. Of 568 samples of herbal teas and aniseed of different companies 6% were positive for *S.* Agona. Subtyping of human, tea and aniseed isolates revealed identical strain patterns.

Conclusions: There is strong epidemiological and microbiological evidence that contaminated aniseed in herbal tea was the vehicle for the outbreak. This important source has not been previously identified in gastroenteritis outbreaks. As surveillance only captures a proportion of cases the true number of affected infants is likely to be higher. Affected tea products were recalled and consumers were advised to only use boiling water for tea preparation. A discussion about improved safety measures in herbal tea production has started among producers.

S-A19

An outbreak detection system for *Salmonella* in British livestock: a risk communication tool

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Early warning systems are commonplace within the public health arena providing governments with information on the timely detection of potential outbreaks of disease in the human population. However, their application to animal health has been limited due to the belief that statistical models for public surveillance data cannot be fitted to animal health surveillance data. This issue was investigated using *Salmonella* as a case study. *Salmonella* is notifiable in the UK, and hence all isolations from livestock are reported. These reports have been collated centrally at the Veterinary Laboratories Agency since 1993. After reviewing this data, it was considered that a previously developed system for public health (Farrington *et al*., 1996) could be applied to a subset of the *Salmonella* data, that of samples submitted for clinical illness with *Salmonella* Typhimuirum. The fitted log-linear regression model accounts for seasonality and past outbreaks in the data set, which are both important criteria for this specific serotype. On a monthly basis, using the fitted regression model an expected and threshold count of *S.* Typhimurium is derived for each animal species in the database. If the current observed count is above the estimated threshold value, a warning is implemented indicating that a potential outbreak of *S.* Typhimurium is occurring in the field. Based on the results, it was concluded that, for this subset of data, the use of an early warning system for animal health is feasible and is an important tool for enhanced surveillance.

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S-A20

Large outbreak of haemolytic uraemic syndrome associated with sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H- in Southern Germany, October to December 2002

Alpers, K.*[1]; Werber, D.[1]; Frank, C.[1]; Koch, J.[1]: Behnke, S.[1]; Morlock, G.[2]; Heissen-huber, A.[2]; Maslo, D.[3]; Diedler, A.[3]; Friedrich, A.W.[4]; Mellmann, A.[4]; Prager, R.[5]; Fruth, A.[5]; Timm, M.[6]; Gallien, P.[6]; Gerber, A.[7]; Zucs, P.[1]; Stark, K.[1] and Ammon, A.[1]

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Background: Haemolytic uraemic syndrome (HUS) and Shiga toxin-producing *Escherichia coli* (STEC)-infections are notifiable in Germany. By November 2002, 6 HUS patients with disease onset in October had been reported from the state of Bavaria (Southern Germany). Concomitantly, the national HUS reference laboratory cultured sorbitol-fermenting *E. coli* O157:H- (sf O157:H-) from stools of 10 HUS-patients during only one month. From 1997 to 2000, this strain had only been isolated from 26 cases. An investigation was started to identify the outbreak's extent, source, and risk-factors in order to implement control measures.
Methods: We contacted all paediatric nephrology units and reference laboratories to timely identify and explore patients. We defined cases as HUS-patients with disease onset between 1 October and 31 December 2002. Cases with culture positive for sf O157:H- and a PFGE-pattern not differing from the outbreak strain were considered as confirmed, probable cases had evidence of O157 infection and possible cases had no microbiological evidence. We conducted a case-control study among probable and confirmed cases with disease onset between 1 October and 10 December 2002. Controls were randomly selected among Campylobacter notifications, frequency-matched on federal state and age-group. Clinical and environmental samples were investigated at the reference laboratories, isolates were subtyped by pulsed-field gel electrophoresis (PFGE). Patients' sera were screened for O157-antibodies.
Results: We ascertained 48 HUS patients between 1 October and 31 December 2002, 22 were classified as confirmed and 13 as probable. There was no corresponding increase in the notification of STEC-patients with gastroenteritis only. Two confirmed and 2 probable cases died during the acute phase of the disease (case-fatality ratio 11.4%). Of the laboratory confirmed cases, 86% lived in Germany's two southern states Bavaria and Baden-Württemberg, 32% were of foreign origin. German patients with sf O157:H- were significantly more often living rurally (place of residence <50,000 inhabitants) than the local census population (p=0.02). 16 cases and 67 controls were enrolled in the case-control study. Seven (44%) cases and 12 (18%) controls had consumed small-scale produced apple-juice (OR_{MH}=3.6; 95% CI=1.1-11.5). Apples came from different orchards and apple-juice from different sources. Likewise, 10 (63%) cases and 21 (31%) controls had consumed a yoghurt product (OR_{MH}=4.2; 95% CI=1.2-14.6). Yet, different brands of yoghurt were involved. All environmental samples were negative. Traceback investigations were difficult and remained inconclusive.
Conclusions: German patients resided predominantly in rural areas. Consumption of small-scale apple-juice and a yoghurt-product were statistically associated with HUS. However, no common source could be identified. More timely and complete reporting of cases is needed and the development of a protocol for traceback investigations. Further investigations are needed to discover the yet unknown reservoir of this virulent strain.

S-A21

A case-control study on risk factors for sporadic illness associated with Shiga toxin-producing *Escherichia coli*-infection in Germany

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Human illness due to Shiga toxin-producing Escherichia coli (STEC) infection remains an important public health problem, associated with considerable illness and death. Most reported outbreaks are associated with STEC O157. Similarly, all case-controls studies, except one, published thus far were restricted to this serogroup. However, the importance of non-O157 strains has been well established. In Germany, where STEC-screening is based on detection of Shiga toxin in stool or their genes in stool enrichment cultures, the majority of statutorily reported STEC-infections with known O-type belongs to non-O157 serogroups.

A case-control study was conducted between 01 April 2001 and 31 March 2003 on risk factors for sporadic STEC-associated illness in Germany. Case-patients were identified via laboratory-based surveillance and defined as persons who showed clinical symptoms, who were not part of a recognized outbreak, and whose stool samples contained a Shiga toxin gene detected by polymerase chain reaction. One age- and telephone-exchange matched control-subject was selected for each case-patient. Interviews of cases and their controls using a standard structured questionnaire were conducted by Health Departments responsible for the patient.

Overall, 202 matched case-control pairs were analyzed. The median age of patients was 2.5 years (range: 3 months-89 years), 101 (50%) patients were female. Among the isolated STEC, O103 was the most frequently typed serogroup (21%) followed by O157 (15%). In single-risk variable analysis, contact to ruminants, consumption of food of ruminant origin, and indicators for person-to-person transmission were associated with illness ($p<0.05$). Of importance is that the risk set strongly depended on the age of patients. For example, contact to ruminants and indicators for person-to-person transmission were more important in young children (<3 years) than in older patients. Conversely, risk of illness due to food-exposures was more elevated in adolescents and adults.
Results of multiple regression analysis are pending and will be presented at the conference.

S-A22

VTEC O157: Changes in the characteristics and epidemiology of the organism Laboratory and enhanced surveillance in Scotland, 1993-2002

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Verocytotoxigenic *Escherichia coli* O157 (VTEC O157) has become a cause of significant human morbidity and mortality since first reported in Scotland in 1984. Higher rates of infection are consistently reported in Scotland, than elsewhere in the UK or Europe. Whilst three large outbreaks of *E.coli* O157 have occurred in Scotland, two of which were foodborne, most cases are sporadic. Animal faecal and environmental exposures were the strongest risk factors for sporadic cases.

Data from routine surveillance for the decade 1993 to 2002, and from enhanced surveillance 1999 to 2002, were analysed for temporal trends or variations. From 1993 to 2002, a total of 2,709 laboratory confirmed cases were reported to SCIEH, identified from faecal isolates. The average annual rate per 100,000 cases was 5.3 (range 2.3 to 9.9 cases per 100,000).

There was a major shift in phage types (PT) over the ten year period. PT21/28 now predominates, whilst PT2 (responsible for major outbreaks in 1994 and 1996, affecting 100 and 500 people respectively) and PT49 have declined in frequency. PT21/28 outweighs other PTs by a greater margin than PT2 previously.

There were substantial variations in age related incidence. Whilst the central Scotland outbreak in 1996/1997 involved large numbers of adult and elderly cases, overall figures for the ten year period reinforced the vulnerability of children under 10. Rates of infection in this group tended to increase, whilst rates for other age groups remained stable.

Seasonality over the ten year period was also affected by the central Scotland and other large outbreaks. There were two annual peaks in most years, with the majority of cases occurring in the third quarter of the year, except for 1996/1997.

Regional rates of infection within Scotland changed over time. The highest rates were consistently reported from the north east, but in recent years the next highest rates were reported from the south west. In some areas outbreaks led to fluctuating annual rates, but other regions not reporting substantial outbreaks still experienced changing rates year to year.

Enhanced surveillance provided additional data, previously unavailable on a national scale, and identified for instance that the proportions of cases hospitalised, or asymptomatic, were largely stable over recent years. From 1999 to 2002 the majority of cases (78%) were sporadic, but the proportions of outbreak and sporadic cases varied from year to year ($P = 0.04$).

Some of these changes were identified from routine reporting; others were identified or confirmed only because enhanced surveillance provided more detailed data. These findings suggest that VTEC O157 may continue to evolve, and highlight the need for ongoing enhanced surveillance on a population basis.

S-A23

Relevance of coordinated international capacity building on surveillance, food contamination monitoring and food control systems in the prevention of foodborne diseases.

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WHO endorsed a Global Strategy for Food Safety in 2002 to decrease the burden of foodborne diseases. The approaches of the new WHO Global Strategy on Food Safety include strengthening of surveillance and food contamination monitoring systems, improved risk analyses, consideration of public health issues by the FAO/WHO Codex Alimentarius, ensuring the safety of new technologies, and support capacity building through international cooperation.

In 2002, FAO and WHO organized the Pan European Conference on Food Safety and Quality
to identify and discuss the ways to strengthen food safety in the Region. Specific recommendations were made on surveillance, including the need for improving data collection and reporting, both at national level and to the WHO Surveillance Programme for Control of Foodborne Diseases in Europe. Additionally, the collection of food microbiology data for risk assessment to assist Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Food (JEMRA) was recommended.

In this context, the WHO Regional Office in Europe is supporting member states to build their capacity on laboratory based foodborne disease surveillance and food contamination monitoring and to strengthen their intervention epidemiological skills and response systems. Particular efforts have been done in the Central Asian Republics under the Multi-country Public Health Initiative on Food Safety in collaboration with FAO, the Centers of Disease Control among other partners. Training on laboratory based surveillance is being provided to Russian speaking New Independent States under the framework of the WHO Global Salmonella Surveillance activities, and the third level of the WHO Global Salm-Surv training courses for Central Eastern Europe, Baltics, and South Eastern Europe will be completed this year. The WHO Global Salm-Surv network provides capacity building on surveillance worldwide.

Technical assistance to strengthen food safety strategies, food control and surveillance systems is being provided by the WHO Regional Office in Europe, in collaboration with the Council of Europe, FAO, the EC, the Food Safety Authority of Ireland and other agencies, to several member states and in particular to South Eastern European countries under the framework of the Stability Pact Initiative for social cohesion in the Balkans.

WHO is also providing countries with guidance to establish and strengthen prevention and response systems to meet the threat of any food safety emergency including terrorist threats to food. The strategy at the regional level previews that actions to respond to the threat from deliberate use of biological agents as weapons should rest primarily on the existing programmes and networks (for example the surveillance programmes, food safety network, drinking water producers networks, etc.).

This paper will further discuss the role of WHO in assisting countries to build their capacity on surveillance, response, food contamination monitoring and control. The need for improving the cooperation and coordination on capacity building at the regional and international level to build on previous experience, to promote synergies and avoid duplication of efforts will be specifically addressed.

S-A24

ObSurv: The surveillance of outbreaks of infectious intestinal disease in Scotland

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In 1996, the Scottish Centre for Infection and Environmental Health established “ObSurv”, a surveillance system for general outbreaks of infectious intestinal disease (IID) in Scotland. For the purpose of ObSurv an outbreak is defined as an incident in which two or more linked cases experience the same illness, or when the observed number of cases unaccountably exceeds the expected number. The system seeks information on general outbreaks, defined as outbreaks affecting members of more than one household or residents of an institution.

Between 1996 and 2003, a total of 1124 outbreaks of IID were identified at SCIEH and completed outbreak report forms returned by the local NHS Boards for 87% of these outbreaks.

Norovirus (confirmed or suspected) has been the most frequently reported agent; 51% of all outbreaks, a further 22% of outbreaks were reported as viral and 6% as unknown aetiology, many of which were probably norovirus.

With respect to outbreaks of bacterial aetiology, the most frequently identified agent was salmonella, accounting for 7% of all outbreaks of IID, *E. coli* accounted for 6% and campylobacter for 2%. Bacterial outbreaks were also identified where the causative agent was *Bacillus cereus, Clostridium perfringens, Clostridium difficile, Shigella sonnei, Staphylococcus aureus* and *Yersinia enterocolitica.*

For outbreaks of salmonella infection, *S.* Enteritidis accounted for 59% of such outbreaks, with the second most prevalent type *S.* Typhimurium responsible for 24%. Restaurants were the most frequently reported location for outbreaks of salmonella infection accounting for 33%, however 11% were hospital associated. Foodborne transmission was reported for 73% of salmonella outbreaks. The numbers affected by outbreaks of salmonella infection ranged from 2 to 204 persons.

All but one outbreak of *E. coli* were associated with serotype O157, with the remaining outbreak associated with O111. Private houses were the most frequently reported locations accounting for 28%, only 7% of *E. coli* outbreaks were associated with restaurants. Foodborne was the most frequently identified mode of transmission reported from 27% of outbreaks, person to person transmission was considered the main mode of transmission in 24% of outbreaks. Outbreaks ranged in size from 2 to 512, the largest outbreak affecting 512 persons, occurred in 1996 and was a foodborne outbreak associated with a butchers shop.

Protozoal outbreaks of IID were also identified through ObSurv. Cryptosporidium was responsible for 14 outbreaks, representing 1% of all outbreaks and Giardia responsible for just three outbreaks.

S-A25

The eXplosie-project: a one-year intensified study of outbreaks of gastroenteritis in the Netherlands.

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In 2002, in the Netherlands a national study of outbreaks of gastroenteritis was performed. An outbreak was defined as the occurrence of diarrhoea and/or vomiting in at least five cases with some common factor. Epidemiological information was collected by the Public Health Services (PHS) and the Food Inspection Services (FIS). Data included a.o. number of cases, number exposed, number hospitalised, number died, setting of the outbreak, most likely transmission route, and contributing factors in causing the outbreak (for foodborne outbreaks only). Stool samples were collected for diagnostic testing. For foodborne outbreaks, also food samples were taken. In total, 281 gastroenteritis outbreaks were reported, mainly from nursing homes and homes for the elderly (57%), restaurants (11%), hospitals (9%) and day-care centres (7%). In total, at least 8,717 cases (range 5 to 151 cases per outbreak) were affected, of which 62 (0.7%) were hospitalised and 16 died (0.2%). Direct person-to-person contact was the most likely route of transmission (78%), followed by foodborne transmission (13%) and a combination of foodborne and person-to-person transmission (8%). However, for restaurant-associated outbreaks, as expected, food was the dominant transmission route (88%). For all outbreaks, norovirus was the most common pathogen (54%), followed by Salmonella spp. (4%), rotavirus group A (2%), Campylobacter spp. (1%) and only incidentally others. Of the 59 foodborne outbreaks, based on stool analyses, 25% was caused by norovirus, 17% by Salmonella, 5% by Campylobacter, 2% by Shigella and 2% by C. perfringens. The remainder of the foodborne outbreaks was unexplained. For 38 of the foodborne outbreaks the FIS questionnaire was available, in which the contributing factors were reported. Most common were, often combinations of, temperature misuse (13 outbreaks; 2 norovirus, 1 *C. perfringens*, 1 *C. jejuni,* 9 unexplained), inadequate processing or preparation, such as cross contamination, insufficient hygiene or use of contaminated raw material (7 outbreaks; 3 *S.* enteritidis, 1 *C. perfringens*, 1 *C. jejuni*, 2 unexplained) and environmental factors, such as contamination by personnel or equipment (5 outbreaks; all norovirus). Contributing factors were reported unclear for 45% of these outbreaks. Of the norovirus foodborne outbreaks, at least 31% was caused by contamination of food by food handlers (no information for 44%). Of the Salmonella outbreaks, for 5 (50%) consumption of food prepared with raw shell eggs was reported, and for an additional two this was suspected (consumption of chocolate mousse). For the remaining three Salmonella outbreaks, meat was reported as suspected vehicle. In conclusion, most gastroenteritis outbreaks were reported from health and residential institutions, with norovirus as dominant agent. Control should target at reducing person-to-person spread. In foodborne outbreaks norovirus was common, due to contamination of food by food handlers. Salmonella, as the second foodborne pathogen, was mainly associated with raw shell eggs. These results stress the continuous need for food safety education, complementary to governmental regulation.

S-A26

Electronic outbreak reporting system for outbreaks of foodborne infections in Germany

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Outbreaks of infectious diseases deserve major attention in surveillance because they are signs for deficiencies in infection control management and often require intervention by health departments and departments for food safety. According to the directive 2003/99/EC of the European Parliament and the Council Member states of the European Union need to report food-borne outbreaks to the European Union. Therefore a system is needed to allow standardised collation and analysis outbreak data on national and international level. We developed a system to allow, up-to-date and easily retrievable epidemiological data management of outbreaks on all administrative levels.

Methods: Case-based data in the German notifiable diseases reporting system are administered and forwarded electronically via a distributed SQL database developed by the Robert Koch Institute (RKI). Outbreak reporting is integrated into this system by linking individual cases to groups. On the group level, standardised descriptive data is entered describing the source of the outbreak (using the Eurocode-2) and the evidence that lead to the identification. The system generates automated reports, based on case specific data. Multiple outbreaks can be linked to meta-outbreaks, allowing multi-state outbreaks to be analysed. For the presented data, only outbreaks with a size of 5 or more cases are taken into account.
Results: In the year 2003 6629 outbreaks were reported through this system (2001 = 4220; 2002 = 7256). Of these outbreaks 2676 (40%) were caused by Salmonella and had a median duration of 3 days, 1499 (23%) by Norovirus (8d), 1105 (17%) by Rotavirus (10d), 488 (7%) by Campylobacter (4d).
Discussion: The electronic outbreak reporting system has already in the fist years of its implementation provided important information relevant for public health interventions. It shows the immense amount of outbreaks already identified by local health departments. A revision of the system in 2003 is expected to further reduce their workload and at the same time improve the ability for flexible retrieval of data. The system could serve as a blue print for multi-national outbreak reporting systems such as required for the European Union zoonosis regulation.

S-A27

Implementation of an electronic food surveillance information system for food law enforcement officers in Dublin, Ireland

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The Food Safety Authority of Ireland was established to ensure the highest standards of food safety and hygiene and compliance with legal requirements in Ireland. The Authority retains ultimate responsibility for enforcement but most activities are undertaken on its behalf by agencies on the basis of service contracts. Ireland has a population of 4 million and more than one third live in the capital city Dublin.

The Environmental Health food control service has recently implemented an electronic food surveillance information system for food law enforcement officers in Dublin, Ireland.
The development of a computerised information system is a collaborative process and the project needs to be managed carefully from the outset. The most common arrangement is to have an information systems strategy group, a steering committee and a systems development team in place prior to the commencement of the selection process.

Communication with all the major food control stakeholders is essential and a lack of user input will cause problems, as expectations often influence satisfaction levels. It is important to embrace the process of change and three key areas namely technical, infrastructure and organisational must be monitored. User feedback may be channelled through the project groups.

The computerised food surveillance information system is modular. The modules include a Food Premises Database, Inspection Planner, Visit History, Sampling, Registration, Licensing, Complaints, Food Alerts, Reports and Statistical Returns. The system enables the user to schedule the inspection frequency and record inspection results in a timely manner. However the selected system will require adaptation because the technology will never exactly fit the user environment.

The introduction of a computerised food surveillance information system will establish and achieve a uniform and standardised approach to food control inspections, reports and EU Returns. Understanding that the development and implementation of a computerised information system is a collaborative process between managers, users and information technology staff at all levels within the organisation is crucial. Ultimately it is how the interaction of people and technology is managed that will mean the difference between success or failure of the introduction of an information system.

S-A28

Foodborne diseases surveillance in Andorra: Implementation, improvements and opportunities.

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Background:
Andorra is a little country in extension and population but there is a high influx of tourists. For this reason, report and investigation of food borne disease outbreaks are specially important. This paper studies the evolution of food borne disease outbreaks reported from years 2000 to 2003 and the consequences that measures taken by the Ministry of Health and Welfare may have on it. These measures include: An early warning system available 24 hours every day of the year for general practitioners and Hospital emergency service, publication of a good practices guide for food handlers and promotion of training courses for food handlers.
Subjects and methods:
Retrospective review of documentation regarding food borne disease outbreaks notified from years 2000 to 2003 in Andorra country.
Results:
Number of notified outbreaks increased sharply when the early warning system was established at the beginning of the year 2002. Specifically, in 2002 it was registered an increase of 1,100% (11 reported outbreaks for each outbreak reported the previous year). In 2003 the number of notified outbreaks decreased a 63.6% compared with the year 2002. Besides the largest part of notified outbreaks (76%) appear during the first trimester of the year.
Conclusions:
These results show that thanks to the establishment of an early warning system, much more food borne disease outbreaks could be detected, specially during winter time with the major influx of visitors. At the same time, this increase in detection allowed better knowledge of food borne disease contributing factors and other features, which were essential to design suitable protection and prevention measures (publication of a food handlers good practices guide and promotion of training courses for food handlers). When these measures were put into practice we achieved a decrease of food borne disease outbreaks.

S-A29

Distribution of *Mycobacterium avium* subsp. *hominissuis* isolates in tissue samples of pigs reared on deep litter or fed with peat and kaolin as a supplement

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In early 1999, there was an increased incidence of tuberculous lesions in the lymph nodes of 4 667 251 slaughtered pigs in the Czech Republic with 10.2 mil. of inhabitants. In the first part of the study tuberculous lesions were detected in 140 (62%) tissue samples collected at routine veterinary-hygienic examinations in abattoirs from pigs coming from 15 farms from 15 of 75 districts. Mycobacteria were isolated from 37 (16%) tissue samples: 34 *Mycobacterium avium* subsp. *hominissuis* isolates (serotypes 8 and 9) and three atypical mycobacteria. In search of infection sources esp. *M. a. hominissuis* was isolated from 38 (79%) samples of peat and from 6 (2.3%) samples of kaolin used as feed supplements. Mycobacteria (esp. *M. a. hominissuis* of serotypes 8 and 9) were also isolated from 11 (91.7%) of 12 sawdust samples of the used bedding and in only two (16.7%) of 12 non-used sawdust samples. Identical IS*1245* RFLP types of *M. a. hominissuis* were detected in two peat and lymph node isolates from one pig farm and in two sawdust isolates and one pig isolate from another pig farm. In the second part of our study, 117 randomly selected slaughtered pigs from one farm with young piglets fed with peat as a supplement were investigated in head, mesenteric and inguinal lymph nodes for mycobacterial infection. From 65 (55.6%) pigs, 76 mycobacterial isolates were identified (56 *M. a. hominissuis*, 12 atypical mycobacteria, five *M. a. avium* and three *M. intracellulare* isolates). IS*1245* RFLP types with more than 20 bands of 45 distinct RFLP types were found in 49 *M. a. hominissuis* isolates from pigs (n = 31) and peat (n = 18) from one farm. Identical RFLP types were found in only four pigs' isolates. Five randomly selected isolates from pigs and peat were subcultured to six independent clones/colonies. Among IS*1245* RFLP types of 30 clones, identical RFLP types obtained from pigs and peat were identified, which confirmed the hypothesis, that peat contaminated with mycobacteria represents a significant source of mycobacterial infection for pigs. In the years 1990 to 2002 *M. tuberculosis* was bacteriologically confirmed in a total of 14 891 patients (relative number of bacteriologically confirmed cases decreased from 14.9 to 7.8 per 100 000 inhabitants). In the same period *M. avium* complex isolates were bacteriologically confirmed in a total of 689 patients (relative number of bacteriologically confirmed cases ranged between 0.28 and 0.95 per 100 000 inhabitants). More than 77% of patients were older than 50 years and during the last 6 year increasing incidence from 0.28 to 0.95 per 100 000 inhabitants was documented.

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S-B01

Experimental studies in pigs on behaviour and diagnosis of different *Trichinella* species

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Trichinellosis in humans is a parasitic zoonosis acquired by the consumption of raw or insufficiently treated meat containing infectious *Trichinella* (*T.*) muscle larvae. Obviously, *Trichinella* is an emerging and re-emerging foodborne agent as well. So far *T. spiralis*, *T. nativa*, *T. britovi* and *T. pseudospiralis* were discovered in the domestic and/or sylvatic cycle in Europe. Besides horse and wild boar meat, pork was identified as most important infection source for man. In Eastern European countries like Serbia, Croatia and Romania thousands of people acquired trichinellosis due to consumption of pork from the beginning of 1990's. More than 1800 trichinellosis cases traced back to outdoor pigs were notified from EU Member States (Spain, France, Italy and Germany) during the last two decades.
TRICHIPORSE is a research project of the EU which deals with current questions of risk assessment with special regard to diagnosis and epidemiology of *Trichinella*. One workpackage is focussed on experimental infection of pigs with *Trichinella* species which occur in Europe with special concern to host-parasite interaction and use for diagnostic purposes. In 4 separate trials 3 SPF pigs per group were inoculated with 200, 1,000 and 20,000 muscle larvae of *T. spiralis* (strain ISS 004), *T. nativa* (ISS 010), *T. britovi* (ISS 002) and *T. pseudospiralis* (ISS 013), respectively. Muscle samples of 50 to 100 g from diaphragm, tongue, masseter, shoulder, foreleg, hind leg, abdomen, intercostal and cutlet were obtained post mortem for counting larval recovery rate (larvae per g of muscle = LpG) by artificial digestion (magnetic stirrer method). Blood samples were taken twice prior and at 5 days and at 10 days intervals up to day 30 and 60 post infection, respectively. Serum samples were examined for anti-*Trichinella*-IgG by means of ELISA based on E/S antigen from *T. spiralis* muscle larvae.
According to post mortem findings in muscle, susceptibility in pigs was high in *T. spiralis*, moderate in *T. britovi* and *T. pseudospiralis* and remained very low in *T. nativa* inoculation. Larval recovery rate corresponded with infection dose. For pigs infected with 200, 1,000 and 20,000 larvae, respectively, the mean recovery rate was 2.8, 15.9, 416.8 LpG for *T. spiralis*, 0.01, 0.07, 3.53 LpG for *T. britovi*, 0.002, 0.043, 9.66 LpG for *T. pseudospiralis* and 0, 0.006, 0.02 LpG for *T. nativa* inoculation. In most cases tongue, diaphragm and masseter were identified as predilection muscles. ELISA results showed a high cross-reactivity between *T. spiralis* E/S antigen and specific serum antibodies induced by the different four *Trichinella* species. Anti-*Trichinella* IgG was detected at days 25 to 40 p.i. in pigs infected with 20,000 larvae and seroconversion occurred later or was not detectable in pigs infected with 1,000 and 200 larvae, respectively. Results emphasise the problem of "diagnostic window" in the early stage of infection and the need to improve such detection methods. Serological methods are not considered as alternative for classical meat inspection but may be useful as diagnostic tool for testing of living pigs in connection with certification of *Trichinella*-free farms.

S-B02

Detection of trichinellosis in swine by ELISA using an excretory-secretory antigen- comparison of antigens from 4 different *Trichinella* species

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Serodiagnostic methods are used mainly for ante-mortem and post-mortem examination of blood or serum samples for *Trichinella*-specific antibodies, and under some conditions may have a higher sensitivity than methods of direct detection. The ELISA method is recommended for farm surveillance programs and may be useful for epidemiological studies in wildlife or for establishing *Trichinella*-free areas. The suitability of a serological detection method depends on specific factors of the test system and characteristics of host immunity. The test antigen is considered to be an important factor for the specificity of the test result.

In this study our interest was to compare the specificity and the time of seroconversion of excretory-secretory (E/S) antigens prepared from four *Trichinella* species (*T. spiralis*, *T. britovi*, *T. nativa* and *T. pseudospiralis*). Groups of pigs were inoculated with 200 or 20 000 *Trichinella* larvae per animal and blood sampling was performed every three days. Antibody responses were detected by any of four different *Trichinella* E/S antigens, but the time of seroconversion and dynamics differed among the experimental groups and correlated with the infection dose. The earliest seroconversion was found in pigs infected with 20 000 *T. spiralis* larvae per animal (12 days post inoculation) and was stable or increased slightly throughout the experimental period (60 days post inoculation). In contrast, pigs infected with 200 *T. pseudospiralis* larvae per animal seroconverted at 57 days post inoculation. Homologous E/S antigens were more sensitive in detecting antibodies to the corresponding *Trichinella* species. Our results point to the potential use of one common antigen for surveillance and epidemiological studies on both domestic and sylvatic *Trichinella* in pigs and represent important data for validation of a serological test, especially if blood samples are taken during early stages of infection.

S-B03

Virulence factors and phenotypic traits of non-O157 STEC isolated from ruminants in Switzerland

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Shiga toxin-producing *Escherichia coli* (STEC) has emerged as a pathogen that can cause food-borne infections and severe and potentially fatal illness in humans. STEC are a cause of human gastroenteritis that may be complicated by hemorrhagic colitis (HC) or hemolytic-uremic syndrome (HUS).

STEC strains causing human infections belong to a large, still increasing number of O:H serotypes. Most outbreaks and sporadic cases of HC and HUS have been attributed to O157:H7 STEC strains (Blanco et al., 2001; Chapman et al., 2001). However, especially in Europe, infections with non-O157 STEC strains, such as O26:H11 or O26:H-, O91:H-, O103:H2, O111:H-, O113:H21, O117:H7, O118:H16, O121:H19, O128:H2 or O128:H-, O145:H- and O146:H21, are frequently associated with severe illness in humans (Beutin, 1999; Boerlin et al., 1999; Blanco et al., 2001). Pathogenicity of STEC is associated with various virulence factors. The main factor is the ability to form Shiga toxins that can be subdivided into a Shiga toxin 1 group (Stx1) and a Shiga toxin 2 group (Stx2). Characterizations of the *stx*1 and *stx*2 genes revealed the existence of different variants in both Stx groups. Apart from the capability to produce Shiga toxins, STEC may possess accessory putative virulence factors such as intimin (*eae*), STEC autoagglutinating adhesin (*saa*) or enterohemolysin (*ehxA*). Characterization of *eae* genes revealed the existence of different *eae* variants.

Ruminants, especially small ruminants, represent the most important reservoir of STEC (Beutin et al., 1997; Djordjevic et al., 2001; Kobayashi et al., 2001; Hornitzky et al., 2002). However, STEC have also been isolated from a variety of animal species (pigs, horses, chicken, pigeons, rabbits, dogs and cats). In Switzerland, our examinations of ruminants showed for cattle a non-O157 prevalence of 14% (cattle at slaughtering) and 5.4% (cattle carcasses) and for sheep a non-O157 STEC prevalence of 29.9% (sheep at slaughtering) and 36.6% (sheep carcasses), respectively.

In view of the high STEC prevalence and in view of the lack of characterization data from bovine and ovine non-O157 STEC in Switzerland, bovine (n=40) and ovine (n=60) non-O157 STEC strains were further characterized by (i) determining the serotypes of STEC strains isolated from ruminants in Switzerland, (ii) further characterizing Shiga toxin subtypes (*stx*1 and *stx*2 variants) and virulence factors (*eae, saa, ehxA*). Moreover, possible associations between these factors and the potential pathogenicity of these strains for humans are discussed.

S-B04

Development of a Triplex PCR System for rapid detection and species level identification of *Mycobacterium avium* subsp. *paratuberculosis*

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Over the past few years *Mycobacterium* subsp. *paratuberculosis* (MAP) a causative agent for paratuberculosis (Johne's disease) in ruminants has also been linked with a possible aetiological role in human Crohn's disease. The causal agent in human Crohn's disease remains unknown but there has been significant evidence suggesting that MAP may be a causal agent in some of the human CD cases (Sanderson et al, 1992; Fidler et al, 1994; Mishina et al, 1996; Cellier et al, 1998; Clarkston et al, 1998; Kanazawa et al, 1999; Bull et al, 2003). This has raised concerns that people may acquire MAP infections through consumption of food such as meat and milk derived from animals with clinical or subclinical Johne's disease. Studies on the aetiological role of MAP in human disease or the role of food of animal origin in its transmission are hampered by difficulties in organism isolation and detection. Therefore development and application of molecular methods in MAP detection methods will aid in resolving the pathogenic role of MAP as well as determining the role foods of animal origin in its transmission to the human population. The PCR based detection of MAP provides a fast diagnostic tool ideal for large scale screening of potential sources of MAP infection. The current PCR detection systems are based on amplifying the IS900 multicopy genetic insertion element found in the MAP genome. However, there are some drawbacks with these methods as highly homologous IS900 like insertion elements are also found in other mycobacteria besides MAP (Vary et al, 1990; Miller et al, 1997; Cousins et al, 1999; Englund et al, 2002). This has meant that interpretation of positive PCR results based on the current IS900 PCR systems are prone to confounding by false positives. Thus, in two recent survey of bulk-tank milk samples in Switzerland using an IS900 based PCR system a prevalence of 19.7% was found (Corti et al. 2002, Muehlherr et al. 2003). This might be indicative of high level of MAP infection in the Swiss dairy herds, which would be of concern. On the other hand it may be significantly confounded by false positive signals from other environmental mycobacteria also present in the raw milk samples analyzed. These concerns warrant development of more specific PCR systems that can clearly distinguish MAP from other closely related environmental mycobacteria species.

To enable subspecies level distinction of MAP in a single PCR assay, a multiplex PCR assay combining three genetic markers and an internal control has been developed. This allows the simultaneous detection of the mycobacterium 16SrRNA sequence, IS 900 insertion element sequence as well as the MAP species-specific F57 genetic element. The potential of this triple genetic marker PCR assay in subspecies level distinction of MAP was assessed through analysis of different MAP strains, several other mycobacterium strains and unrelated bacterial strains. In all cases, a clearly distinguishable MAP-specific triple band pattern was obtained for the three genetic markers clearly differentiating it from other closely related mycobacterium strains. Moreover this system was used in the routine screening of bovine and small ruminants raw milk samples for the presence of MAP. Using this simple multiplex PCR step, we show that MAP can readily be identified down to subspecies level thereby enhancing both the speed and reliability of PCR based approaches in the diagnosis of MAP.

S-B05

VTEC O157 in slaughter animals in the Gauteng Province of South Africa

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Verotoxin producing *E. coli* (VTEC), including O157 (H7/H-), have emerged as an important public health problem all around the world. The bacteria have been implicated in severe human diseases, including bloody diarrhea (hemorrhagic colitis) and hemolytic uremic syndrome (HUS) which is the main cause of renal failure in children. Outbreaks of *E. coli* O157 have been observed in many countries such as USA, Canada, Scotland, Spain and Japan. Ruminants, especially cattle and sheep, are considered the primary reservoir of both O157 and non-O157 VTEC bacteria. Cattle frequently carry VTEC without suffering from any pathological symptoms.

We conducted a study to determine the presence and prevalence status of *E. coli* O157 in South African red meat (bovine, ovine and porcine). Fecal material from the rectum and post chilling carcass swabs were collected from 1203 slaughter animal (330 cattle, 383 sheep and 490 pigs) at abattoirs in the Gauteng Province of South Africa. The study was conducted over four seasons between April 2002 and February 2004. Fecal material was collected directly from the rectum immediately after evisceration and the carcasses tagged to ensure that the same carcasses are sampled the next morning after chilling. The fecal samples were tested for the presence of *E. coli* O157 using a dynabeads immunomagnetic separation technique (Dynabeads® anti-*E. coli* O157, Dynal) followed by streaking onto Sorbitol MacConkey agar. Suspicious colonies were confirmed by a PCR assay for a gene within the O-antigen biosynthesis loci (*rfb*) of *E. coli* O157. *E. coli* O157 was detected in 83 of 330 (25 %) bovine fecal samples, 26 of 383 (7%) of ovine fecal samples and 129 of 490 (26%) porcine fecal samples. After the presence of O157 was established in the first two seasons, the corresponding carcass swabs of O157 positive fecal samples in the third and fourth seasons were also tested for the presence of the bacteria. Results show that carcass contamination with *E. coli* O157 was 9%, 2% and 4% in bovine, ovine and porcine respectively. There was a seasonal variation in the isolation rate of *E. coli* O157 with more isolation in the summer than in winter.

Prior to this study, the prevalence of *E. coli* O157 in South Africa was not known. Data obtained from this study indicate that slaughter animals shed this organism and it is transferred onto the carcass. The potential impact of this finding on public health needs further investigation.

S-B06

Fecal shedding of *Escherichia coli* O157 in Swiss cattle at slaughter

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Experts estimate that 76 million cases of human diseases, 325,000 hospital admissions and 5,000 mortalities are annually caused in the USA by the consumption of contaminated food (Mead et al. 1999). The importance of latent zoonoses has increased in recent years in view of foodborne diseases: (i) the "healthy" animal represents a reservoir for specific pathogens; (ii) no pathological-anatomical changes in the carcass and its organs show the presence of these pathogens; and (iii) these pathogens may enter the food chain via weak points in the slaughtering or milking process. Worldwide, *Salmonella* spp*., Campylobacter* spp. and Shiga toxin-producing *E. coli* (STEC) are among the three most important pathogens of foodborne diseases. The number of gastro-intestinal infections in humans associated with these pathogens has increased in the recent years (Rodrigue et al. 1990, Armstrong et al. 1996, Rautelin et al. 2000*)*.

Strict maintenance of good practices of slaughter hygiene in meat production is of central importance because microbiological hazards are not eliminated in the slaughtering process. Furthermore, to estimate the risks involved and to take appropriate measures, analysis of the slaughtering process should be complemented by collecting data related to the carriage of the animals of latent zoonotic pathogens.

Fecal samples from 2,930 slaughtered healthy cattle were examined with the aim: (i) to monitor the shedding of *E. coli* O157 in cattle; and (ii) to further characterize isolated strains. The percentage of the 2,930 samples testing positive for *E. coli* O157 by polymerase chain reaction was 1.6%. Thirty-eight strains from different animals agglutinating with Wellcolex *E. coli* O157 were isolated. Of the 6 sorbitol-negative strains, 5 strains tested positive for *stx*2 genes (twice *stx*2c and three times *stx*2) and one strain for *stx*1 and *stx*2c genes. All sorbitol-negative strains belonged to the serotypes O157:H- and O157:H7, and harboured *eae* type γ1 and *ehxA* genes. The 32 sorbitol-positive strains tested negative for *stx* genes, and belonged to the serotypes O157:H2, O157:H7, O157:H8, O157:H12, O157:H19, O157:H25, O157:H27, O157:H38, O157:H43, O157:H45 and O157:H-. The finding of a high number of sorbitol-positive *stx*-negative O157 strains with other H type than H7 was striking. However, these data agree with the results in one of our recent studies, where in minced meat samples (minced beef and minced pork) taken throughout Switzerland we found O157:H38, O157:Hru, O157:H2 and O157:Hnt strains, and none of these strains was positive for *stx* genes, too. Interestingly, all O157:H45 strains harboured *eae* subtype α1, and therefore seem to be enteropathogenic *E. coli* (EPEC) strains. The present study is the first, to our knowledge, that documents the detection of the *eae* α1 intimin variant gene in bovine *E. coli* strains.

S-B07

The occurrence of *Arcobacter* in living poultry and on poultry meat

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Arcobacters are gram negative bacteria and *Arcobacter butzleri, Arcobacter cryaerophilus* and *Arcobacter skirrowii* are the animal and human related species. Arcobacters are frequently present on food of animal origin, including poultry. Foods of animal origin are considered as a main source of *Arcobacter* infection in humans and, therefore, arcobacters are considered emerging foodborne pathogens.
Arcobacters are common on broiler chicken carcasses, skin and poultry meat samples. During slaughter, even before evisceration, hundreds to several thousands of arcobacters were detected per gram neck skin. Investigation revealed that *A. butzleri* and *A. cryaerophilus* are commonly present on slaughter equipment. However, contamination of poultry carcasses through slaughter equipment alone can't explain the high contamination levels (10^2-10^3/g neck skin) found on Belgian poultry products. The purpose of the present study was to examine if arcobacters belong to the natural chicken flora and to clarify if chickens themselves are responsible for the *Arcobacter* occurrence on poultry products.
Ten living birds, 120 cloacal swabs and ten swabs from the transport crates were collected from one flock before the start of the slaughter procedure. During slaughter, twenty neck skin samples from birds of the same flock were taken after defeathering along the processing line. The samples were transported to the laboratory under cooled conditions and processed within 4 h after sampling. *Arcobacter* selective isolation broth and *Arcobacter* selective isolation agar was used for the isolation.
Euthanasia was carried out on the ten living chickens and was followed by dissection. Direct isolation and enrichment cultures were performed on feathers, neck-, breast- and thigh skin, skin around cloaca and cecal content. Moreover, enrichment was performed on internal samples of gall, spleen, liver, cloaca, stomachs, crop and small intestine. Arcobacters were isolated from the swab samples after enrichment. Direct isolation and enrichment were performed on one gram of the twenty neck skin samples. All colonies on the plates were counted, picked and subcultured onto blood agar plates. Bacterial growth was harvested and identified at species level using a multiplex-PCR assay.
From the samples of the living chickens, *A. cryaerophilus* and *A. skirrowii* were isolated out of one cloacal skin sample. *A. cryaerophilus* was also found in the cloacal swab from the same bird, only after enrichment. No arcobacters were recovered from the 120 cloacal swabs. In contrast, arcobacters were found in seven of the ten swab samples of transport crates. Twenty isolates were identified with m-PCR as *A. butzleri* and *A. cryaerophilus*. *Arcobacter* was isolated from all the twenty neck skin samples collected along the processing line. By direct isolation, *A. cryaerophilus* was isolated from six samples and *A. butzleri* and *A. cryaerophilus* were simultaneously present in the fourteen other samples. After enrichment, *A. butzleri* was isolated from seventeen samples, and *A. butzleri* and *A. cryaerophilus* were simultaneously present in the three other samples.
In conclusion, results suggest that arcobacters do not belong to the natural poultry flora. The carcass contamination must therefore have another unknown source.

S-B08

Human brucellosis in Germany: Clinical and laboratory observations

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Brucellosis is a ‘re-emerging’ zoonosis causing abortion in infected animals and a severe, potentially life threatening multiorgan disease in humans. Approximately 500,000 new cases of human brucellosis are reported worldwide every year. In Germany up to 35 patients suffering from brucellosis were notified each year during the last decade. Currently, brucellosis in Germany is a travel-associated infection or may occur as an occupational disease. In endemic areas, i.e. the Mediterranean countries, the Middle East, Africa, Asia, Central and South America, the infection is mainly transmitted by ingestion of contaminated raw milk and unpasteurized cheese or direct contact with the animal reservoir. Only four out of six nomen species of the genus *Brucella* (*B.*) are pathogenic for humans: *B. melitensis*, *B. abortus*, *B. suis* and *B. canis*.
Punctate, nonpigmented and nonhemolytic colonies and microscopically tiny, faintly stained, gram negative coccobacilli which are oxidase- and urease-positive should be suspected of being *Brucella*. Definite diagnosis of the disease is based on ‘direct’ isolation of the bacteria but culturing of *Brucella* is time-consuming and often not successful. Therefore, elevated or rising titres of anti-*Brucella* antibodies and a history of possible exposure are generally accepted as an indirect proof of brucellosis.
In Germany, 35 and 27 cases of human brucellosis were notified in 2002 and 2003, respectively. 31 *Brucella* strains could be recovered mostly out of blood samples. They were sent to the German Reference Laboratory for Brucellosis in Berlin for further differentiation. CO_2 requirement, urease activity, growth in the presence of basic fuchsin and thionin dyes, agglutination with specific sera and phage sensitivity were investigated. Most of the isolates were identified as *Brucella melitensis* bv 1 or 2. Only one *Brucella suis* strain was detected. *Brucella* spp. were also correctly identified using the species-specific AMOS-PCR.
Because of the low incidence of human brucellosis (<0.03 per 100,000 citizens) in Germany and the unspecific clinical signs and symptoms, most physicians are not aware of the disease and consequently diagnosis is often delayed. The most frequent symptoms at the time of presentation were fever, weakness, back pain and arthralgia, myalgia, headaches, weight loss, scrotal pain and depression. The major clinical signs were arthritis, splenomegaly, hepatomegaly, lymphadenopathy and epididymo-orchitis. Abnormal haematological findings and liver function tests were seen in variable frequency. Neurological and cardiac complications were rarely described. Different combinations of rifampicin, doxycycline and aminoglycosides were used for antibiotic therapy.
In patients with fever of unknown origin human brucellosis has to be taken into consideration. As brucellosis is a ‘re-emerging’ zoonosis which is endemic in a lot of tourist areas and is the most frequent bacterial laboratory-acquired infection, the awareness of the clinician has to be increased especially in ‘brucellosis-free’ countries.

S-B09

Isolation and characterization of toxigenic *Aeromonas* from seafood in Germany

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Certain strains of *Aeromonas* species are known to produce potent cytotoxins and were described as agents of diarrhea, renal failure and extraintestinal infections in humans. *Aeromonas* is known to be associated with an aquatic environment and we were interested in the significance of toxigenic *Aeromonas* as a contaminant in seafood. In 2000, we have purchased 84 samples of seafood from retail traders in Berlin, Germany and have investigated these for the presence of cytotoxic *Aeromonas* species. The samples were grouped as follows: raw fish (n=21), smoked fish (9), young salted herring „Matjes"(13), fresh salmon (11), sushi (12), deep freezed fish products (5), boiled fish (1), mussels (4), fish and seafood salad (5), herring products (2) and algae (1). *Aeromonas* were detected in 27 samples originating from raw fish (90.5% positive), mussels (75.0%), smoked fish (22.2%), „Matjes" (15.4%) and algae (100%). A total of 130 *Aeromonas* strains were isolated on plating media such as Blood-agar, GSP-agar and Ryan's Aeromonas medium, and the strains were further characterized by their biochemical reactions. *Aeromonas hydrophila* was found as most frequent (68.5%), followed by *Aeromonas caviae* (26.9%) and *Aeromonas sobria* (4.6%). All *Aeromonas* strains did grow at 37°C and 28 (21.5%) of these produced cytotoxins at 37°C when examined with the Vero cell toxicity test. The 28 cytotoxic *Aeromonas* strains originated from 17 samples: raw fish (14), „Matjes" (2) and mussels (1). Toxin associated genes such as aerolysin A and hemolysin A were detected in 26 of the 28 toxin producing *Aeromonas* strains. Our data indicate that fish and fish products can harbor potential human pathogenic, cytotoxin producing *Aeromonas* strains.

S-B10

Comparative study among *V. alginolyticus* strains isolated from bivalve mollusks retailed from markets in Venice (Italy) and Rio de Janeiro (RJ-Brazil)

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Vibrio alginolyticus is widely distributed in marine environment. Despite the ability to recover this species, often in high number from marine environment and seafood, until the 80´s it was infrequently associated with human infection, because many of these sites (wounds, ears, ...) yield polimicrobic flora upon laboratory analysis. Nowadays in several well documented cases, its role in noninvasive diseases cannot be disputed. Cutaneous infections from marine vibrios are common among those, who are exposed to seawaters, such as fish and molluscs breeders. Cases of dermatitis in tourists who bathed in the Venice Lagoon have been noticed for some years. Those *V. alginolyticus* infections result, in healthy people, in localized vesciculae or bullae filled up with serosanguineous fluid, ulcerae and local tissue necrosis spreading from a pre-existing wound, generally located on the extremities. Numerous factors which potentially contribute to enhanced susceptibility to its infections include neutropaenia, chemotherapy, liver diseases, alcoholism, *diabetes mellitus* and other immunosuppressive diseases. This work aimed to compare the pathogenicity of *V. alginolyticus* strains isolated from bivalve molluscs from markets from 2 highly polluted regions: the Venice Lagoon, (North eastern Italy) and the Guanabara Bay (RJ, Brazil). In both areas, breeding and fishing of bivalve molluscs are present and well exploited, even if the waters around these densely populated, touristy areas are heavily polluted, both chemically and microbiologically. From 30 samples of live molluscs (*M. galloprovincialis* and *R. semidecussatus*) collected in Italy from September to November 2003, 6 strains of *V. alginolyticus* were isolated. In Brazil we collected 13 samples of mussels (*P. perna*) heat treated and prepared. From these molluscs we isolated 10 strains of *V. alginolyticus*. The virulence of both Italian and Brazilian strains, was analyzed using BHI Agar plus 1% collagen to detect collagenase; BHI Agar plus 1% ellastin (ellastase) and BHI Agar plus 0,04% condroitine sulfate, 1% bovine serum albumine (condroitinase). The studying strains are inoculated by spots. The assay was positive when a translucent, luminous halo appeared on the border of the colonies. Although it´s patogenicity have not been elucidated, the production of ellastase and collagenase have been detected among strains isolated from human wounds and low percentage from environment and seafoods. Among italian strains we identified 3 patterns of enzyme production: 4 strains produced the complete set of enzymes: collagenase, ellastase and condroitinase. The others had just one enzyme: one ellastase, the other, condroitinase. Among Brazilian strains we found a higher variability: 3 strains had the three enzymes; 6 strains just had 2 enzymes (3 had ellastase and condroitinase, 2 had collagenase and condroitinase, 1, collagenase and ellastase). Just 1 strain had one enzyme (collagenase). No strain had just ellastase or chondroitinase as the Italians had; no strain possessed any enzymes. The presence of these virulence markers indicates the inherent pathogenicity of these strains, and the risk to cause severe wound or ear infections in both healthy and immunocompromised individuals, whose wounds are exposed to warm seawater and who consume raw or undercooked shellfish.

S-B11

Food and feedborne bacteria: Risk factors as carriers of antibiotic resistance?

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The use of antibiotics has been accompanied by increasing antibiotic resistance problems in non-pathogenic, commensal bacteria and can be regarded as a major cause of resistance development in bacterial pathogens. Moreover, this situation has obviously contributed to the selection of resistant bacteria within the commensal bacterial community, followed by horizontal gene transfer of antibiotic resistant determinants to pathogenic bacteria.

Concerning the food chain ("farm to fork concept"), feeds and foods from different sources (animals, plants, fermented products) often contain a complexity of micro-organisms involving drug-resistant gram negative and gram positive bacteria. Following the SCAN (Scientific Committee on Animal Nutrition) document "Opinion of the Scientific Committee on Animal Nutrition on the Criteria for Assessing the Safety of Micro-organisms Resistant to Antibiotics of Human Clinical and Veterinary Importance" (April 18th 2002), in general a lack of useful data is getting evident. In particular, (a) the definition of breakpoints needs more experience, and (b) there is an urgent need for having available standard procedures for the assessment of Minimal Inhibitory Concentrations (MIC). Since a multitude of data is necessary to be able to decide whether a detected antimicrobial resistance level is of intrinsic nature or due to acquired resistant determinants, further information based on the characterisation of more bacterial isolates is needed.

In a recently started EU-project (Priority 5) entitled "Assessment and Critical Evaluation of Antibiotic Resistance Transferability in Food Chain" (Acronym ACE-ART; www.ace-art.net), non-pathogenic bacteria representing an important part of the feed and food microflora are focused on. The strains considered belong to the genera *Lactobacillus*, *Bifidobacterium*, *Lactococcus* and *Streptococcus*. It is well-known that these bacteria can be found in a wide range of habitats, but also possess some considerable relevance as starter cultures for fermented foods. Data regarding the level of antibiotic resistance, the transmission of resistance and the genetic mechanisms will be examined and evaluated, besides their modes of transmission. The results obtained will be disseminated via "consumer-related associations" and "industrial platforms" and form the scientific basis for the development of a strategy for a tailor-made application of antibiotics as well as for the selection of well-defined starter cultures in order to inhibit further spread of resistance properties in commensal and pathogenic bacteria.

S-B12

Resistance situation in indicator bacteria isolated from bulk milk samples

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The development of antibiotic-resistant strains of bacteria in livestock is not only a major concern of veterinary medicine but is also relevant for human health, because they are considered to be a source of resistant pathogens in human medicine. According to "Good Veterinary Practice (GVP)" the responsibility of veterinarians for consumer health is ranging from diagnosis, selection and administration of adequate drugs, in compliance with dosage regulations and documentation of drug use to the monitoring of therapeutic success.

The sampling system in this bulk milk study was based on a representative sampling model, which involved the taking of random samples. In total 761 Enterococcus and 184 *E. coli* strains were isolated from these samples and tested for resistance to 13 and 16 different antibiotics, respectively. The resistance test and determination of MIC (minimum inhibitation concentration) values was done by using Sensititre^{R} (MCS-Diagnostics, Swalmen), a commercially available test system using dehydrated antimicrobials in microtitre wells.

All results of the resistance analysis from bulk milk samples were assigned to the respective farms by means of their LFBIS (information system for agricultural and forestry enterprises) numbers and evaluated using a geographical information system (VETGIS$^{©}$-Styria). The percentage of multiresistant isolates, 18% for *E. coli* and 15% for Enterococcus, was quite similar for both bacterial species. The highest resistance rates in enterococci were found for tetracycline (52%), streptomycin (21%), kanamycin (18%), flavomycin (15%) and chloramphenicol (13%), in *E. coli* for sulfamethoxazole (48%), amoxicillin & clavulanic acid (25%), trimethoprim (23%) und streptomycin (21%).

The test results of the Styrian Resistance Monitoring Programme (REMOST) are published on an annual basis and are fed into a central database, which is linked to the VETGIS$^{©}$ Styria geographical information system. In order to assess the risk of transmission of resistance factors these data are combined with current analysis results from human medicine and with international animal production data. Continuous data acquisition allows to identify possible changes in the resistance behaviour of bacteria at an early stage.

Resistance testing in bulk milk samples represents an efficient method for supervising the resistance situation of indicator bacteria and zoonotic pathogens of cattle. In future risk management actions (e.g. prohibition of the use of certain antibiotics) should be considered, if fixed resistance rates are exceeded.

S-B13

Multidrug resistance of *Salmonellae* isolates from chicken in central Ethiopia

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A total of 679 samples consisting of chicken meat (n = 452), gizzard (n = 88), heart (n = 59) and liver (n = 80) were examined for the presence of *Salmonella* from January 2001 to April 2002. Salmonellae were isolated in 132 (19.4 %) of the samples analysed. Fourteen different serovars were identified of which the most prevalent were *Salmonella* Braenderup (39.4 %), *S*. Typhimurium var. Copenhagen (18.2 %), S. Anatum (15.2 %), *S*. Saintpaul (6.1 %) and S. Uganda (4.5 %). All *Salmonella* strains isolated were examined for antimicrobial resistance to a group of 24 selected antimicrobial agents. Eighty-two (62.1 %) *Salmonella* isolates were resistant to one or more antimicrobials. Of the tested strains, 51.5% were resistant to sulfamethoxazole, 37.1% to spectinomycin, 34.1% to ampicillin, 30.3% to tetracycline, 27.3% to amoxicillin-clavulanic acid and 25% to trimethoprim-sulphamethoxazole and to trimethoprim. Less than 25% of the strains showed resistance to carbadox, chloramphenicol, florfenicol and streptomycin. Out of 82 resistant *Salmonella* isolates, 59 (44.7 %) exhibited multiple resistance up to different antimicrobials (ampicillin, streptomycin, trimethoprim, sulfamethoxazole, trimetoprim-sulfamethoxazole and spectinomycin). *Salmonella* Typhimurium var. Copenhagen, *S.* Anatum, *S.* Braenderup, *S*. Typhimurium and *S*. Saintpaul showed multiple antimicrobial resistance to up to eight antimicrobials. None of the strains tested were resistant to amikacin, apramycin, gentamicin, kanamycin, neomycin, tobramycin, quinolones, cephalosporins and nitrofurantoin.

S-B14

Antibiotic resistance and rare serotypes in food related verotoxigenic *E. coli*

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Verotoxin (VT) producing *E. coli* (VTECs) are naturally occuring in the intestines of cattle but can be found in most other animals too. Originating from raw, unproperly heated meat or other food, besides other environmental sources, EHEC, as a group belonging to the VTECs, can lead to a life-threatening complex called haemolytic uraemic syndrome (HUS). While the serotype O157:H7 has emerged to a foodborne pathogen of considerable public health importance, a diverse range of serotypes are recognised also capable of causing serious human disease. Rare serotypes or variants have been found in HUS outbreaks recently. To reliably control VTEC in the food production chain it is important to improve information about the variety and occurrence of this bacteria. Apart from that, resistance patterns are of major interest for further risk assessment strategies. In many countries only fragmentary surveillance information is seen. It is therefore essential to collect further data on the occurrence of different serotypes and the antibiotic susceptibility of VTEC strains.
Of 130 *E. coli*-samples from healthy cattle, cattle slaughter houses or beef and beef products in Bosnia and Herzegovina 7 samples were identified as verotoxigenic *E. coli* (VTEC). Further PCR and serological investigation was done to identify pathogen genes and different serotypes. VTEC strains from cattle (7 strains; faeces and minced meat) and pigs (23 strains; faeces, carcasses and products) in Germany were investigated in the same manner. After the serotyping all VTECs underwent a MIC (minimum inhibitory concentration) detection according to NCCLS. The susceptibility of the isolates was tested against the following antibiotics: ampicillin, apramycin, ceftiofur, cephalothin, chlortetracycline, clindamycin, enrofloxacin, erythromycin, florfenicol, gentamicin, neomycin, oxytetracycline, penicillin, spectinomycin, sulphachloropyridazine, sulphadimethoxine, sulphathiazole, tiamulin, tilmicosin, trimethoprim/sulfamethoxazole, tylosin tartrate.
Serotyping and PCR detection resulted in the following variants: of Bosnian VTECs 4 strains were identified as O157:H7 (*vtx*1+ and *vtx*2+, *eae*+ and EHEC-*hly*+), the other 3 as the predominantly unknown serotype O96:H19 (*vtx*1+ and *vtx*2+, EHEC-*hly*+ but *eae*-). The VTECs of cattle in Germany were all of different rare serotypes (O1:H2, O3:H-, O56:H-, O113:H21, O116:H21, O126:H21), though O157:H7 (*vtx*1-, *vtx*2+, *eae*+ and EHEC-*hly*+) was also found. In the investigated 23 VTECs from pigs or pork products no O157:H7 or O96:H19 was seen. O138 and O139 variants were found in 10 samples as well as O6:H10, O8:K87, O9:H-, O65:H- and O121:H10, being *vtx*2+ only, O65:H- and O6:H10 solely detec-ted positive for *vtx*1, respectively. Apart from the different serotypes susceptibility against the tested antibiotics was varying in the examined bacteria, however the resistance patterns revealed a high percentage of susceptible strains compared e.g. to *E. coli* isolates in general. Information about antibiotic resistances of VTEC strains isolated from pork is of special interest, as the application of antibiotics in pig production is more relevant than in cattle. Gathering more information on VTEC, also from non-EU-countries, is essential for suitable future european risk assessment strategies.

S-B15

Detection of antibiotic resistance genes in various *Salmonella* strains by thematic micro-array analysis

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Due to the use of antibiotics the prevalence of antibiotic resistant microorganisms has increased all over the world. In the case of pathogenic bacteria antibiotic resistance causes a major public health issue especially the emergence of multi-drug resistant strains, such as *Salmonella enterica* serovar Typhimurium DT104. *S.* Typhimurium DT104 possesses a gene cluster located within the *Salmonella* Genomic Island 1 (SGI1) that is responsible for resistance to the commonly used antibiotics ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (so-called R-type ACSSuT, Boyd *et al*., 2001). To assess the applicability of the microarray technology as a screening tool for the presence of antibiotic resistance genes a pilot micro-array was designed based on 60-mer oligonucleotides directed against the 5 resistance genes present on SGI1. Furthermore a second microarray containing a larger set of oligonucleotides was designed to screen more than 20 different *Salmonella* strains.
Two sets of *Salmonella* strains were tested: set 1 consists of strains with known antibiotic resistance profiles and genotypes (determined by PCR); set 2 contains strains of which only phenotypic data were available. The thematic microarrays were spotted on silylated slides using a Microgrid spotter (Biorobotics). Fluorescently labelled DNA fragments were either generated by PCR or by direct labelling of total DNA using the BioPrime DNA labelling system (Invitrogen). After hybridisation the microarrays were scanned using a confocal laser scanner (ScanArray 3000 (General Scanning)).
The microarray results of the first set of strains, all with variant SGI1 MDR (multi-drug resistant) regions, perfectly matched with the phenotypes and genotypes as determined by Boyd *et al*., 2002. The microarray data of the second set of strains were almost completely in concordance with the available phenotypic information. For instance in strains resistant to gentamycin the presence of the *aacC2* gene was demonstrated and strains with a doxycycline resistance phenotype (i. e. tetracycline) the presence of a *tet*(A), *tet*(B), or *tet*(G) gene was proven, whereas *dfrA1* or *dfrA14* was demonstrated in the trimethoprim resistant serovars. On the other hand, also discrepancies between the microarray data and the phenotypes of the strains were found, for instance the results obtained with the *sul* gene specific oligonucleotides could not always be linked to the phenotypic information of the investigated strains. Furthermore, hybridisation signals were also found with oligonucleotides corresponding to resistance phenotypes that had not been determined in the investigated strains.
From the obtained data it can be concluded that microarrays based on oligonucleotides can serve as a rapid screening technique for the presence of antibiotic resistance genes in resistant bacteria.

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S-B16

Trends in antimicrobial resistance in *Campylobacter* from Norwegian poultry and human cases

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The objective of this study was to assess the occurrence of antimicrobial resistance among *Campylobacter jejuni* isolated from imported and indigenous human cases as well as from Norwegian broilers in 2003, and to compare the results to similar data from 2001 and 2002.
A systematic sample of all *C. jejuni* isolates of human cases of campylobacteriosis in 2003 resulted in a total of 144 (imported) and 63 (indigenous) *C. jejuni* isolates. In addition, 108 *C. jejuni* isolates from Norwegian broiler flocks (one isolate per flock) and 31 isolates of Norwegian broiler meat products were included. The isolates were tested for susceptibility to a variety of antimicrobials using Etest (human isolates) or VetMIC (poultry isolates). The Norwegian AFA breakpoints were used for classification of resistance.
For year 2003, 84% of the indigenous human cases were susceptible to all antimicrobials included, as opposed to 16% of the isolates from imported human isolates. Furthermore, 98% of the isolates from Norwegian broiler flocks and 97% from the Norwegian broiler meat products were susceptible to all antimicrobials included. Among the imported human isolates, 70.8 % were resistant to ciprofloxacin and 62.5 % to tetracycline, as opposed to 7.9% and 9.5% respectively, for the indigenous isolates. Among the poultry isolates, 1.4% was resistant to enrofloxacin.
These data are in accordance with similar Norwegian data from 2001 and 2002. Also, for these years antimicrobial resistance, in particular fluoroquinolone resistance, was more widespread in *C. jejuni* from imported human cases than among *C. jejuni* from indigenous human cases. Moreover, the occurrence of antimicrobial resistance in *C. jejuni* from Norwegian broilers was very limited.
In Norway, antimicrobial formulations for use in animals are available by prescription only. The Norwegian drug usage monitoring programme shows that antimicrobial usage in animal production is relatively low, and that the prescription patters are favourable. As concern fluoroquinolones, the usage in food animals, including poultry, is very limited. No quinolone preparations are licensed for use in broilers. Also in humans, the usage of antimicrobials is relatively low, and quinolones represents only a minor fraction (2.6% in 2002) of the total usage. The results described above are likely a reflection of the favourable situation as regard usage of antimicrobials in Norwegian food animals and in humans in Norway. The relative high prevalence of resistance among imported human isolates is likely a reflection of the resistance situation in *Campylobacter* in the food chain for those countries Norwegians frequently travel to.

S-B17

Prevalence of multiresistance to antimicrobials in *Campylobacter spp.* isolated from poultry and humans in Berlin, Germany

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Campylobacters from retail poultry products and from human infections sampled in 1991 and in 2001-2002 in one region of Germany were tested for simultaneous resistance to antimicrobial agents. Aim was to identify resistance combinations and to study the occurrence of multiresistant isolates over the ten-year period lying between sampling data. Further, the prevalence of multiresistant isolates in retail turkey and chicken products in 2001-2002 was determined.

251 *C*. spp. isolated in 1991 (82 from humans, chicken: 139, turkey: 30) and 253 strains from 2001/2002 (human: 82, chicken: 134, turkey: 37) were tested by microdilution for susceptibility to erythromycin, gentamicin, ampicillin, ciprofloxacin, tetracycline and trimethoprim-sulfamethoxazole.
The analysis of combinations of antimicrobial resistances in *C*. spp. revealed significant species and origin specific differences. Some combinations occurred frequently, while others were not found. Only 23.2% (117) of the 504 isolates were susceptible to all 6 antimicrobials tested. 40.7% (205) were resistant against one and 22.6% (114) against two of the antimicrobial substances.

68 isolates (13.5%) were resistant to 3 or more antimicrobials and thus defined as multiresistant. Multiresistance increased significantly (p=0.001) in *C. jejuni* of human origin over the ten year period lying between sampling dates (1991: 2.9%, 2001-2002: 21.5%). In comparison, *C. jejuni* from retail poultry products displayed high multiresistance rates in 1991, already. Multiresistant *C. coli* were found only in 2001-2002.
41.2% of multiresistant isolates carried simultaneous resistance to ampicillin, tetracycline and ciprofloxacin. The number of isolates with this resistance combination increased significantly (p=0.002) from 1991 to 2001-2002, giving evidence for a spreading multiresistance mechanism.

The prevalence of *C*. spp. in retail turkey (sampling from september to december 2001) was as high as 82.0%, whereas 57,8% of retail chicken products (sampling from october 2001 to april 2002) carried the pathogen. Multiresistant *Campylobacter* strains were present in 15.5% of turkey and in 8.6% of chicken products.

In conclusion, retail poultry products seem to be an important source for human infections with multiresistant *C*. spp.

S-B18

The role of efflux pumps in antimicrobial resistance in *Campylobacter jejuni/coli*

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The contribution of *Campylobacter* multidrug efflux pump, *cmeABC*, and nine other putative efflux pump genes or operons to the antimicrobial susceptibilities of *Campylobacter* species were investigated. Mutant strains were constructed by inserting a *Campylobacter* chloramphenicol or kanamycin resistance cassette to target efflux genes, thus disrupting expression of the genes. When comparing the susceptibilities of the mutant and parent strains to four antimicrobial agents (chloramphenicol, ciprofloxacin, erythromycin, and tetracycline) by agar dilution, insertional mutations in *cmeB* resulted in 4 to 128-fold decreased minimum inhibitory concentrations (MICs) to chloramphenicol, ciprofloxacin, erythromycin and tetracycline, with erythromycin being the mostly affected. In addition, *cmeB* mutants completely changed the susceptibility category by reversing a resistance phenotype to a susceptible phenotype in two *C. coli* strains co-resistant to ciprofloxacin and erythromycin. In contrast, mutants of all other putative efflux pumps did not show decreased MIC to any of the four agents tested. Our finding indicates CmeABC is the only efflux pump tested that is important to antimicrobial resistance in *Campylobacter* species, and further studies are under way to characterize the gene expression regulation of this efflux pump in *Campylobacter* strains.

S-B19

Differences in the survival potential and survival mechanisms of selected *Campylobacter jejuni* strains encountering various stressors

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Campylobacters are the most common bacterial form of human infectious intestinal disease in most temperate countries. A majority of human campylobacteriosis cases are associated with food of animal origin. During slaughter and the subsequent processing of meat and meat products bacteria encounter several adverse environmental stressors. When evaluating the impact of environmental/technological stressors on *Campylobacter* spp. one has to take into account the broad genetic strain variability and their different responses to environmental factors. Genotypic differences that result in phenotypic changes could allow specific genotypes to be favoured when encountering adverse environmental situations. To improve food safety, it is insufficient to observe bacterial phenotypic changes during stressor application only, but a deep knowledge of the underlying mechanisms is needed to optimize technological procedures to kill pathogenic bacteria successfully.

Recent studies demonstrated that genetically different avian *C. jejuni* strains vary in their ability to survive technological stressors that occur during the slaughter process at poultry abattoirs (such as scald bath temperature, drying, chilling and oxygen contact). Some strains exhibited a higher potential to survive these adverse environmental situations.

In our study, phenotypic observations were combined with expression analysis to characterize the heat shock response. In the first step we characterized the heat shock response of *C. jejuni* NCTC 11168 on the mRNA level. A whole genome cDNA microarray was used. Additionally, to quantify the microarray date, quantitative PCR (qPCR) analysis of the cDNA samples was carried out on selected genes encoding heat shock proteins, metabolic markers and housekeepers. Global changes in expression patterns were already detectable after 2-3 min. Whereas an intense and swift increase of the transcript level of many heat shock genes is detectable after temperature elevation, components of major metabolic pathways are downregulated (such as parts of the tricarboxylic acid cycle, fatty acid biosynthesis and gluconeogenesis). After 20 min a return to the initial transcript level of many genes was observed. When combining phenotypic methods (cell counts and fluorescence assays to visualize bacterial viability by staining with SYTO 9 dye and propidium iodide) and expression analysis, a rudimentary heat shock response with a simultaneously decreasing cell count was detectable even after 20 and 40 min at 50°C.

Secondly the heat shock response of genetically unrelated *C. jejuni* strains was compared phenotypically as well as on the expression level. As expected, genotype specific phenotypic differences in the ability to survive heat stress were observed. Additionally, after applying heat shock the expression patterns varied between different genotypes. Some strains appeared to respond earlier and more intense to the heat shock on the mRNA level than other strains.

These findings indicate that genetic diversity can be linked to different potentials to respond to environmental stressors. Microarray analysis proofed to be an excellent and promising tool to characterize the stress adaptive response of *Campylobacter* to changing environments.

S-B20

Genetic relationship between *Campylobacter* spp. from retail chicken products and humans in Berlin, Germany

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An important source of infection with *Campylobacter* is considered to be raw poultry meat. A lot of broiler flocks are infected with *Campylobacter* spp. and case-control studies have identified the handling and consumption of poultry as a major risk factor for *Campylobacter* infections. To assess the impact of poultry products as vectors for sporadic Campylobacter infections in Germany, we analysed the relatedness of Campylobacter spp. strains occurring in a 3-month period within the city of Berlin on retail chicken products and in cases of human campylobacteriosis. To evaluate the possible genetic relationship between isolates from poultry products and human isolates of sporadic cases DNA fingerprint techniques are needed. PFGE is one of the most discriminatory genotypic typing methods currently available for subtyping *Campylobacter* species.

We analysed the relatedness of Campylobacter spp. strains occurring in a 3-month period within the city of Berlin on retail chicken products and in human campylobacteriosis by PFGE and antimicrobial resistance patterns. *Campylobacter* spp. strains were isolated in Berlin, Germany from January to March 2002. 31 *C. jejuni* and 6 *C. coli* from chicken product samples and 40 *C. jejuni* and 7 *C. coli* from cases of human campylobacteriosis were subtyped. Poultry food samples (chicken) were taken at retail level. Sampling included different meat samples (breasts, drumsticks, and wings), liver, gizzard and heart, presenting the broad range of chicken products consumed regularly by German consumers. Human isolates originated from ambulant cases of diarrhea (stool samples) occurring in Berlin. The 84 isolates were subtyped by PFGE using the restriction enzyme SmaI. Strains with identical SmaI profiles were additionally analysed with KpnI. In addition, the strains were subtyped by their antimicrobial resistance pattern. The antimicrobial susceptibility to erythromycin (ERY), tetracycline (TET), ciprofloxacin (CIP) and nalidixic acid (NAL), gentamicin (GEN), ampicillin (AMP) and ampicillin/sulbactam (SAM) was tested by the microdilution technique.

Genotyping by PFGE revealed a high degree of diversity, which is a well-known phenomenon for bacteria of the genus Campylobacter. But despite the weak clonality of *Campylobacter* spp., 10.6% of human isolates were genetically identical with isolates found in the same geographical region and time frame on retail chicken products. Thus, it can be concluded that retail chicken products are an important source for sporadic human infections with *Campylobacter* spp. in Germany. *Campylobacter* spp. with the same SmaI/ KpnI PFGE-profile did not always have identical resistance phenotypes, but the genetically identical strains found in human and chicken products displayed the same antimicrobial resistances.

More research is needed to evaluate whether subtyping of C. spp. by antimicrobial resistance patterns may offer an interesting alternative as a phenotyping method, e.g. for identification of small outbreaks. In general, analysis of larger numbers of isolates is needed to fully assess the impact of poultry products as vectors for sporadic Campylobacter infections.

S-B21

Combating *Campylobacter* spp. in Norwegian broilers

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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 and consists of ten swabs from fresh faecal droppings. Positive flocks are slaughtered at the end of the day, and the carcasses from these flocks are either heat treated or frozen for a minimum of five weeks before being marketed. All flocks are retested at the slaughter plant by sampling of ten cloacal swabs per flock.

Since the implementation of the action plan, more than 10 000 flocks has been sampled, of which approximately 6% have been positive for *Campylobacter* spp. By the end of 2003, more than 3 million positive carcasses have been prevented from entering the market fresh. Significant seasonal and regional variations in prevalence are observed. There was a significant reduction in the proportion of positive flocks from 2002 to 2003, as well as in the proportion of positive farms. This reduction can probably be attributed partly to the advisory service and general improvement of hygienic practices in the Norwegian poultry industry. There are indications of a positive public health effect, but this is still to be evaluated.

S-B22

Assessing exposure to BSE infectivity in Great Britain throughout the BSE epidemic

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There have been 146 cases of variant Creutzfeldt-Jacob Disease (vCJD) in the United Kingdom (to 1st March 2004), which are presumed to have been caused by exposure to the infective agent of BSE present in meat or meat products from infected animals. A series of studies has been carried out to review the likely sources of infectivity in food, to assess whether butchery practices in use through the 1980s may have contributed to exposure and to provide an estimate of the exposure.
Representatives of organisations involved in meat production from 1980-1995 were interviewed, covering all stages of the production chain including; abattoirs, meat cutting plants, producers of mechanically recovered meat (MRM), meat brokers, producers of processed meat products, butchers, retailers and institutional users. The study focussed on those tissues known to carry the most infectivity and the products or processes through which consumers may have been exposed.
The latest data on the infectivity of bovine tissues, the development of infectivity through the incubation period and the cattle to human species barrier are reviewed. All the possible routes by which infective material could be included in food for human consumption, including contamination with infected tissues in the abattoir, embolism following slaughter, dorsal root ganglia (DRG) in meat, MRM and failure of specified risk material (SRM) controls are examined over the period of the BSE epidemic.
The exposure from one fully infected animal slaughtered for food in 2002 is estimated to have been about 27 bovine oral ID_{50} units. This compares to a peak value of about 1900 early in the epidemic. At present the exposure is primarily due to DRG, but over the course of the epidemic the main contributor to total infectivity is estimated to have been MRM. However, it is also shown that the potential exposure to infectivity in a typical meal containing MRM would have been small.
By combining these values with estimates of the numbers of infected animals by incubation period and year it is estimated that a total of some 54 million bovine oral ID_{50} units would have been consumed from 1980 to date, reaching a peak of about 11 million units in 1993, but falling rapidly following the introduction of SRM controls. The study provides an insight into the overall exposure and demonstrates the effectiveness of the controls put in place in the UK. It also provides a basis for assessing possible changes to those controls.
The work described here was funded by the UK Food Standards Agency (FSA).

S-B23

Prion proteins detected in fish – What are the chances of Transmissible Spongiform Encephalopathies in fish and what are the potential risks for the consumer

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cDNA sequences coding for homologues of mammalian prion proteins (PrP) were first reported for teleost fish species in 2003. Since teleost fish genomes encode prion proteins, they fulfil the basic precondition for developing transmissible spongiform encephalopathies (TSE).
In the past, animal meal, which was potentially contaminated with infectious prions, was processed for the production of fish feed. Animal meal was banned from use in fish feed by the EU by the end of the year 2000.
The fish cDNA sequences coding for homologues to mammalian PrP provide a basis to make predictions concerning the likelihood of fish to develop a TSE after exposure to Prions from a mammalian source.
An important factor controlling interspecies transmission of prion infections is the PrP homology between source and recipient species. Although comparisons indicate that for example the C-terminal part of one of the Fugu rubripes PrPs is surprisingly most closely related to the bovine and human PrP with 25.0% and 24.8% amino acid identities, respectively, based on the sequence homology alone, it seems unlikely that fish could contract a TSE by the uptake of prions of mammalian origin. However, the multiple factors responsible for the so-called species barrier are neither fully investigated nor completely understood. Moreover, the situation appears to be more complex than previously anticipated because as shown for Fugu rubripes fish genomes encode more than one protein with homologies to mammalian PrPs. Thus, although fairly remote, for the time being the possibility of prion infections of fish cannot be ruled out entirely.

S-B24

Assessment of beef carcass contamination with spinal cord tissue and/or infectious BSE spinal cord tissue and exposure of the Swiss population

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Using the usual post-slaughtering method that consists of sawing carcasses in two with a band saw, bone meal and spinal cord tissue gets on to sides of beef as saw dust and can cause contamination and also rub off on further sides of beef.
According to current understanding it can be assumed that BSE can be transmitted to humans through contaminated food. To assess the degree of contamination of sides of beef with infectious BSE spinal cord tissue and the likelihood of infectious material getting into the food chain, the Swiss Federal Veterinary Office (FVO) conducted a risk analysis addressing the following topics:
The degree of contamination of sides of beef with CNS tissue using current methods in abattoirs.
The proportion of CNS tissue originating from BSE cattle (classified in the legislation as Specified Risk Material [SRM]) that is viewed as infectious.
Exposure of consumers to infectious spinal cord tissue.
The risk assessment was conducted in line with international standards and the fundamental principles established at the FVO for such risk analyses[1].
Using a calculation model established by the FVO, it was estimated that 48 BSE cattle were sent for slaughter in 2002. Using the BSE rapid test eight animals would have been identified as infected with BSE, and the sides of beef incinerated. According to the model for BSE, CNS tissue of 6 further animals would be infectious, but these animals would not be identified as BSE cases. Again according to the model, CNS tissue would not be infectious at the time of slaughter in the remaining 34 infected animals.
Further calculations indicate that 1.357 million sides of beef would have been contaminated with a total of 76 g of infectious spinal cord tissue. Assuming further production measures reduce contamination of meat with infectious material by a factor of 100 (through removal of obvious bone splitters, saw dust and the vertebrae of animals more than 30 months old) the Swiss population would be exposed to approximately 0.76 g of infectious CNS tissue, corresponding to an ID_{50} (bovine oral) value of 38.
According to the model and the assumptions made, 68 sides of beef remained contaminated with some infectious material. Presuming that approximately 160 kg of meat per slaughtered animal reaches consumers, the remaining rate of infection of 38 ID_{50} is distributed over 5,440 kg of meat, corresponding on average to 0.00699 ID_{50} per kg of meat.
This risk assessment took account of the release of the hazard and consumer exposure to the hazard. As far as food safety is concerned the consequences for consumers cannot be assessed because there is no dose and effect curve available for human variant Creutzfeldt-Jakob disease, which, according to our current understanding, is caused by BSE. According to present scientific findings it can be assumed that the probability of humans contracting vCJD through contact with CNS tissue released during cutting of the spinal cord is negligibly low.

[1] Federal Veterinary Office (2002) Basic Principles for Risk Analyses conducted at the Swiss Federal Veterinary Office (FVO) (http://www.bvet.admin.ch/tiergesundheit/e/berichte_publikat/risikoanalysen/risikoanalysen_policy.pdf)

S-B25

Real time RT-PCR for species-specific detection of central nervous system tissues as BSE risk material in meat and meat products

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This work is a part of a project including three German veterinary institutes at the Justus-Liebig-University Giessen and is founded by the Federal Ministry of Consumers Protection, Food and Agriculture (AZ.: 01HS022/1). With regard to an efficient preventive health protection of consumer´s European legislation prohibited specified bovine offals (SBOs), e. g. central nervous system (CNS) tissues, from food chain. The identification of bovine-specific CNS tissues is an essential prerequisite for effective control of a potential source of human bovine spongiform encephalopathy (= new variant of Creutzfeld-Jakob disease). Till now several chemical and immunological methods for detection of tissues of the CNS in meat products have been published to be used in food safety control.

The aim of the own project was to identify the bovine-specific BSE risk material in meat and meat products. For this reason, a novel molecular method based on real-time reverse transcriptase (RT) Real Time-PCR was developed. A specific small region of Glial fibrillary acidic protein (GFAP)-mRNA has been selected and employed to reach the goal of detection of illegal use of bovine, ovine and caprine CNS.

S-C01

Pre-processed bovine milk quality in Trinidad: Prevalence of aerobic bacterial pathogens and antimicrobial residues

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In November 2000, the major milk processing plant in Trinidad and Tobago changed the milk collection system from a 16 – centre system involving transportation of milk in stainless steel churns to the processing plant at ambient temperature to a 5 – centre system with chilling facilities and transportation to the plant in an insulated truck. At the collection centres, from randomly selected dairy farmers, approximately 20 ml each of pooled milk from churns was collected for laboratory analysis. Milk samples were also collected from the chiller during the visit. The pH and temperature of all milk samples collected were determined immediately. In the laboratory, subclinical mastitis was assayed using the California mastitis test (CMT), the total aerobic plate count (TAPC), staphylococcal and *Escherichia coli* counts per ml were also enumerated. Antimicrobial residues in milk were detected using the Delvo test.

Of the 266 samples of milk from churns tested, 255 (84.6%) were CMT-positive and the mean ± sd temperature and pH were 20.35 ± 7.91 and 6.80 ± 0.13 respectively. All (100.0%) 266 samples contained staphylococci with mean $\log_{10}$ ± sd colony forming unit (cfu) per ml of 4.67 ± 0.92, 230 (86.5%) samples were positive for *E. coli* with mean $\log_{10}$ ± sd cfu per ml of 4.15 ± 1.97 and the mean $\log_{10}$ ± sd TAPC per ml was 6.3 ± 1.09. Of the 20 milk samples from chillers tested, all (100.0%) were CMT-positive and the mean ± sd temperature and pH were 15.10 ± 2.73 and 6.84 ± 0.09 respectively. All (100.0%) samples contained staphylococci with mean $\log_{10}$ ± sd cfu per ml of 5.29 ± 0.42 , *E. coli* with mean $\log_{10}$ ± sd cfu for ml of 5.58 ± 0.72 and aerobic bacteria with $\log_{10}$ ± sd cfu for ml of 7.04 ± 0.33. For truck samples, 34 (94.4%) of 36 were CMT-positive with mean ± sd temperature and pH of 13.02 ± 4.44 and 6.85 ± 0.04 respectively. All 36 (100%) samples were positive for aerobic bacteria, staphylococci and *E. coli* with mean $\log_{10}$ ± sd cfu for ml of 6.97 ± 0.43, 5.38 ± 0.41 and 5.52 ± 0.46 respectively. The difference in the microbial load of milk was significantly different ($p< 0.05$) across collection centres and for various temperature ranges. Of a total of 192 churn milk samples tested, 8 (4.2%) were positive for antimicrobial residues compared with 1 (5.0%) of 20 chiller samples and 7 (19.4%) of 36 truck samples. The difference was statistically significant ($p< 0.05$). It was concluded that despite the change in milk collection system, the microbial load of raw milk was still high suggesting poor sanitary practices at the farm level and a high prevalence of subclinical mastitis. The presence of antimicrobial residues in milk cannot be tolerated because of the potential health hazard to the consumer. It is imperative that measures be instituted to ensure that the farmers practise good hygiene during cattle rearing, milking and transportation to the collection centres.

S-C02

The contribution of chicken consumption to the problem of quinolone-resistance in micro-organisms causing human infection

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Antimicrobial resistance is currently a major topic in veterinary medicine and public health around the globe. In many countries and including the UK, it has been hypothesised that the use of antimicrobials in livestock production has caused a rise in the resistance of micro-organisms. In the early Nineties, coinciding with the licensing of quinolones for food animal use in several countries, there was an increase in the resistance to this class of antimicrobials in micro-organisms found in poultry and humans, and in particular *Campylobacter spp.* (Endtz *et al.*, 1991; Threlfall *et al*., 2000; Luber *et al.*, 2003). However, a direct causal link between the use of veterinary antimicrobials in food producing animals and an increase in antimicrobial resistance in humans has never been established. Furthermore, there is uncertainty relating to the contribution of other sources of quinolone-resistant *Campylobacter spp*., such as foreign travel, clinical treatment and the environment. Therefore, in order to address this issue, a risk assessment is being developed to assess, relative to other pathways, the contribution of the food chain to the problem of quinolone-resistant *Campylobacter* infections in humans.
This paper presents results from the chicken section of the food chain model, and includes many different types of chicken including conventional, free-range, organic and non-UK chicken. The risk assessment model was a further development of an existing farm-to-consumption risk assessment model that estimates the risk of *C. jejuni* illness from broiler consumption (Hartnett, 2001). Using the adapted model, for each production type considered, the number of human QRC illnesses (with associated uncertainty) was estimated. This was then used to assess the contribution of chicken to the number of human QRC cases in the UK.

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S-C03

Risk factors for *Campylobacter jejuni* (and Salmonella) infections in the Netherlands: a case control study

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Introduction: Campylobacter and Salmonella infections are the most common causes of bacterial gastro-enteritis in the Netherlands. A case control study of sources, risk factors and transmission routes for human campylobacteriosis (and salmonellosis) was conducted by the National Institute of Public Health and Environment (RIVM) during April 2002- April 2003, in collaboration with the Regional Public Health Laboratories and the Research Institute of Animal Husbandry.
Methods: For each laboratory-confirmed case basic information (age, sex, species and antibiotics resistance) was registered and a questionnaire regarding risk factors was sent to the physician with a request to forward it to the patient. Besides, the Campylobacter isolates were sent to the Research Institute of Animal Husbandry for determination of the species. Controls (two per case) were selected from the population registries of 25 municipalities by frequency matching for age, sex, degree of urbanization and season. Each first working day of the month questionnaires were sent to controls. Numbers of approached controls varied according to the expected number of cases based on historic data. Factors associated with *Campylobacter jejuni* infection with a p-value ≤ 0.20 in the univariate analyses were considered in the logistic regression analysis. Submodels were developed for food consumption, occupational exposure, animal contact, water exposure, kitchen hygiene and food processing and finally combined in a final model.
Results: The study comprised 2811 culture-confirmed patients with *Campylobacter jejuni* infection of which 1292 (46%) patients completed the questionnaire. Of the 10250 approached controls 3409 (33%) completed the questionnaire. The following risk factors were found to be independently associated with endemic *Campylobacter jejuni* infection: consumption of chicken, undercooked meat, meat prepared at a barbecue and undercooked fish, eating in a restaurant, ownership of dogs or cats, especially puppies and kittens and occupational exposure to raw meat as a cook or butcher.
Discussion: This study confirms some well-known risk factors for campylobacteriosis but, in contrast to other studies, consumption of raw or unpasteurised milk was not a risk factor in our study. As the answer “I don’t know” for several variables was highly associated with illness, information or recall bias might have played a role. The recall period was 7 days prior to illness (cases) or completion of the questionnaire (controls). However, for cases the median time interval between the onset of disease and completion of the questionnaire was 20 days.

S-C04

Exogenous and endogenous contamination of German retail chicken with *Campylobacter* spp. - Consequences for an exposure assessment

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Infections with the zoonotic bacteria *Campylobacter* (*C.*) *jejuni* and *C. coli* are the second most common cause of diarrhea in humans in Germany [Robert Koch-Institut, 2003]. Several investigators worldwide have found high prevalences of campylobacters in retail chicken products and most of the case-control studies have identified consumption and handling of chicken as an important risk factor. Thus, it was decided to carry out a national microbiological risk assessment focusing on thermophilic *Campylobacter* spp. in German chicken products.
Data on the dose-response relationship of *Campylobacter* infections are spare. The lowest dose reported leading to symptoms of illness was 500 cells only [Robinson, BMJ 1981], demonstrating that even ingesting small quantities can be hazardous.

To estimate the exposure of German consumers to *C*. spp. through chicken products we exemplarily analyzed chicken legs. Due to the protective effect of the skin these parts of the chicken are supposed to carry an increasing number of *C*. spp. Moreover, drumsticks are presumed to be the chicken part mostly eaten undercooked due to failures during preparation.
71 chicken legs were sampled on retail level from June to September 2002 in one region of Germany to test for *C*. spp. prevalence. The chicken legs were analyzed qualitatively following the guideline ISO 10272 for exogenous (surface swab sample) and endogenous (10g inner muscle) contamination with *Campylobacter* spp.
67.6% (n = 48) of the surface samples were positive for *C*. spp. Eight of the chicken legs (11.3%) were as well endogenously contaminated. Molecular typing of strains isolated from the outside and inside of contaminated drumsticks revealed different PFGE-types in 5 out of 8 cases. Possible causes for endogenous contamination of the chicken legs will be discussed.

As *C*. spp. are very sensitive to heat treatment, those located in the chicken skin will be killed easily by standard cooking procedures. An exposure of consumers via consumption of undercooked chicken meat needs to be taken into account only for products which are contaminated inside. Handling of contaminated chicken products in the private kitchen is supposed to be the most important factor for consumers exposure with *C*. spp. Pathways range from a direct contact to mouth with contaminated hands to cross-contamination of ready-to-eat foods in the kitchen.
We intend to model transfer and spread of *Campylobacter* for various cross-contamination pathways in the private household. Assessment of the relative importance of pathways will be based on a survey of German consumers behaviour. Future labwork will focus on quantification of *C*. spp. inside and outside of retail chicken leg samples and on determination of transfer rates for *C*. spp. in typical German household settings.

S-C05

Uncertainty in the use of consumption studies for exposure assessment in foodborne infections and intoxications

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The risk in the context of microbiological risk assessment is the result of the probability of the occurrence of an impairment of health as a consequence of the consumption of food contaminated with pathogenic microorganisms or the contact with such food. With the help of exposure assessment, model parameters and model interrelationships are used to determine the type, frequency and intensity of the contact of the population with a bacterium.
To quantify the model parameters and model interrelationships, the two aspects uncertainty and variability are taken into account. Consumption studies constitute a central source of data for the assessment of microbial exposure. In the following, uncertainty and variability in the use of consumption data are systematized, and the uncertainty is quantified in examples.
For exposure assessment, exact knowledge into the means by which pathogenic germs are transmitted to the consumer is necessary. On the one hand, this can occur as a result of the consumption of contaminated products; on the other hand, however, it can also happen through cross-contamination on kitchen appliances and other food. For both scenarios, consumption data form a central component for the assessment of the parameters of the exposure. Consumption studies can be differentiated into several types that provide various information for the quantitative estimation of the relevant parameters. In addition, information about the food consumed and the pattern of consumption are collected in different depth depending on the study. Study type and degree of detailling in the collection of consumption data determine decisively the quality of the exposure assessment.
By means of various study types regarding investigations of consumption, the exactitude of essential parameters such as consumption frequency and amount is affected. The concepts available for the determination of portion sizes also vary. In addition to the use of standardized values, portion sizes can be obtained directly by questions using illustrations or calculated with weight journals on the basis of the entire consumption amount. In addition to the varied methodology for the calculation of portion sizes, additional sources of variability have an influence on the quality of the estimation of consumption amounts, frequencies and portion sizes. Among these are, besides sociodemographic characteristics, regionality and seasonality.
The assessment of seldom assessed foods and the estimate of the intraindividual variability is dependent on the length of the registration period. The currentness of the data and the exactness of the coding of the foods also have a significant influence.
The use of separate scenarios for typical and special dietary habits like diets and consumption away from home offers possibilities for the reduction of uncertainty and variability.
Depending on the selected food-bacterium-combination for which an exposure assessment is to be carried out, differentiated additional information regarding preparation (raw consumption), storage (expiration date), packaging, brands and origin (e.g. bio-conventional import-export) must be taken into account. Moreover, for the modeling of cross-contamination paths, information about simultaneously prepared or consumed foods is necessary.
The sources of variability and uncertainty are specified, and by means of a few examples presented. The extent of the influence on the exposure assessment is described. Proceeding on this basis, requirements as to the quality of consumption data for use in microbiological risk assessment are formulated.

S-C06

Microbial risk assessment applied to the issue of antimicrobial resistance

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There is widespread concern to understand the epidemiology of antimicrobial resistance, its sources and influential factors. Many debates have centred around the use of antimicrobials in livestock, which may increase the levels of resistant bacteria in the animal population, and subsequently effect the probability of resistant bacteria being consumed by humans.

Microbial risk assessment (MRA) is a scientific tool that can be used to evaluate the level of exposure and the subsequent risk to human health due to a specific pathogen. Results can be used to provide decision-makers and industry with information on which to base policies and codes of practice relating to antimicrobial resistance. This paper will give an overview of the antimicrobial resistance MRAs that have been carried out, focussing on the assumptions and methods used. Furthermore, common data issues experienced during the development of such risk assessments will be highlighted and discussed. By highlighting the data issues, and in particular data gaps, to the antimicrobial resistance research community it is hoped that these gaps in knowledge will be addressed by future research work.

S-C07

Cross-contamination of foodborne pathogens in (household) kitchens

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A quantitative analysis was carried out to estimate the probability of contamination and the levels of *Salmonella* and *Campylobacter* on salad as a result of cross-contamination from contaminated chicken carcasses via kitchen surfaces. This study illustrates how the cross-contamination during food preparation in the domestic kitchen can be modelled by linking experimental data and currently available data.

The survival of microorganisms on dry(ing) surfaces, resulting in exposure to low water activity (a_w), may affect the morphology and physiological activity of the cells. In a study the morphological changes and cell viability of *Salmonella enterica* serovar Enteritidis, challenged to low a_w surfaces, were analysed. Viability was determined using the Live/Dead BacLight bacterial viability kit with epifluorescence microscopy and flow cytometry. The results indicated that exposure to reduced a_w surfaces induced filamentation of the cells. The amount of filamentous cells at a_w 0.94 was up to 90% of the total number of cells. Cellular damage was evidenced by the inability of a proportion of the stressed cells to form colonies on selective medium. Surviving filamentous cells maintained their membrane integrity after exposure to low a_w surfaces for a long period of time, and were able to split in single cells under favourable conditions, resulting in the instantaneous appearance of a large number of viable cells. Furthermore, both short and elongated cells pre-challenged to low a_w surfaces demonstrated better tolerance against sodium hypochlorite than control cells. These tolerant cells are able to survive disinfection and therefore could be a source of contamination of foods coming in contact with surfaces. This points to the need of increased attention in the disinfection procedures of surfaces in processing plants, catering plants or household environments.

More and more evidence is available that infection outbreaks in the home, particularly infectious intestinal disease (IID) and respiratory infections, are a significant concern. Much of the cross infection that occurs in the home probably arises from direct person-to-person interaction and can only be controlled by changes in social behaviour, if at all. We know that many gastrointestinal infections result from consumption of contaminated raw food purchased from retail outlets, which has been improperly cooked or inadequately stored. Increasingly, however, it is acknowledged that IID in the community is by no means all food-borne, and that person-to-person spread within families via hands and other surfaces is often a factor, especially with viral infections. Such evidence suggests that a significant proportion of IID is preventable through improved standards of food handling coupled with better hand and surface hygiene practices.

The International Forum on Home Hygiene (IFH) evaluated the potential for infection and cross infection in the home. This data was used in the production of a set of “Guidelines for the prevention of infection and cross infection in the domestic environment” (www.ifh-homehygiene.org). The guidelines follow a risk-based or “targeted” approach in which sites, surface and situations in the home, which carry a significant risk of exposing family members to infectious microbes are identified, and hygiene procedures targeted at these sites and surfaces at the appropriate time. Another document, the “Recommendations for selection of suitable hygiene procedures for use in the domestic environment”, details the procedures to be used where a hygiene risk is identified. The data presented in this review paper shows that the effectiveness of a hygiene procedure in interrupting the chain of transmission of infection depends on a whole range of factors which include the efficacy of the procedure, the nature of the site or surface, the manner by which it is applied, the facilities available, and the knowledge of hygiene practice of family members.

Overall however it can be concluded that application of good hygiene practice in the home can have a significant impact in reducing the impact of infectious disease.

S-C08

Survival of foodborne pathogens in (household) kitchens

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Infectious diseases are known as serious health risks since many centuries. The increasing awareness of the importance of personal hygiene as well as the introduction of safe water supplies and sewage systems, milk pasteurisation, population wide vaccination schemes and the use of antibiotics resulted in successful control of acute infections in the course of the twentieth century. However, epidemiological data indicate that infectious diseases remain globally a serious threat for public health. Previously unknown infections (emerging infectious diseases) and the reappearance of known diseases after a significant decline in incidence (re-emerging infectious diseases) cause enormous public health problems both locally and internationally.

With respect to foodborne disease it was particularly during the 1980s and the early 1990s that the international incidence increased considerably as a result of infections by (re-) emerging pathogens. Several factors contribute to the emergence and re-emergence of infectious diseases, but most can be linked with the increasing number of people living and moving around on the globe, including changes in human demographics and behaviour, changes in food production systems, rapid increases in international travel and commerce, microbial adaptation and change, and the breakdown of public health measures. Understanding the route(s) of an infectious disease is critical in order to identify accessible targets for control strategies. For example, person-to-person transmission may be inhibited by proper hygiene and sanitary conditions and education. Vector-borne diseases may be prevented by control measures that either kill the vector or prevent its contact with humans. This contribution presents an overview of potential aspects in kitchen environments implicated in the transmission of infectious diseases. Although there are different organisms present in the kitchen, including bacteria, viruses, protozoa and fungi, as causal agents of diseases, this study only deals with the bacterial contaminations.

The retention of bacteria on food contact surfaces increases the risk of cross-contamination of these microorganisms to food. The risk will be lower when the surfaces are dry, partly because bacterial growth and survival would be reduced. However, some non-spore forming bacteria might be able to withstand dry conditions on surfaces for an extensive period of time. The survival of *Salmonella* Enteritidis, *Staphylococcus aureus* and *Campylobacter jejuni* on stainless steel surfaces at different initial levels was determined at room temperature. The transfer rates of these pathogens from stainless steel surfaces to foods were also investigated. *S. aureus* was recovered from the surfaces for at least 4 days when the contamination level was high (10^5 cfu/cm^2) or moderate (10^3 cfu/cm^2). At low levels (10 cfu/cm^2) the surviving numbers decreased below the detection limit (4 cfu/100cm^2) within 2 days. *S*. Enteritidis was recovered from surfaces for at least 4 days at high contamination levels, but at moderate level the numbers decreased below the detection limit within 24 hours and at low level within 1 hour. *C. jejuni* was the most susceptible to slow-air-drying on surfaces; at high contamination levels the numbers decreased below the detection limit within 4 hours.

S-C09

Reduction of bacteria from hands with different hand drying methods

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Hand hygiene is an important measure to control and prevent the transfer of pathogens. This holds true for the medical field as well as for the food and catering industry. Just as much hand hygiene issues must be considered in the private household.

In this study the effectiveness of three hand drying methods was compared as to its potential to remove bacteria from artificially contaminated hands. After contamination with *E. coli* the reduction of the bacterial load was separately investigated for hand washing and hand drying using single use paper towels, single use textile cloth, and hot air drying on eight volunteers. Through washing a reduction of 0.20 to 1.98 logs (average: 1.04 logs) was achieved. Statistically virtually no further reduction could be achieved with hand drying. With paper towels the reduction comprised between -0.68 and 1.18 logs (average: 0.31 logs), with textile cloth it was between -0.66 and 0.52 logs (average: -0.13 logs), and with hot air drying it was between -0.16 and 0.29 logs (average: 0.06 logs).

Our results demonstrate that the hand drying method does not play a significant role as to the effectiveness of hand drying. The improvement in this study is that reductions in bacterial load for either hand washing and hand drying were calculated separately. This shows that hand washing is responsible for the major part of the reduction in bacterial load whereas drying scarcely removes bacteria, regardless of the used drying method.

From a microbiological point of view the tested hand drying methods are equally effective.

S-C10

Risk Ranger, a food safety risk assessment tool

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Quantitative risk assessment (QRA) is often an expensive, labour-intensive and technically demanding process which may take many person-years to complete. Even then, the assessment may conclude that the data were insufficient to estimate risk within narrow confidence limits. For risk managers this type of outcome raises questions, rather than providing answers, further prolonging an already long assessment process. Risk managers would also like to develop “what-if” scenarios to assist in decision making and QRAs often cannot supply information to support such scenarios.

Risk Ranger is a simple, easy-to-use spreadsheet tool developed in Microsoft Excel and designed to support decision making. It is a risk-ranking tool where the user is required to answer 11 questions related to: severity of the hazard; likelihood of a disease-causing dose of the hazard being present in the meal; and the probability of exposure to the hazard in a defined time. As well as ranking risks of hazard:product combinations, the tool can be used to explore the effects of different risk reduction strategies.

Within Australia the tool has been used by risk managers in government and in industry sectors as means to prioritise risk and to allocate funding for food safety R&D. In the present paper the role played by Risk Ranger in a risk profile for the meat industry will be illustrated.

S-C11

HACCP / CCP-Finder – the alternative to the decision tree when searching for the Critical Control Point

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The CCP-Finder is a new, simple and free of contradiction means for finding the CCPs when implementing HACCP. It is applied to the process steps of the flow-diagram and consists of a three-part, never changing, sequence of questions. This method avoids all the disadvantages of the so far recommended decision trees.

The CCP-Finder is a sequence of three questions:

QUESTION 1 (from top downwards in the flow diagram): **"Can you find at least one (not belonging to good hygienic practice) measures for controlling a given hazard in this process-step of the flow-diagram?"**
Answer YES: Mark it as a possible CCP; proceed to the next step in the flow diagram and start again with Qu 1; when completing all the process-steps continue with Qu 2.

Answer NO: No CCP for a given hazard, continue with the next step in the process of the flow diagram and start again with Qu 1; after the last process-step proceed to Qu 2.

QUESTION 2 (following the flow diagram upwards at all the marked steps): **"Is it possible to control the given hazard at this process-step of the flow-diagram adequately?"**
Answer YES: This step is the CCP. You can start the discussion of the next hazard.
Answer NO: This step is no CCP, but it may be part of a CCP for the given hazard. Proceed to the next potential CCP and start again with Qu 2; when finished with the last potential CCP continue with Qu 3.
QUESTION 3 (affecting the complete flow diagram**): "Do combined measures of various process-steps of the flow-diagram make an adequate control of a given hazard possible?"**

Answer YES: Define CCP. It is a combination of process-steps of the flow diagram (corresponding to the definition of a CCP as step). Discuss next hazard.
Answer NO The given hazard cannot be controlled in this particular buisness. Either a) the product or the process of its production need to be changed and then the implementation of HACCP has to be started again from the point of creating the flow-diagrams or b) make sure that the given hazard is controlled either by a preceding or following business in the chain of production or c) do not produce this product at all.

ADVANTAGES of the CCP-Finder compared to conventional decision trees:

1. It does deliberately not consider aspects that belong to good hygienic practice.
2. It does not contain questions that actually cannot be answered at the time they are formulated.
3. Of several possible CCPs the one that is situated last in the productionline is always the definite; therefore this method reduces the possibility of recontamination.
4. Controlling of hazards by the "hurdle technology" is taken into consideration.
5. Automatically process-steps, in which hazards in foods are created (e.g. development of toxins of micro organisms), are realized.

S-C12

Fundamental principles for risk-based planning of random sampling to trace hazards in imported meat

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The Swiss Federal Veterinary Office's Border Veterinary Service checks whether certain categories of commodity of animal origin comply with Swiss animal disease and food safety regulations. In 2002, imports of approximately 145,500 tonnes of meat and meat products of all types were inspected by the service. If there is any strong suspicion, the Border Veterinary Service directs that the necessary tests be performed on an ad hoc basis. Additionally, based on a sampling plan established by the Federal Veterinary Office (FVO), random sampling is used in connection with food hygiene testing (foreign substances and ingredients, additives, pathogenic agents and other sources of infection). In view of the present financial shortages and the demand for even higher standards of food safety, the FVO developed fundamental principles for risk-based planning of random sampling to test for residues in imported meat; this work was done in cooperation with the cantonal laboratories that are responsible for implementing food safety legislation. The prototype scientific model was used for the first time to plan sampling for 2004.
In this model all the various imported commodities are assigned to five risk categories with respect to country of origin and the various potential impurities that might be found. Nationally and internationally available data from 2002 were gathered and assessed in line with standard procedure and combined in a database.

- Quantities of commodities imported from a specific country of origin.
- Local legal requirements in the country of origin compared to Swiss regulations.
- The availability of monitoring programmes in the country of origin and results obtained.
- The significance of the hazard (substance) for food safety and the means of identification used for the substance in the matrix.
- The proportion of the commodity category from a specific country of origin in relation to the total consumption of this commodity category in Switzerland (exposure).
- Information (analyses) from Border Veterinary Service tests and from food safety inspections of retail goods.

Difficulty was encountered in combining data generated by different agencies that had been gathered using different criteria. For example information customs officials had gathered about meat imports was collected from the standpoint of tariffs, a substantially different criterion to that used by food safety inspectors to categorise imports. A further difficulty arose because internationally available data is not standardised.
Evaluation of data led to establishing 19 combinations of factors (product, land of origin, substance) that were perceived to be "high risk" and as a consequence all imported commodities falling into this category were tested at the border in 2004. Commodities perceived to present the next highest risk were tested using a selection of methods.
On the basis of our experience with the prototype model and in view of the difficulties encountered in combining data from various sources the model will be optimised and a standard method for gathering data introduced. This model could also be used to provide a foundation for planning similar testing programmes.

S-C13

When is *Campylobacter* in Poultry not a public health problem?

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A major challenge in risk management is how to determine and reach consensus on cost-effective mitigation strategies. Total mitigation costs as well as the impacts on human health are often difficult to measure. It is imperative that the authorities gain enough information to set the food safety acceptance levels such that mitigation efforts really do improve human health, but do not add unnecessary costs to food products. The challenges in balancing cost-benefit calculation are illustrated through Campylobacter in Norwegian poultry production. Food poisoning caused by Campylobacter is clearly a food safety issue, and poultry meat has been shown in epidemiological studies to be an important risk factor in human cases. This has led to comprehensive national Campylobacter programs in many countries, including Norway where there is a national action plan aimed at reducing the “human exposure to *Campylobacter* through Norwegian broiler meat products.” From 2002 to 2003, the number of Campylobacter positive flocks fell by 24%, and positive retail samples fell by 45%, and the level of bacteria in the positive samples tested was low (75% < 100 cpu/g). However, these reductions are not reflected in the numbers of reported cases of human illness, which increased by 5%. This leads to the question of the benefit of reducing the level of Campylobacter in poultry below current levels, and if the efforts are cost-efficient, or if the efforts should be moved to reducing the level of Campylobacter from other sources, or even targeting other food problems.

We have estimated the annual societal cost of campylobacteriosis in Norway, based on the number of reported cases. The calculation includes direct medicine and hospitalisation costs, indirect costs and the number of unreported cases for each reported case. Monte Carlo simulation was used to represent uncertainty in input data. We then estimate the contribution from poultry to total societal costs of campylobacteriosis. This is the basis for a discussion of acceptable risk level in products, how businesses can document their risk levels, the use of cost-benefit calculations, and how to facilitate communication between the authorities and producers.

S-D01

Erroneous concepts and beliefs in foods in the twentieth century

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The Twentieth century has been a brilliant period of progress for the biomedical sciences including food sciences and nutrition. In fact nutrition has become a scientific discipline during this century. Inspite of this remarkable progress the field of food and nutrition remains heavily vitiated by mistaken beliefs on the part of the general public and even some scientists. This remains a fertile field for exploitation by quacks and charlatans.

There is a widespread belief among the sophisticated public in the west that irradiated food is harmful for health and they reject it categorically. Thus a perfectly safe and useful method of eliminating foodborne pathogens remains only partially exploited. There is a similar but less prevalent prejudice against microwave cooking.

Some people believe that cholera is caused by eating unripe fruits in the summer rainy season. In the first 3/4th of the twentieth century they have been advocating withholding solid food in all diarrhoea diseases including cholera. During the same period people have been restricting the amount of water and other drinks in infantile diarrhoea although now oral rehydration is a standard procedure for reducing mortality due to these diseases.

"Slimming diets" are a huge industry but most of them are outside the scope of this paper which deals primarily with foodborne infections. However, treatment of obesity with thyroid extracts has been a vehicle of Salmonella transmission. Also foods rich in proteins and low in carbohydrates with fruits withheld totally have been seen to favour gastrointestinal infections.

Some highly placed scientists have exaggerated the role of food supplements and have, for example, recommend the taking of large quantities of vitamin C to prevent or cure colds and influenza. There is no scientific basis for this recommendation.

Some false proverbs or sayings:

- Man ist, was man isst (one is what one eats)
- An apple a day keeps the doctor away
- Starve the fever and feed the cold
- Chew each morsel 32 times before swallowing
- Do not drink water after eating fresh fruit

S-D02

Reasons of foodborne diseases and their consequences in the food safety situation in Mongolia

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The Mongolian Government has approved the "Food Law" and other associated legislation to create a control structure for its enforcement to provide the population with quality food and assured hygiene and safe conditions at food production, service and consumption levels and some results have been achieved.
But the economic crisis and poverty of the population (which arose during the first phase of the transition to the market economy), frequent climatic fluctuations relating to drought and hazardous winter conditions (dzud), and insufficient assessment of the situation resulted in only partly meeting the objectives of the Food Program in the country.
Approximately 30 from 60 types of transmitted food borne and diarrheas diseases are diagnosed in the livestock of our country and occurrences of contradictions to the veterinary and plant quarantine requirements have been registered in the procurement and purchase of meat, milk, potato, vegetables, fruits to the public trade and distribution system still. The problems of inadequate coverage of raw food products for control analysis and certification process, direct selling of major part of meat and milk to the consumers without passing any processing treatment, insufficient quality control at food production level, low professional level of technology personnel and lack of proper attitude and knowledge about nutrition issue among the population are still existent, too.
During the last years, the average microbiological contamination of food products was recorded as 22.7 percent, chemical contamination was 14.1 percent and heavy metal's contamination was 6 percent. The 35.5 percent of the total samples were food products, which fail to comply with the national food quality and safety requirements. More than 150 species of molds are known to be carcinogenic, mutagenic and genetic effects and only thirteen fungi species can be analyzed in today's food control laboratory. The prevalence of cancer is increasing during 1995-2000, especially liver cancer rate, which is first in prevalence rates of cancer in Mongolia. Health reports show that gastrointestinal infectious diseases and diarrheas diseases related with food and environment contamination and unhygienic conditions contribute to 30 percent of the total infectious diseases.
There is not a comprehensive system to report and register food borne diseases in Mongolia. Only extensive outbreaks of food borne diseases are reported and diagnosed. Single cases of diarrhea diseases are usually not reported. Clinical laboratory analyses data (2002) of the Infectious Disease Center showed that from 51 cases of clinically confirmed cases as food borne intoxication.
As mentioned to improving the situation the country has developed the National Plan of Action for Food Security, Safety and Nutrition. The objective of the program on Food safety action is to harmonize the national food safety measures with the international standards and establish a system to build capacity to conduct risk analysis and safety control measures; to strengthen food safety control laboratories and improve national resources capabilities; to conduct training and advocacy on food safety issues for the producers, consumers in order to improve proper culture in food consumption and public participation; to ensure the quality of water supply and sanitation.
As food supply, food safety and nutrition issues influence and sustain the society and so are important assurances promoting the political and economic independence of the country.

S-D03

Occurrence of foodborne pathogens in meats: The Malaysian legislations approach and challenges

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Food legislations are to ensure the safety and wholesome quality of food for human consumption. To ensure these legislations are useful, they must be properly implemented, monitored and enforced.

Several studies reported on the isolations of foodborne pathogens from meats such as beef, poultry meat and pork at processing plants and retail outlets. These pathogens include *Salmonella, Campylobacter, Listeria monocytogenes*, *E. coli* O157:H7 and vancomycin resistant enterococci (VRE). In some of these studies, the resistance of the isolates to a number of antibiotics were also determined. A large number of these isolates were found resistant to more than two antibiotics, as many as five to twelve antibiotics.
Some of these foodborne pathogens, in particular *Salmonella* and *Campylobacter* were reported to be quite prevalent, particularly in poultry meat. The different types of processing procedures and retailing conditions of these meats contributed to the contamination with and proliferation of the pathogens. This is especially so with meats retailed under warm conditions at wet and night markets. In Malaysia, acts, regulations, guidelines and codes of practice related to food safety and wholesome quality are eastablished and monitoring and enforcement are reported to be carried out regularly. The authorities in the country, mainly the Food Quality Control Division (Ministry of Health) and Department of Veterinary Services and Department of Fisheries (Ministry of Agriculture) are promoting food hygiene and quality programmes, such as, GHP, GMP, GRP, veterinary inspection and accreditation programme for livestock farms and livestock products and HACCP system. In 2003, the Ministry of Health launched National Food Safety Policy and announced two new regulations related to food safety; with two more regulations are in the pipe line.

Despite efforts undertaken by the regulatory agencies, the problems of high microbial contamination with coliforms and pathogens in meats continue to occur. This is particularly so with small to medium meat producers and retailers.
The “profit-making first and safety comes second attitude” of the producers and retailers and the socio-economic status and attitude of the public play a role in allowing the conditions under which the meats are produced and retailed.

S-D04

International Education in Veterinary Public Health: 13 years of experience

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Recent events such the BSE-crisis and the West Nile virus outbreaks highlight the challenges that veterinarian must embrace in public health. Nowadays it has become evident that diseases and different conditions are not limited to specific locations. This demands for a global perspective that needs to be reflected in veterinary (and medical) curricula.
Different veterinary curricula from many different countries were compared. It was shown that the degree of attention given to the area of Veterinary Public Health varied significally from curriculum to curriculum. A revision and harmonization of these curricula and a closer cooperation between the different faculties is recommended.
In this view we will discuss the historical development of the teaching strategies, which have been used during a joint ERASMUS course in Veterinary Public Health and Animal Production that has been held at Utrecht University since 1990. This 3 months course has been held on a yearly basis and is open for last year veterinary (undergraduate) students or recently graduated veterinarians originating from Western and Eastern European faculties as well as faculties from Latin America, Africa and Asia.
Critical issues facing intercultural higher education were examined. First of all the course programme has to be adapted to the international dimension. Further, issues such as classical lecturing, the problem-solving approach, project training, group assignments, round-table discussions, computer-based learning social programme outside of the classroom have been discussed. It can be concluded that especially in international courses the efficiency of the learning process is very much related to the background of the students. It has to be considered how students learn and the use of innovative training methods has to be adapted consequently.

S-D05

Kitchen HACCP in the public health curriculum

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The expanding field of Public Health places new demands on the knowledge and skills of students. Therefore, there is a need for the veterinary curricula to adapt to this new profile. Through the introduction of case studies dealing with up-to-date issues, students are being trained to solve (real-life) problems and come up with realistic solutions. At the Department of Public Health and Food Safety of the Veterinary Faculty, University of Utrecht, the Netherlands, positive experiences have been obtained confronting students with HACCP based risk analysis of the kitchens of the University Utrecht, The Netherlands. In the training period Public Health in the fifth year of the Veterinary Medicine study, students visit one of the 11 university kitchens. They check the HACCP plan and will take samples for hygiene and product control at identified critical control points. Other points that are examined are clean and dirty routes, personal hygiene, last in first out, refrigerator temperatures and separation of raw and processed products. Every kitchen is visited at least twice a year. Results of the exam and comments on the HACCP procedure are discussed with the quality manager of the kitchen.
Conclusions after implementing the training period for two years are:
Students understand far more of HACCP in kitchens if they actually check and implement HACCP procedures and HACCP procedures are more stringently implemented if checks are made on a regular basis. Furthermore hygiene and product quality levels have risen.

S-E01

Ozonated wash-water for the quality guarantee of pre-packaged salads

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Outbreaks of food-borne diseases are frequently attributed to raw or minimally processed foodstuffs. Over 300,000 cases of food-borne illness were reported in the EU in 1999 alone. Contamination of produce with plant pathogenic organisms may also have a marked effect on productivity with losses due to microbial spoilage being reported as being as high as 30% per year. In many countries contamination effects are minimised through the use of chlorine in order to kill the contaminants present on the surface of produce. Chlorine may leave behind odour and flavour residuals that may affect consumer acceptance and the formation of halogenic by-products in the waste water after use is also undesirable. Hence the fact that in Germany the use of chlorine is forbidden. An elegant alternative for disinfection is the use of ozone (O_3). Ozone is a strong oxidant with bactericidal, fungicidal, and virucidal effects. It has a very short half-life in aqueous solution, thus during the wash process ozone will rapidly decompose to O_2. Therefore there is no waste water produced that requires treatment prior to disposal. Ozone has been used in water treatment for approximately a century, and there are also cases of use in the treatment of butter, cheese, eggs, potatoes, and fruits such as apples and citrus fruits. The use of ozone in the treatment of minimally processed produce such as lettuce is a relatively new development.

In co-operation with BWT (ozone generator manufacturer) a wash step incorporating ozonated water into a pre-existing wash system at Havita (fresh vegetable producer) is being developed in order to guarantee produce quality to the end of product life time. To facilitate this, investigations were performed on model systems to quantify the efficacy of various ozone concentrations at differing times on the survival of selected contaminating microorganisms (*E. coli* DSMZ 1116, DSMZ 5923, *S. choleraesuis* DSMZ 554, *B. cereus* DSMZ 31, *L. monocytogenes* DSMZ 20600). All test organisms showed considerable sensitivity to the effects of ozonation at concentrations < 1.0 ppm within 2 minutes. This effect generally persisted up to 20 minutes. In some cases the bacterial survival rate increased over this time period, particularly in the case of *E. coli*. In the case of lettuce leaves inoculated with a bacterial cocktail that was washed under commercial practice conditions, bacterial reductions following the use of ozone at 1.5 ppm for 2 minutes were observed. Strains of *B. cereus*, *L. monocytogenes*, and *S. choleraesuis* displayed higher levels of sensitivity to the effects of ozonation than *E. coli*.

Further investigations considered the effects of ozone on product quality. Sugar content (HPLC analysis) and vitamin C content (Reflectoquant test kit, Merck) of lettuce was measured. With ozone levels up to 2.0 ppm for 2 minutes, no changes in contents were detectable.

The installation of the wash system incorporating ozonated water at Havita will commence early 2004. The effects of the ozonated wash step on fresh packaged lettuce will then be examined and the results evaluated according to the recommended and warning limits for mixed salads from the DGHM.

This project was funded by the BMBF (Grant no. 0330092).

S-E02

The effect of power direct ultrasound on microbial count of date syrup

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Date fruits are mixed with water at 50-60 degrees Celsius for about 50 minutes in the date syrup industry. This amount of heating is not sufficient for killing of micro-organisms. Therefore date syrup carries always the risk of causing health problems. Ultrasound power can be used for decontamination and provides food safety in date syrup.

In this research, various methods of extraction were applied for obtaining the best processing procedure with the highest effect on microbial death:
1) Two ratio of date fruit and water: “1,3“ and “1,9“.
2) Three levels of direct ultrasound intensity: 150, 90 and 0 Watt.cm^{-2} in 20 kHz.
3) Two levels of temperature: 15 and 35 degrees Celsius.

The results showed that direct sonicated samples with high intensity (150 Watt.cm^{-2}) at 15 degrees Celsius with 1,9 mixing ratio had the best results in decreasing the microbial count. We noticed there were anti-microbial and anti-fungal substances in date syrup which decreased the microbial count in samples. So the combination of ultrasonication and anti-microbial substances in date syrup complete the action of each other in removing the pollution of product.

S-F01

Microbiological quality of organically produced German meat products

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Currently, there are no representative data on the microbiological safety and quality of organic meat products such as spreadable raw sausage and pasteurized, sliced and prepackaged convenience products such as Bologna-type sausage and cooked ham which are made available to the consumer under cold storage with minimum durabilities of 15 to 30 days. Because of an increasing market share of organic meats and extensive differences in livestock husbandry, meat production and processing without or with reduced concentrations of nitrite and other “chemical” additives compared to conventional meat processing it is important to fill this knowledge gap.
In this study, financed by the Federal Program on Organic Farming, we investigated in regular intervals from October 2002 - October 2003 products from six cooperating German manufactures of nationwide importance as well as products from organic retail outlets. The results are compared to current data from the official food control kindly provided by six laboratories from the regional authorities.

The analysed samples of spreadable raw sausage (n=208) were negative for Salmonella. Shigatoxin producing *Escherichia coli* (STEC) were found in 0.5 % of the samples. *Listeria monocytogenes* was always below 100 cfu/g, i.e. within the permitted limits. Levels of coagulase positive staphylococci and *Enterobacteriaceae* were with some exceptions within tolerable limits below 10^3 cfu/g respectively below 10^4 cfu/g.
The sliced cooked meats (n=326) also did not contain *Listeria monocytogenes* in excess of 10 cfu/g, neither fresh nor at the end of the best-before-date. The numbers of lactic acid bacteria and *Enterobacteriacae* were comparable to conventional products and are indicative for unsolved recontamination problems during slicing and packaging.

The results show that meat products manufactured according to the guidelines of the approved associations like Demeter and Bioland do not represent an increased health risk to the consumer as compared to conventional products.
In general, hygiene of processing and self-control mechanisms should continuously be improved to avoid potential health risks and to minimize losses through microbial spoilage.

S-F02

Microbiological investigation of retail helva produced in Turkey

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Introduction: Helva (also known as halva or halvah) is a Middle East and Eastern-Mediterranean dessert made from sesame seeds paste called tahin, sugar and flavour. It is prepared by mixing tahin and acidified heated glucose syrup. After adding flavour or other ingredients like vanilla, pistachio or chocolate, the hot mass is poured into jars. An international outbreak of multiresistant *Salmonella enterica* serotype Typhimurium (*S*. Typhimurium) definitive phage type (DT) 104 infection associated with consumption of helva has been reported in Sweden, UK, Australia, Norway and Germany since early June 2001. The strains were resistant to the antimicrobials ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracyclines (R-Type ACSSuT). The information disseminated through the Enter-net, *Eurosurveillance Weekly*, Global Salm-Surv, and ProMED allowed to identify epidemiological association with helva imported from Turkey. In this consideration, the aim of this study was to present microbiological properties including coliform, faecal coliform, *Escherichia coli* and *Salmonella* in several brand and type of helva produced in Turkey.

Material and Methods: Ninety and three helva samples from 14 different brand were collected from different supermarkets and factories in Turkey. Of the 93 samples, 30 were plain, 29 were flavoured with chocolate, 17 with pistachio, 10 with vanilla, six with orange and chocolate, and one with pistachio and chocolate. The most probable number technique (Fishbein et al. 1976) using Fluorocult LMX broth followed by confirmation with EC broth was used to enumerate coliforms and faecal coliforms. Positive EC broth cultures were streaked on EMB agar and incubated at 37°C for 24 h. One or two colonies showing green metallic sheen were subcultured and differentiated by biochemical tests and production of gas at 44.5°C in EC broth. Standard culture method was used for detection of *Salmonella*. 25 g of each helva sample was pre-enriched in 225 ml buffered peptone water at 37°C for 16 to 18 h and then 10 ml of pre-enrichment broth was inoculated into 90 ml Selenite Cysteine broth for selective enrichment and incubated at 37°C for 48 h. XLT-4 were used as selective plating media. Presumptive *Salmonella* colonies were identified biochemically and serotyped.

Results: Of all samples, 64 (69%) were containing coliform group bacteria, but only 2 (2.2%) were at high level according to the Turkish Food Codex. Faecal coliforms were not present in 31.18% of all samples and 59.14% contained faecal coliform less than 3 cfu/g. Of all samples, 6 (6.45%) have faecal coliforms between 3-10 cfu/g. From remaining samples; one sample flavoured with pistachio had $4.3x10^1$ cfu/g, one flavoured with vanilla had $1.5x10^2$ cfu/g and another one flavoured with chocolate had $4.6x10^2$ cfu/g faecal coliform. Two *E. coli* strains isolated from samples flavoured with chocolate and vanilla. One *Salmonella* strain was isolated from sample flavoured with chocolate. It was serotyped as *S.* Typhimurium and R-Type ACSSuT was determined by antimicrobial susceptibility test. Two *E. coli* and *S.* Typhimurium strains were isolated from same manufacturers' samples.

Discussion: In recent years, several reports show an increase of *Salmonella* infection from outbreaks and from food samples associated with different types of seeds and spices from several countries. Sesame seeds and sesame seeds products, including helva, tahin, and hummus should always be considered as the potential contamination sources for *Salmonella*. Because these products are consumed as ready-to-eat, hygiene and good manufacturing practices through the all production steps are highly important.

S-F03

Milk as a potential source of food-borne disease in The Gambia, Senegal and Guinea-Conakry

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Milk produced in the countries of the region is usually consumed raw or fermented. There is little information about the quality either at farm's level (milk already infected with pathogenic agents or contaminated because of unhygienic handling) or at market level. As milk can, under certain conditions, pose a potential health hazard, particularly when consumed raw, it is not only the quantity but also the quality that needs to be investigated in order to improve the nutritional base of an increasing population in urban and peri-urban areas. The objectives of this study are the identification and quantification of bacterial contaminants and zoonotic agents at producer's, trader's and vendor's level and associated risk for the consumer. Additionally, the study intends to assess the improvement in milk quality and hygiene at pasteurisation units in Senegal.

The microbial contamination of 915 milk samples from The Gambia, Guinea and Senegal was investigated. Samples were tested for Coliform bacteria, *E.coli*, coagulase-positive *Staphylococci* spp., *Salmonella* spp., *Bacillus cereus*, *Listeria* spp. and H_2S- reducing *Clostridia*. All methods used for culturing and identification of microorganisms comply with the International Standardization Organization (ISO).

54.3% (463/852) of all non-pasteurized milk samples were highly contaminated with coliform bacteria with counts above $5x10^4$ colony forming units per millilitre (cfu/ml). Many of the samples contained potentially pathogenic bacteria. Counts of *E.coli* above $1x10^5$ cfu/ml were found in 22.6% (113/501) of the raw milk and in 17.7% (62/351) of the sour milk samples. Counts of coagulase-positive *Staphylococci* spp. above $2x10^3$ cfu/ml were more frequent in raw (30.5% (153/501)) than in sour milk (10.5% (37/351)). *Bacillus cereus* and H_2S-reducing *Clostridia* spp. could be isolated in 26.3% (132/501) resp. 18.0% (90/501) of the raw milk samples and in 33.3% (117/351) resp. 31.1% (109/351) of the sour milk samples. Less frequent was the presence of Listeria spp. (2.9% (25/852)) and *Salmonella spp*. (0.6%(5/852)) in both raw and sour milk samples.

Results further show that pasteurisation clearly reduces risk for consumers. Samples collected from small pasteurisation centres in Senegal had lower counts of *E.coli* and coagulase-positive *Staphylococci* spp. Only 3 out of 63 samples (4.8%) showed counts of *E.coli* above $1x10^5$ cfu/ml and 5 samples (7.9%) contained more than $2x10^3$ cfu/ml of coagulase-positive *Staphylococci* spp. Neither *Salmonella spp.* nor *Listeria spp*. have been isolated from pasteurized milk samples. However, 38.1% (24/63) of pasteurised milk samples contained *Bacillus cereus* and 11.1% (7/63) H_2S-reducing *Clostridia* spp.

These results clearly indicate that the consumption of milk that is offered at local markets as raw or fermented milk poses a health risk. Pathogenic microorganisms present in milk could be one of the causes for the frequent occurrence of diarrhoeal diseases, especially in children (45,644 cases of diarrhoea in children under 5 years in 2002 in The Gambia.) Though the direct link between the high contamination of milk and cases of milk-borne diseases in the population could not be established in the framework of this study, it can be assumed that the consumption of such milk might lead to mild to severe symptoms of food infection and/or -intoxication.

S-F04

Occurrence of *Staphylococcus aureus* enterotoxins in food in Norway

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Staphylococcal food poisoning, caused by *S. aureus* enterotoxins (SEs), is one of the most frequent foodborne microbial diseases worldwide. In Norway, *S. aureus* is one of the most common forms of bacterial foodborne disease and for the period from 1993 to 1998 has been the causative agent in 24.2% of food-borne disease outbreaks reported. The number of cases reported is probably underestimated because the symptoms usually develop quite rapidly, normally 1–6 hours after ingestion of food containing SEs, and are over within 24 to 48 h. To incriminate food as the causal agent it is necessary to detect eneterotoxin in the suspected food or show that isolated *S. aureus* produce SEs. Altogether nine serologically distinct SEs have been identified. SE A-E represents classical types, while SE G-J are newly described enterotoxins. Recently, other SE genes (*se*); *sek*, *sel*, *sem*, *sen*, *seo* and *seu* have been described, which point to the possible existence of new SEs.
National Veterinary Institute in Oslo is the Norwegian reference laboratory for *S. aureus* and SEs in food. During 2000-2003 we have characterised 237 *S. aureus* isolates from humans and foods and analysed 235 food samples that have been involved in suspected human cases of staphylococcal food poisoning. Samples analysed included milk, meat, fish, and their products as well as ready-to-eat products were analysed for presence of SEs.
From 2001-2002, 6.6% of 121 food samples tested were positive for SEs (SEA 2, SEB 3 and SEC 3). In 2003, 15% of 106 food samples tested were positive for SEs. From all these tested samples coagulase positive staphylococci were isolated only from eight food samples.
SET-RPLA test was used for detection of SEs A-D from 27 human and 210 food staphylococcal isolates. 67% of human *S. aureus* isolates produced SEs and most of them were characterised as SEA (67%). *S. aureus* food isolates also mostly belonged to SEA (52%) followed by SEB (27%), SEC (26%) and SED (9%).
The results of occurrence of SEs in human and food isolates as well as food products that have been linked to staphylococcal food poisoning in Norway are rather low.

S-F05

Application of HACCP to chef self-control in catering operations

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To assure that safe food is consumed, food safety scientific information must be translated into hazard control rules that cooks can use to prevent, eliminate, or reduce a hazard in the food that they prepare to a tolerable level. The cook may be in a process plant, a foodservice facility, or the home, but the rules for cleaning food contact surfaces, pasteurizing a product, or disinfecting a product with washing should be based on science and should be uniform among all processing facilities, because the pathogenic substances in the food are very similar. At the same time, the retail HACCP system that the cook uses must be kept very simple, with a minimum of records. Record keeping is a major barrier and a major cost burden to the widespread use of HACCP by the retail food industry.

If one follows the principle that HACCP is a pre-control process, records of times, temperatures, bacterial counts made "after the fact" are not critical controls. They only prove that the HACCP program is stable. This author has developed a retail-cook-level HACCP program for restaurants with minimum records. It consists of: 1) a scientifically correct hazard control checklist, 2) an employee training form, 3) a HACCP team meeting form, and 4) a corrective action form. In small operations, one does not need a multi-page HACCP manual. The policies are defined by the hazard control checklist and the training form used by the chef to train the employees in the kitchen. When a kitchen becomes increasingly sophisticated, more people will be hired, and there will be more resources for documentation. However, if the checklist accurately identifies all of the hazards and provides validated controls, and employees are trained to perform the controls, the pre-control basis for HACCP is being met.

This presentation will describe the successful application of HACCP as a pre-control process to three restaurants and the Rochester school system in Olmsted County, Minnesota. It will discuss problems that were encountered and how they were solved, so that the checklist is an accurate list of hazards in each facility, and controls are validated to be effective.

S-F06

HACCP: efficient prevention of catering-borne outbreaks?

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The significance of food safety enhanced worldwide in the last decades. According to the estimation of WHO, the number of diseases possibly related to food and water consumption, shows continuous increase all over the world, concerns yearly 10-30% of the population.

The number of food borne outbreaks and cases shows gradually increasing tendency since the early nineties in Hungary as well. According to the statistics, although most foodborne outbreaks occurs in private households but the greatest number of illnesses (cases) is shown among the guests and consumers of public catering, concerning mainly children. In the last decades the main causative agent was the *Salmonella* Enteritidis. The major foodborne outbreak of Hungary occurred in 1996 in numerous schools of Budapest, when more than 5000 school children became seriously ill and more than 800 of them were taken to hospital on the same day. The outbreak was connected with the consumption of a broad-wide distributed mass-catering food heavily contaminated by *Salmonella* Enteritidis. The presentation analyses the reasons and consequences of this catastrophic outbreaks in connection with the HACCP analysis of the incriminated product.

The level of food hygiene and food safety in catering outlets significantly influences the national food safety records, and its importance is enhancing. We are living in a rapidly changing world, and our lifestyle regarding food preparation and food consumption changed more in the last twenty years, than centuries before. The presence of new technologies, new equipments, services, as cook-chill, Sous-Vide technology, take-away and delivery to the homes doors, mass preparation of hot and cold meals, exotic foods could be hazardous for large number of people.

In the changing world of catering the traditional food hygiene measures do not provide enough safety. The HACCP system theoretically offers an efficient tool for prevention, but its implementation and maintenance means a lot of difficulties for catering sector. Risk-based, catering-tailored, cost-effective food safety management system is necessary.

The presentation gives a short SWOT analysis (**S**trengths, **W**eaknesses, **O**pportunities, **T**hreats) of HACCP application in catering.

S-G01

Joint European - Asian postgraduate education in response to global trade and regional needs in food safety: The 1st MSc Course in Veterinary Public Health between the Freie Universitaet Berlin and the Chiang Mai University in Thailand

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The pivotal importance of veterinary public health in general, and food safety in particular in the global trade of livestock and livestock products is addressed in international standards and regulations as laid down in the Sanitary and Phytosanitary Agreement (SPS-Agreement) of the World Trade Organisation (WTO) as well as in the Codex Alimentarius. To put these regulations and agreements into meaningful action skilled and specialized professionals are increasingly in demand in Southeast Asia countries - such as Thailand and Vietnam - targeting European markets. Thus, region-based as well as Europe-linked postgraduate education is to be delivered through mutual partnership in joint projects.

As part of its efforts to promote regional and multilateral networking between higher education institutions in EU Member States and South-East Asia the Asia Link Programme of the European Union is granting substantial co-funding to the 1st Ioint MSc Course in Veterinary Public Health of the Chiang Mai University / Thailand, Freie Universitaet Berlin / Germany and the University of Veterinary Medicine Vienna / Austria.

Course curriculum and concept of the MSc Course in Veterinary Public Health (MSc VPH) as implemented in Chiang Mai, Berlin and Vienna are described; experiences gained during the first year of this 24-month “Joint Degree”-Programme with 14 young veterinarians from Southeast Asia are reported.

S-G02

Developments of food hygiene in Latvia as consequence of EU accession

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1. According to the needs of global trade and arrangements of WTO, particularly SPS agreement, there is request in place to harmonize, minimize and unify globally trade requirements, including food hygiene, animal health, and plant health as well as environment protection. Although EU takes active part in development of globally harmonized requirements in the frame of Codex Alimentarius and O.I.E. recommendations, makes efforts to harmonize EU rules with them, there are still differences in several areas. As Latvia is joining EU on the 1st of May 2004, the most essential was to harmonize all legislation with aquis communitaire.
2. Latvia, as other Eastern and Central Europe countries, during last decade has gone through enormous changes.

- First it concerns market organization. A lot of new small-scale establishments spread up; old export markets were lost; due to introduction of more liberal trade, import increased and so also internal competition. Previously existing establishments had to be reorganized and they lost their production volumes and traditional marketing possibilities; there was an urgent need to develop skills for marketing in new circumstances. This leaded to the situation that Latvia from the exporting country in several areas became an importing one (e.g. meat and meat products about 50%).
- The food safety legislation in Latvia was changing even more - from Soviet rules to the first Latvian ones, then the harmonisation with EU ones, after direct full transposition of EU legislation. At the end there was no much room left for discussion with stakeholders - producers and consumers on the new legislation, they just had to accept harmonization process. Especially dual situations occur when EU newcomers have to implement existing EU legislation, when new one, substantionally modified was close to finalization and would be more acceptable due to more flexibility. Sometimes rules became weaker (some limits for acceptable contamination levels; import requirements; border control of all kind of foodstuffs) other times stronger (food hygiene structural requirements, implementation of HACCP in the whole food chain). Often for industry in transition is was difficult to adapt to changes in short time, it was especially disappointing for them to be required for expensive structural changes, which no doubt improved the possibility to produce safety food, but were luxury relating to the many local consumer demands and financial situation.
- And then question rises - is the main aim of the legislation to stimulate quality and safety of products, as well as complex development of each society, or just to promote free global trade? At the moment WTO recognises only science-based minimalized rules. But there is limitation of science abilities - equipment, prolongation of experiments, and combination of different conditions? And the whole methodological process of investigation - good food is - what is proved to be a good or good is - what is not proved to be bad? We should, perhaps be more strategic –to take in serious consideration consumer perceptions and sustainable development principles.

3. The most important for development of global trade in food is not the trade it self, but the need to create the mechanism, which avoids the reduction of food quality and safety in the situation of tuff competition as well as keeps local food operators to be able to serve consumers according to their perceptions, needs and demands.

S-G03

Response programme for countering food terrorism in Spain

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Recently, WHO has warned that "the malicious contamination of food for terrorist purposes is a real and current threat". Similarly, CDC's infectious disease experts have concluded that "sabotage of food and water is the easiest means of biological or chemical attack largely because such attacks have been successful in the past". These warnings have acquired a special relevance since the terrifying attack undergone in Madrid the past 11th of March. As a result, the Public Health Authorities must be prepared to suitably respond to the deliberate contamination of water and food with terrorist aims.
In Spain, the Spanish Food Safety Agency, has the responsibility in leading the planning and coordination of procedures that reduce the vulnerability of the food chain and to develop answer protocols that diminish the effects that the deliberate sabotage of food would have on illnesses and death, in addition to the economic, social and political effects.
To achieve this aim it has been created a work group made up of members of the Spanish Food Safety Agency in cooperation with personnel belonging to the General Inspectorate of Health of the Spanish Armed Forces. The former will contribute with their experience in the management of food alert; the latter provide knowledge on radionuclear, chemical and biological weapons. The main points we have focused on are the following ones:

- Identify and characterize the chemical and biological agents capable of being used with terrorist aims and make an evaluation of the risk of undergoing an attack with these agents in any point of the food chain.
- Select and review the specialized bibliography in the subject, highlithing the main aspects of each one of the compiled documents.
- Establish contacts with similar work groups in surrounding countries, to interchange information and experiences.
- Design a decision taking algorithm for foodborne outbreaks to allow an early recognition of the terrorist nature of the event, based on its credibility, symptomatology and epidemiological features.
- Implement response protocols to facilitate the management of the food alert, once recognized the deliberate character of the outbreak.
- Establish a network of available laboratories for the rapid diagnosis of causative agents, with effective contacts to laboratories and standardised protocols for reagents and analytical methods.
- Promote cooperation with the food industry to implement security measures in their food safety management programmes.

S-G04

Sensitive detection of ricin, a potential weapon for bioterrorists, from food

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Ricin is a protein of 62 kDa size and is composed of two polypeptide subunit chains called ricin A (31.6 kDa) and ricin B (31.4 kDa). Ricin is present in quantities of 1-5% in the seeds (castor beans) of the plant *Ricinus communis* which is worldwide cultivated for production of castor oil. The Ricin A-subunit functions as a potent cytotoxin which belongs to the family of RNA *N*-glycosidases, enzymes which block protein synthesis in mammalian cells leading to cell death. The B-subunit is a lectin which enables binding of the holotoxin to galactose residues on the surface of mammalian cells. Due to its high toxicity for humans, its resistance to physiochemical stress and to the lack of adequate therapeutic measures after intoxication, ricin became of interest as a weapon for bioterrorists. Data from accidental ingestion of castor beans show that an amount of 4-5 mg ricin orally ingested may cause death in humans. Because of the bioterrorist threat, there is a growing need to establish quick, reliable and sensitive detection system for toxins such as ricin. We have developed and compared biological (cytotoxicity) and immunological detection systems (ELISA and western blot) for ricin as a contaminant of food. The cytotoxicity test was found as most sensitive detecting 0.01 ng/ml purified ricin A and of 1 ng/ml of partially purified ricin. However, this biological assay takes 24-72 h for detection and requires a neutralization test for specificity and is therefore not suitable as a rapid detection system. An ELISA was developed using monoclonal antibodies directed to ricin A and ricin B. The ELISA detected ricin in milk (25 ng/ml), coca cola (10 ng/ml) and vegetable broth (1 ng/ml). Because the sensitivity of detection varied according to the food we have developed enrichment procedures for ricin using immuno magnetic separation (IMS) followed by Western blotting. IMS takes 1h to perform and results in a dramatic increase in sensitivity: using IMS the detection limit of ricin in milk could be increased about 250-fold up to 0.1 ng/ml. The combination of IMS and Western blot detection allowed the sensitive detection of ricin from different food samples in 6-8 h of time.

Poster

P-A01

Occurrence of *Listeria monocytogenes* in the industrial processing line of spreadable raw sausages

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In this study the occurrence of *L. monocytogenes* in the industrial processing line of spreadable raw sausage was examined.
A total of 1772 swab samples were taken from relevant comparable locations in the sanitary and processing area of two German plants (A and B) and analysed for the presence of *L.monocytogenes*.
In parallel the *L. monocytogenes* plate counts of samples including raw meats (n=385), raw sausage batters (n=1223) and corresponding ready-to-eat products (n=1180) were evaluated.
All samples were examined at regular intervals during November 2000 and March 2002.

The overall frequency of *L. monocytogenes* in environmental swab samples were markedly different between plant A and B (2 % and 49 %).
41 out of 48 selected strains isolated from different places in plant B were confirmed to be serovar 1/2a, 1/2b, 1/2c, 4b and 4d, whereas strains belonging to serovar 4b were most often detected and were present at all examined places.
The *L. monocytogenes* plate counts in all samples of raw sausage batters and ready-to-eat products never exceeded 100 cfu per gram. Moreover, 97 % of all product samples had plate counts of less than 10 cfu per gram.

Results of the study show, that in spite of the possible high overall occurrence of *L.monocytogenes* as demonstrated in plant B, spreadable raw sausages did in no case contain *L. monocytogenes* in numbers representing a high risk for the not vulnerable consumers.

P-A02

Occurrence of emetic toxin producing *Bacillus cereus* in the dairy production chain

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Spores of *B. cereus* are common contaminants in raw milk but the occurrence of emetic strains of *B. cereus* in the dairy production chain is not well known. The vast majority of emetic food poisoning cases by *B. cereus* have been attributed to rice and rice dishes but milk and milk ingredients may have been implicated in some food poisoning episodes involving pasteurized cream, vanilla slices, reconstituted infant formula, and in one case UHT-milk.

We have screened a large collection of *B. cereus* isolates for the presence of emetic strains, based on phenotypic traits (starch and salicin negative isolates which exhibit a narrow zone of hemolysis, or no hemolysis at all, on TSA blood agar), RAPD-PCR (specific RAPD pattern) and a sperm motility test. The sperm test was the most specific, followed by RAPD and the phenotypic tests the least specific. However, looking for colonies with a small zone of hemolysis may be a useful presumptive test for emetic *B. cereus* in foods.

No emetic strains were found among 423 raw milk isolates, 374 soil isolates and 122 isolates from feed, grass, dung and rinsing water from the milking equipment taken at a farm during the grazing seasons over two years. Three emetic strains were identified among of 279 milk isolates from two out of 7 farms during the stall period. No emetic strains were found among 498 environmental isolates (rinsing water, used bedding, feed, air and dung) from the same seven farms. However, at an additional farm, emetic isolates were identified in milk samples, rinsing water and used bedding material at a frequency of 47%. This farm had cubicles with deep sawdust bedding, which allowed extensive growth of *B. cereus*.

Only one emetic strain was found among 2031 isolates taken along the processing lines for pasteurized milk of 4 dairy plants at 8 sampling days. Monthly samplings of silo tanks at eight dairy plants over a year only resulted in one emetic strain out of 1370 tested. However, intensive sampling during shorter periods at the positive dairy revealed that 89% of the isolates over a winter period were emetic. Clonal development of certain *B. cereus* strains was observed in silo tanks at all dairy plants.

Emetic strains were rare in the farm to food chain for pasteurised milk production. With exception of one farm the frequency of occurrence of emetic strains in raw milk at farms was less than 0.2-1%. Likewise, the occurrence in silo tanks and in the production chain at dairy plants was in general less than 0.1 to 1%. However, evidence for clonal development of emetic *B. cereus* was found on a farm as well as in a silo tank at a dairy plant. Some of these strains were as strong producers of toxin as known food-poisoning strains. Emetic strains are not psychrotrophic so they will not affect the quality of pasteurised milk. On the other hand, the occurrence of emetic strains will be of concern for the production of milk powder and other milk based products that may become ingredients in other types of food, including milk replacers for babies. It may be important for the dairy industry to further optimise the cleaning routines of silos.

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P-A03

Prevalence of *Salmonella* in eggs and other samples from laying flocks vaccinated for *Salmonella* Enteritidis

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In most developed countries contaminated table eggs are considered to be the predominant source of *Salmonella enterica* serovar Enteritidis (*S.*Enteritidis) for humans. Control of the organism in the breeding sector of the poultry industry has been largely successful but in many countries this has had little impact on the level of infection in commercial laying flocks because of persistent contamination of farms and infection of *Salmonella* free pullets placed in the contaminated environment of the laying house. In some countries, particularly Germany and UK, vaccination has been extensively used to control this. It is apparent however that vaccination is not fully protective. A study of *Salmonella* infection and contamination was carried out in 20 cage layer flocks, 17 free-range flocks and 11 barn egg flocks where *S.*Enteritidis had been found. In 15 of 17 free-range flocks vaccination with a killed vaccine was associated with disappearance of infection within one flock cycle and the organism was eliminated from the rest in two cycles. In 4 of 11 barn egg flocks infection disappeared in one flock cycle and was eliminated from the rest in two flock cycles. Vaccination led to disappearance of infection in 2 of 20 cage flocks within two flock cycles but the remainder were still infected after three flocks, although the level of *Salmonella* was reduced in faecal, environment and spent hen post-mortem samples. Eggs were collected from 12 cage layer flocks which had remained persistently infected despite vaccination. 24 batches of 6 egg shells of 13,652 individual eggs tested (0.18%) were positive for *S.*Enteritidis and 54 (0.40%) for other serovars. 6 x 6 contents pool batches of 13,640 (0.02%) individual eggs tested contained *S.*Enteritidis. In total 33 batches / 13,682 eggs (0.24%) from vaccinated flocks were contaminated with *S.*Enteritidis. This contrasted with results of previous studies where 1.0% of eggs from non-vaccinated flocks were contaminated. *S.*Enteritidis was found in 67/699 (9.6%) of spent hens from vaccinated flocks and 64/562 (11.4%) of pooled fresh faeces samples. Failure to adequately clean and disinfect cage laying houses and to control mice appeared to be a common feature on the persistently infected farms whereas mice were not a problem in the two cage flocks where *S.*Enteritidis did not persist, even though no disinfection was carried out. It is likely that poor disinfection increases the risk of persistence of infection by adding moisture which enables *Salmonella* to multiply in poorly cleaned feeders and other equipment.

P-A04

Prevalence of *Salmonellae* and their resistance to antibiotics in slaughtered pigs in the Czech Republic

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Protection of swine herds and pork against contamination with *Salmonellae*, of which some strains can be resistant to one or more antibiotics and can transmit resistance genes to other human or animal bacteria, is a serious problem from the stand point of both health and economy (Council Directive 92 117/EEC, Directive 2003/99/EC). The objective of a pilot study conducted in the Czech Republic (2001 – 2004) is the evaluation of *Salmonella* prevalence in slaughtered pigs, the analysis of isolates` resistance to antibiotics, and the assessment of the level of health risk in pork contaminated with salmonellae. *Salmonella* prevalence was evaluated in 816 pigs from 15 herds which were slaughtered in 10 slaughter-houses from June 2001 to December 2002. Samples from cecum contents, mesenteric lymph nodes and smears from carcass halves were collected approximately once a month from 40 to 55 slaughtered pigs of the same herd. Simultaneously, 10 to 12 smears from the slaughter line environment were collected. The samples were cultured using a standard ISO 6579 method. After serotyping and phage typing of *Salmonella* Typhimurium strains (according to CPHL Colindale, UK), salmonella isolates were tested by the disc diffusion method NCCLS, 1999 for sensitivity to 14 antibiotics: Ampicillin (AMP 10 µg), Amoxycillin/Clavulanic acid (AMC 30 µg), Apramycin (APR 15 µg), Colistin (CT 10 µg), Sulphamethoxazole/Trimethoprim (SXT 25 µg), Cefotaxime (CTX 30 µg), Enrofloxacin (ENR 5 µg), Gentamicin (CN 10 µg), Neomycin (N 30 µg), Streptomycin (S 10 µg), Tetracycline (TE 30 µg), Chloramphenicol (C 30 µg), Nalidixic acid (NA 30 µg) and Sulfisoxazole (Su 300 µg). Further, the isolates were subtyped using five gene specific PCRs for identification of genes encoding resistance to antibiotics.

Salmonellae were isolated in 27 (3.30%) samples of the slaughtered pigs; the most frequent site being cecum (2.45%). This finding is statistically significant ($P< 0.01$) compared to the findings from mesenteric lymph nodes (0.73%) and smears from carcass halves (0.12%). No salmonella was found in slaughtered pigs from 8 herds. *Salmonella* prevalence in pigs from the other herds ranged between 2.0% and 12.0%. *Salmonellae* were not found in smears from the slaughter line environment (n=197). A total of 27 salmonella isolates were classified into 7 following serotypes: *S.*Iinfantis (n=8), *S.* Typhimurium (n=5), *S.* Agona (n=4), *S.* Kaapstad (n=4), *S.* Derby (n=3), *S.* Bredeney (n=2), and *S.* London (n=1). All *S.* Typhimurium DT 104 strains were resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (phenotype ACSSuT). Resistance genes bla_{PSE-1}, *floR*, *aadA2*, *sul1*, and *tetG* were identified in all pentaresistant strains of this phage type. One strain of *S. derby* was resistant to gentamicin, streptomycin and sulfisoxazole. The other salmonella isolates were sensitive to all antibiotics used. With respect to a sporadic occurrence of *S.* Typhimurium DT104 ACSSuT in swine carcasses, health risk of pork contamination with salmonella was assessed to be low.

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P-A05

Foodborne pathogens in foodstuffs of animal origin in the Czech Republic

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The study targeted on foodborne pathogens in raw materials and foodstuffs of animal origin in the period of 1999 – 2002, was created as an activity of Scientific Committee on Veterinary Measures relating to Public Health. Diagnostic results of competent authorities were analysed. The choice of pathogens followed the valid national legislation. The findings documented that *Salmonella spp*. are the most frequently isolated pathogens. Most cases of food poisonings are caused by these types of bacteria. A sharp decrease in the findings of salmonellas in foodstuffs and reduction of salmonelloses prevalence, was also noted. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Listeria monocytogenes* were also often isolated from foodstuffs. No verotoxin-producing E. coli strains were found in foodstuffs and *Campylobacter spp.* was diagnosed very rarely. The prevalence of campylobacteriosis, however, increased. Industrially processed foodstuffs were not the principal source of human infection. The cases of campylobacteriosis are associated with the handling of raw poultry or eating raw or undercooked poultry meat. Generally, a similar way of infection takes part in salmonelloses and listerioses. A decrease in the findings of major food borne pathogens was noted in foodstuffs during period of interest.

P-A06

Seroprevalence of *Toxoplasma gondii* during the breeding amd fattening period of pigs and in pork

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Toxoplasma gondii is a protozoan (single-celled) parasite found in muscle and other tissues of many warm-blooded animals including pigs and humans. An important source of *Toxoplasma gondii* infection in humans is the consumption of raw or undercooked pork. Exposure of healthy adults to this parasite generally results in either an asymptomatic infection or a mild “flulike” illness. A major health problem is the transmission of the parasite from a pregnant women to her unborn baby, whereas at risk are women who acquire a primary infection during pregnancy.

The aim of this work was to determine, by the Enzyme-Linked Immunosorbent Assay, the prevalence of antibodies to *Toxoplasma gondii* in naturally infected pigs in four farms with different management systems (one organic and three conventional) in the Federal States Brandenburg and Saxony. In total we examined blood samples of 700 pigs. In each pigsty serum samples were taken four times from 100 pigs: at first from newborns (a few days old), then just before they were taken off the sows, then shortly before they were taken over to the fattening pigsties and at last during the slaughtering. Additionally we investigated 1013 serum samples of fattening pigs and sows of different livestock farmings in the region of Halle/Wittenberg, 240 minced pork samples, 400 pork samples (200 of organic sources and 200 of conventional sources) and 262 shortly ripened raw sausages (129 organically sausages and 133 conventionally produced sausages) on *Toxoplasma gondii*-antibodies. All positiv meat samples and sausages were bioassayed in 8 week old, female NMRI-mice.

Investigations in Brandenburg and Sachsen show very different results. Within the three conventional systems we detected seroprevalences against *Toxoplasma-gondii*-antibodies between 0-15.2%. Additionally the seroprevalences not only differ between the different management systems but also within the same system during several breeding phases. At the organic system we were not able to detect any *Toxoplasma-gondii*-antibodies during the whole breeding and fattening time.

Examinations in the region of Halle/Wittenberg resulted in seroprevalences on an average of 20.4%. The highest prevalences were found in individual livestock farmings (up to 52%) with up to twenty pigs. Also in older animals (seroprevalence in older sows: 25-30.8%) the seropreva-lence of antibodies to *Toxoplasma-gondii* was higher than in fattening pigs. Serological in-vestigations of 240 minced meat samples resulted in a seroprevalence rate of 5.4%. The 400 pork meat samples showed similar results. 23 samples contained antibodies to *Toxoplasma gondii* (5.75%). Pork samples of organic sources were nearly four times more frequent positive (9.0%) than meat samples of conventional sources (2.5%). In ripened raw sausages we found much fewer antibodies to *Toxoplasma gondii.* All conventional sausages were negative and only two organic sausages (1.6%) showed antibodies against *Toxoplasma gondii.* None of the NMRI-mice got an *Toxoplasma gondii* infection because no mice showed tissue cysts in their brain (detected biologically) or rather antibodies to this parasite (detected by ELISA).

Conclusion: In comparison with older German data it could be shown that the prevalences seem to increase. The risk of the presence of *Toxoplasma gondii* in pork should be controlled by monitorings at the slaughter level.

P-A07

The survey of frequency of *Brucella melitensis*, *Escherichia coli* and *Staphylococcus aureus* of ewes fresh traditional cheese in Shahrekord, Iran

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Over the six-month period, 200 samples of ewes fresh traditional (non pasteurized) cheese were bought in retail shops in shahrekord town ship, were cultured in specific bacteriological media for detection of *Brucella*, *E. coli* and *Staphylococcus aureus*. Out of 200 samples, 1 sample (0,5%) and 48 samples (24%) and 114 samples (57%) were positive for *Brucella melitensis*, *E. coli* and coagulase positive *Staphylococcus aureus*, respectively. It was found that most of ewes fresh traditional cheese were contaminated with these bacteria. Therefore it is recommended to use pasteurized ewes and goats milk in the production of traditional cheese. Attention to animal health is important to prevent contamination of cheese with *B. melitensis*, *E. coli* and *Staphylococcus aureus*.

P-A08

Control of *Salmonella* Enteritidis in laying hens by use of lactobacilli as probiotics

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The majority of cases of salmonellosis in Belgium is caused by *Salmonella* Enteritidis. Contamination of eggs seems to be the major source of *S.* Enteritidis infection. One of the most probable routes of contamination of eggs by *S.* Enteritidis is an ascending infection from the cloaca to the vagina and lower regions of the oviduct. Specific actions are necessary to control the contamination of industrial poultry with *S.* Enteritidis. The presence of lactobacilli in the vagina and cloaca of laying hens is important to maintain a microbial ecosystem that prevents the growth and invasion of pathogens such as *Salmonella* spp. The use of lactobacilli as probiotics for laying hens seems an interesting option to reduce *S.* Enteritidis infection.
About 200 lactobacilli were isolated from the cloaca and vagina of 35 laying hens. This strain collection was typed through repetitive element PCR using the $(GTG)_5$-primer and divided into groups of similar profiles (“rep-types”). Representatives of each rep-type were identified using partial 16S rDNA sequence analysis, SDS-PAGE of cell proteins and AFLP. More than 95% of the isolates belonged to the *L. reuteri*, *L. acidophilus*, and *L. salivarius* phylogenetic groups. The rep-type representatives were also tested *in vitro* for probiotic properties. The inhibitory activity towards the growth of 20 different *Salmonella* strains was evaluated under aerobic and anaerobic conditions, showing a high heterogeneity between the different strains. However, a clear correlation was found between the size of inhibition zone and *Lactobacillus* phylogenetic group. Furthermore, susceptibility of the strains to the antimicrobial agents enrofloxacin and oxytetracyclin, two antimicrobial agents which are generally used for breeding of laying hens, was tested. Finally, strains were analysed for tolerance to low pH and bile salts, properties which are important for survival and colonization in the gastro-intestinal tract. From these results, a selection of strains will be made that will be tested *in vitro* for their ability to protect epithelial cells from invasion with *S.* Enteritidis.

P-A09

Shedding of norovirus in a food handler during a restaurant outbreak of norovirus gastroenteritis

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In January 2004, an outbreak of norovirus gastroenteritis occurred among participants of a restaurant dinner. Nine of 18 persons felt ill, approximately 24 hours after the event. Cultures of 5 stool specimens of ill guests did not yield *Salmonella*, *Shigella*, or *Yersinia* species. Four of five specimen showed norovirus by RT-PCR. Two stool specimen of food handlers in the kitchen of the restaurant were examined because local health office authorities suspected food handlers as a potential source of infection. Both food handlers reported not having been ill or having gastroenteritis during the last weeks. However, one specimen showed norovirus by RT-PCR.

All five second-round PCR products of 338 bp were sequenced directly in both directions with the second round PCR primers using an ABI Prism 310 DNA sequencer and BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). The nucleotide sequences showed identity for the four guests, but, interestingly, the isolate of the food handler showed significant differences (figure). Therefore a transmission from food handler to guests through contamination of food during preparing or serving could be excluded, despite the fact that the food handler excreted norovirus.

This data highlights the difficulties in tracking norovirus outbreaks to their sources. After establishing RT-PCR for stool specimens, RT-PCR procedures have to be established for foods. Proper collection and storage of implicated food items will be a next necessary step to improve recovery of norovirus. However, sequencing of isolates has to be added to make sure that transmission from the suspected source has occurred. Description and examination of the various components of norovirus outbreaks is important in understanding outbreak situations and is crucial in developing prevention strategies for the future. This study shows not only the possibilities in routine outbreak investigations, but also highlights the traps and the significance of each component in understanding and assessing norovirus outbreaks.

P-A10

Characterisation of *Staphylococcus aureus* enterotoxin type H from a food outbreak

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Staphylococcal food poisoning is one of the most common foodborne diseases worldwide. Disease is usually caused by consumption of foods containing one or more “classical” staphylococcal enterotoxins (SEs: SEA, SEB, SEC_1, SEC_2, SEC_3, SED and SEE) that can be identified by commercial immunoassay kits. In developed countries, SEA is the most commonly involved in staphylococcal food poisoning outbreaks and accounts for about 75% of the cases, followed by SED, SEC and SEB.
Recently, new enterotoxins (SEG, SHE, SEI, SEJ, SEK, SEL, SEM, SEN, SEO) has been described and only one reported case of intoxication where one of these new enterotoxins (SHE) has been involved.
In 2003 in Norway, a series of suspected staphylococcal intoxications occurred during a Christmas dinner and lunch served in a kindergarten using the leftovers from dinner. Altogether, five children aged between 2 to 5 years and three adults become ill with quite rapid onset of symptoms (within 1 hour) and recovery within 24 hours. The major symptoms were vomiting, diarrhoea and abdominal cramping. Samples of the meal obtained for examination included hot dogs and mashed potatoes. Records of the meal showed that mashed potatoes were prepared from unpasteurised milk. Enterotoxins was neither detected in the food samples nor recovered from *S. aureus* strains isolated from food samples using a commercial immunoassay kits (SET-RPLA and Transia).
Altogether 10 isolates from the mashed potatoes, nonproducers of SE A-E, were tested for presence of newly described SE *genes* (*seg*, *sei*, *seh* and *sej*) using a multiplex PCR-technique. The expected PCR product for SEH was obtained from all 10 isolates tested.
Furthermore, molecular typing of these 10 *S. aureus* isolates by Pulsed Field Gel Electrophoresis (PFGE) and sequencing of SEH will be performed. The results will give more information about SEH produced by *S. aureus* and possible rare involvement of SEH in food poisoning.

P-A11

Method for detection and specific isolation of Shigatoxin-producing *Escherichia coli* (STEC) in vegetarian foods

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Background

Enterohemorrhagic *E. coli* (EHEC) as subgroup of Shigatoxin-producing *E. coli* (STEC) has been recognized as a major cause of hemorrhagic colitis and the hemolytic-urämic syndrome worldwide. Most outbreaks of EHEC e.g. serotype O157:H7 infections have been linked to foods of bovine or in general of ruminants origin, such as undercooked ground beef and dairy products. But other food items of non-ruminant origin, such as water, vegetables, and apple juice or apple cider have been associated with infections mentioned above, too.

Aims

Vegetarian foods contaminated with STEC/EHEC by smearing, fecal pollution or organic manuring were often discussed in literature to be associated with EHEC outbreaks. In most cases it was a conclusion of epidemiological data, only. The EHEC microorganisms could not be isolated. So an optimized method for detection and spezific isolation of STEC/EHEC in vegetarian foods (apple juice, lettuce) is given in this paper. Parameters like nutrient media, supplements, pH-value and incubation conditions were optimized.

Material, Methods, Results

Apple juice was freshly squeezed from apples bought in supermarkets of Dessau. Several STEC/EHEC strains were used for artificial contamination of the juice samples. The infectious doses were between 0 and 10^3 cfu / 25 ml juice. In contrast to the two step enrichment procedure for STEC/EHEC in foods of animal origin the cultivation steps for these microorganisms in vegetarian foods have to be the following: 25 ml juice were mixed with 100 ml modified tryptose soy broth (mTSB) supplemented with Mitomycin C. It was cultivated for 18hrs at 37°C with agitation. 50 cfu / 25ml juice were detectable by using ELISA or PCR as described by Timm et al and Gallien et al.. When using PCR for screening an additional cultivation step was necessary for removing PCR inhibitors (e.g. pectines).

25 g of each lettuce sample bought in supermarkets of Dessau was contaminated with STEC/EHEC artificially. 100 ml mTSB containing Mitomycin C was added. The cultivation was done for 18 hrs at 37°C with agitation. 50 cfu / 25 g lettuce were detectable by using ELISA or PCR. 43 natural lettuce samples bought in supermarkets of Dessau were STEC/EHEC negative when using the optimized method described above.

The specific isolation of STEC/EHEC from enriched cultures of artificial contaminated juice or lettuce samples was successful by applying VT colony immunoblot. The sensitivity was 50 cfu / 25 g (ml) sample, respectively.

Conclusions

An optimized method for detection and specific isolation of STEC/EHEC in apple juice and lettuce is given. The detection limit is about 50 cfu in 25 g (ml) food. A method for a sensitive detection of STEC/EHEC in sprouts is not available at present. First investigations showed a detection limit of 10^4 cfu to 10^5 cfu in 25 g sprouts when using the method described in this paper. The concomitant flora is completely different in relation to other vegetarian foods. It contains e.g. moulds and fungi, too. An optimization of the method described above using fungicidal substances has to be done.

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P-A12

DNA fingerprinting of *Clostridium botulinum* types A, B, E and F by amplified fragment length polymorphism (AFLP) analysis

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Amplified fragment length polymorphism (AFLP) is a high-resolution PCR-based DNA fingerprinting method, which inspects the entire genome for polymorphism. AFLP has been used to genotype many bacterial species in both epidemiological and contamination route studies. In addition AFLP has proven to be a useful tool in bacterial taxonomy. Although AFLP has been applied to characterise related species, such as *Clostridium difficile* and *C. perfringens*, it has not been used to genotype *C. botulinum*. The aim of this study was to examine the applicability of AFLP analysis in the characterisation of *C. botulinum* types A, B, E and F.

An AFLP protocol, based on infrared detection of AFLP patterns on an automated sequencer, was applied in the characterisation of group I (proteolytic) type A (*n*=4), B (*n*=4), and type F (*n*=2) and group II (nonproteolytic) type B (*n*=4) and E (*n*= 11) *C. botulinum* strains. Similarities between normalised AFLP patterns were calculated using the Pearson product-moment correlation coefficient. Clustering and construction of dendrograms were performed by the unweighted pair group method using arithmetic averages (UPGMA).

All strains included in the study were typeable by AFLP. The numerical analysis of AFLP profiles yielded two distinct group-specific clusters, with 10% similarity between *C. botulinum* groups I and II. Group I was further divided into two main clusters, one containing *C. botulinum* type A, and the other, types B and F. In group II, two distinct clusters were also formed, the first cluster consisting of strains of *C. botulinum* type B and the second of strains of *C. botulinum* type E. Strains displaying >89% similarity were considered as same AFLP type. By this criterion, 20 different AFLP types of *C. botulinum* were identified.

AFLP analysis was found to be a fast and highly reproducible method with typeability of 100% and it was also promising regarding discriminatory power. In addition to strain typing AFLP may also be a suitable tool for *C. botulinum* group identification.

P-A13

Typing and toxigenic profile of *Bacillus cereus* strains isolated from a fatal food intoxication in Belgium

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Bacillus cereus is a spore-forming bacterium that causes two types of food intoxications known as the emetic and the diarroeal types. For the emetic type, a heat-stabile emetic toxin named cereulide, that is preformed in the food, is responsible for the symptoms. Heat-labile enterotoxins, produced in the gut by vegetative cells, cause the diarrhoeal type.
In August 2003, a Belgian family had met with a serious food intoxication, by which several children fell ill and the youngest among them died after some hours. In the vomit of this patient and in various food and food-related products present in the kitchen of the family, *B. cereus* was detected. A total of 21 *B. cereus* strains were isolated from the different sources and subjected to further research in order to track the food product and the agent that had caused the intoxication. The genetic similarity among the strains was determined by the molecular typing method Rep-PCR, using the $(GTG)_5$ primer, and by pulsed-field gelelectrophoresis. By Rep-PCR, four groups (1-4) and one subgroup (1a) were detected. With the exception of rep-type 2, all rep-types were confirmed as *B. cereus* with species-specific PCR tests within the *B. cereus* group. The fast Rep-PCR method seems a valuable tool to discriminate between different *B. cereus* strains. By PFGE using two different enzymes, four different patterns (A-D) were identified. All three strains isolated from the vomit of the patient showed the same pattern by Rep-PCR (rep-type 1) as well as by PFGE-analysis (PFGE-type C). One of the strains isolated from pasta salad belonged also to rep-type 1 and PFGE-type C. A selection of the strains was subjected to cytotoxicity assays to identify their potential to produce emetic and/or enterotoxins. It was shown that both strains of rep-type 1 (one from the vomit and one from the pasta salad) and the strains from rep-type 4 are capable to produce the emetic toxin. The only strain of rep-type 3 was demonstrated to be able to produce enterotoxins. These results strongly indicate that the emetic toxin produced by *B. cereus* in the pasta salad has been the causative agent of this severe food intoxication.

P-A14

Characterization of pathogenic *Vibrio parahaemolyticus* isolates from clinical sources in Spain and comparison with Asian and North American isolates

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In spite of the potential risk involved with contamination of seafood with *Vibrio parahaemolyticus*, there is a lack of information on the occurrence of pathogenic *V. parahaemolyticus* in Europe. This organism was isolated in 1999 from a large outbreak (64 cases from a single hospital) associated with raw oyster consumption in Galicia, Spain, one of the most important regions in shellfish production worldwide. Two *V. parahaemolyticus* isolates from the 1999 Galicia outbreak, three additional clinical isolates obtained in the same period from hospitals in Spain, 2 reference strains from clinical sources and 5 Spanish environmental isolates were examined. Additionally, 17 isolates belonging to the pandemic clone isolated in Asia and North America were included in the study for comparison. All isolates were characterized by serotyping, PCR for virulence-related genes, PFGE, and plasmid analysis. Four of the five clinical isolates from hospitals in Spain belonged to the serotype O4:K11; the remaining isolate was O4:K untypeable. All five isolates were Vp-*toxR*, *tlh* and *tdh* positive, and they were negative for *trh* and GS-PCR. PFGE analysis with *Not*I and *Sfi*I discriminated the European isolates in two closely related PFGE-types included in a homogeneous cluster, clearly differentiated from the Asian and North-American isolates. Isolates from Asia and the USA, belonged to serotypes O3:K6, O1:KUT and O4:K68, showed a high degree of clonality. However, PFGE distinguished successfully among isolates belonging to different serotypes. The 5 environmental isolates belonged to serotypes O2:K28, O2:KUT, O3:K53, O4:KUT and O8:K22, and were negative for all virulence genes. The five isolates were discriminated into five different PFGE-types unrelated with any other isolate included in the study. This is the strongest evidence to date associating seafood produced in Europe to human *V. parahaemolyticus* infections. While the virulence characteristics (*tdh* positive, *trh* negative) of the Spanish clinical isolates matched those of the O3:K6 clone from Asia and North America, they were clearly excluded from this clone by GS-PCR, PFGE and serotyping results.

P-A15

Both raw and processed fish share the identical *Listeria monocytogenes* pulsotypes

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A total of 281 raw fish samples sampled at the three different sampling phases were examined for the presence of *Listeria monocytogenes*. A total of 41 isolates of 11 *L. monocytogenes* positive samples were further typed with pulsed field gel electrophoresis (PFGE) typing. Nine different pulsotypes were recovered. From 86 fish product isolates of *L. monocytogenes* from the culture collection of the Department of Food and Environmental Hygiene 31 *L. monocytogenes* pulsotypes were found. Two pairs of the raw fish and fish product pulsotypes were indistinguishable from each other.

From one to two isolates of each pulsotype were serotyped. Six serotypes were recovered. The total number of isolates that represented serotypes 1/2a, 4b, 3a, 1/2c, 4c and 1/2b were 89 (70%), 19 (15%), 9 (7%), 5 (4%), 4 (3%) and 1 (1%), respectively, with no statistically significant difference in the division between raw and processed fish (Chi^2 test, $p>0.01$).

Even though the prevalence of *L. monocytogenes* in raw fish is low, high genetic diversity of the bacteria enables a plenty of different *L. monocytogenes* strains to enter the fish processing plants with raw material. This indicates that raw fish is a source of fish processing plant contamination by *L. monocytogenes*.

P-A16

Real-time PCR for the detection of *Salmonella* in animal faecal and environmental samples

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Current methods for the detection of *Salmonella* spp in faecal and environmental samples require enrichment, selective culture and identification. These are time consuming and lengthy methods, which can take several days to obtain a definitive result. There are various reports on the use of PCR for detection of *Salmonella* in faecal material. However not all of the published methods are sensitive or specific. Traditional PCR methods require amplification and product separation by gel electrophoresis, both of which are time consuming. Also the need for post-PCR processing facilitates the potential for carry-over contamination, and therefore false-positive results.

We describe here a method for the detection of *Salmonella* in faecal samples based on a Real-time LightCycler PCR protocol. The Roche High Pure *Salmonella* sample preparation kit was evaluated for the extraction of DNA from enriched faecal samples. Along with the Roche *Salmonella* detection kit which provides specific primers and probe for the detection of *Salmonella*. The detection limit of the Real-time PCR was 3.4 CFU ml^{-1} in overnight broth cultures, and on average 21 CFU g^{-1} in spiked faeces following 16h enrichment. Naturally contaminated animal faeces and environmental samples were also tested (n=80). In seventy-three (91%) of the samples tested there was agreement between the culture and Real-time PCR results.

Our results demonstrate the potential of this molecular technique to provide a rapid (2 h or less) and sensitive method for the detection of *Salmonella* in enriched faecal samples. Animal samples often present low numbers of *Salmonella* accompanied by a high background of other competitive flora. This represents a dramatic difference from human stool samples from food-poisoning cases, where more than 10^6 bacteria / g is usually present. For this and other reasons, this protocol for processing animal samples requires an overnight enrichment step to achieve adequate sensitivity.

P-A17

Molecular epidemiological characterisation and antimicrobial susceptibilities of *Salmonella* Enteritidis in Turkey between 2000 and 2003

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Introduction: *Salmonella enterica* serotype Enteritidis (*S*. Enteritidis) is the most common non-typhoidal *Salmonella* serotype isolated from humans as a major cause of food poisoning in many countries, including Turkey. The primary objective of this study was to characterise isolates of *S*. Enteritidis from two outbreaks and sporadic cases by using antimicrobial susceptibility test and molecular methods.
Material and Methods: Twenty and one strains of *S*. Enteritidis from different regions of Turkey between 2000 and 2003 were characterised by antimicrobial susceptibility test, plasmid profile typing and pulsed field gel electrophoresis (PFGE). Thirteen isolates had been associated with two outbreaks, seven were from one outbreak and six from the other. Remaining *S*. Enteritidis strains were isolated from several sporadic cases. All isolates were from human feacal samples. Isolates were screened for resistance to ampicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, tetracyclines, trimethoprim, trimethoprim/sulfamethoxazole, ciprofloxacin, nalidixic acid, amikacin, cephalothin, cefuroxime, cefoperazone, ceftizoxime, and cefotaxime using disc diffusion test of Kirby Bauer. Antimicrobial susceptibility was assessed following NCCLS criteria. Plasmid DNA was isolated by the method of Kado and Liu. Genomic DNA by PFGE was prepared according to standard procedures and agarose plugs were digested with *Xba*I. An isolate with one band difference was considered as a different PFGE type.
Results: Of all isolates, ten were susceptible to all studied antimicrobials. Nine isolates were intermediate resistant and one was resistant to tetracycline. Resistance to nalidixic acid was determined in two strains which were isolated in 2001 and 2003. Six plasmid types and three closely-related pulsed field types were identified in the 21 isolates studied. Isolates from each outbreak have identical PFGE, plasmid profile and antimicrobial susceptibility patterns which were also found in the isolates from some of the sporadic cases.
Discussion: These findings demonstrate that the *S*. Enteritidis strains isolated in Turkey have highly clonal nature using PFGE. Plasmid profile typing and antimicrobial susceptibility tests are useful methods to differentiate the isolates from various cases for epidemiological purpose. All these methods are particularly valuable to identitify outbreak and sporadic cases. Antimicrobial resistance is not an emerging problem among these *S*. Enteritidis isolates. However, decreased susceptibility to ciprofloxacin may be investigated in case of nalidixic acid resistant strains.

P-A18

Molecular fingerprinting evidence of the contribution of wild-life vectors in the maintenance of *Salmonella* Enteritidis infection in Layer Farms

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This study aims to provide molecular fingerprinting evidence of the contribution of wild-life vectors in the on-farm epidemiology of *S.* Enteritidis infections. Persistence of *Salmonella* in the environment is an important characteristic in its epidemiology. Most *Salmonella* strains can survive for long periods of time in water and in dry materials such as dust. Low numbers of micro-organisms surviving in the environment in a dormant state can multiply rapidly if suitable conditions are present. This special ability, together with the contribution of wild-life vectors represents a real problem to the control of this infection in poultry environments.

Salmonella Enteritidis isolates were obtained from wild-life and from farm environment samples collected in 10 egg layer farms (5 cage layer farms, 2 barn egg farms, 1 free-range farm, 1 mixed cage/free range farm, 1 breeding unit). Isolates were typed using plasmid profiling, *Xba*I-pulsed field gel electrophoresis and *Pst*I-*Sph*I ribotyping.
In all ten farms we were able to identify the same *S.* Enteritidis clones[defined as genetic combined types (plasmid type/ribotype/PFGE type)] in wild-life vectors and farm environment. On several occasions the same clones were found before and after cleansing and disinfecting the farm premises. Also in some instances the same clones were present in mice samples, egg contents and spent hens. This is an indication that these particular strains are invasive and able to colonise birds and to infect eggs. It has been previously suggested that mice constantly re-introduce unstable orally invasive phenotypes back into the environment of birds

Definitive molecular evidence for the involvement of several wild-life species (mice, rats, flies, litter beetles, foxes) in the maintenance of *S.* Enteritidis infection on farms has been presented. Failures in biosecurity seriously compromise the control of this pathogen on laying farms. This work reports on the use of molecular tools for the study of the epidemiology of *S.* Enteritidis. It gives useful information to be considered in control programs for this organism on poultry farms.

P-A19

Molecular characterization of *Salmonella* Senftenberg isolates from mussel processing facilities in Spain and comparison with clinical, feed and environmental isolates

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S. Senftenberg is frequently isolated from animal sources. However, this serotype is not very often described as a human pathogen, and most of the reported cases are represented by nosocomial infections. Recently, *S*. Senftenberg has been revealed as one of the predominant serovars isolated from marine environments and seafood, especially in temperate and tropical zones. It has also been detected as a persistent contaminant of fish feed factories.
In Spain, persistent contamination by this serovar was detected in mussel processing plants from 1998 to 2001, causing a serious disruption of the industrial activity. This study describes the use of molecular methods in the investigation of the epidemiology of Salmonella contamination within mussel processing plants. We also included in the analysis isolates from diverse sources in an attempt to identify the possible sources of contamination. A total of 110 *S*. Senftenberg isolates from 8 facilities were subjected to molecular typing by Pulse-Field Gel Electrophoresis (PFGE), and to antibiotic sensitivity testing. Additionally, a selection of epidemiologically unrelated isolates of this serovar originating from human, animal, feed and environmental sources was included in the study.
PFGE analysis proved to be a useful tool for studying the persistence and dissemination of *S*. Senftenberg in these factory environments. Results from our study indicate that facilities using brine in their processing lines presented greater genetic diversity in their *S*. Senftenberg populations. This observation supports the hypothesis that low-quality imported salt used for brine preparation could be the origin of the contamination. The XbaI-PFGE type X19 was the most prevalent among the panel, and it persisted exclusively in one facility during the 5-year study. Isolates from mussel processing plants were clearly different from those of clinical and environmental sources. One of the human isolates showed a restriction pattern well differentiated from the rest of the clinical isolates, and indistinguishable from a processed-mussel isolate from one facility. Although we do not have supporting epidemiological data to prove it, molecular fingerprinting evidence suggests that consumption of contaminated mussels could potentially contribute to food-borne salmonellosis in humans.
The results obtained in the current study showed that the XbaI-PFGE typing technique was sensitive enough to discriminate clonal diversity, and helped to trace contamination inside and between facilities.

P-A20

Molecular characterisation of group O:7 (C_1) *Salmonella* veterinary isolates using pulsed field gel electrophoresis

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The Veterinary Laboratories Agency (VLA) routinely receives and serotypes *Salmonella* isolates from poultry companies originating from various feedmills, hatcheries and farms from Great Britain. During 2002-2003, numerous *Salmonella* isolates were received that exhibited the somatic 6,7,14 structure (C1 group serotype) but no detectable flagellar antigens, even after numerous phase conversion passages. These were subsequently reported as *Salmonella* structures i.e. untypable, non-motile isolates. It has also been observed that most *S.* Virchow isolated from hatcheries are predominantly phagetypes 2 and 4 but *S.* Virchow isolated from breeding farms consist of more diverse phagetypes (PT26, PT2A, PT53, PT17, PT4VAR). Genotypic characterisation of these isolates was undertaken to determine similarities with other Group C_1 *Salmonella* serotypes and the potential existence of clonal relationships.

A total of 65 poultry Salmonella isolates from the Kauffman-White Scheme Group O:7 (C_1) were included in this study; *S.* Virchow (n=25), *S.* Thompson (n=3), *S.* Montevideo (n=1), *S.* Mbandaka (n=4), *S.* Livingstone (n=11), *Salmonella* [6,7,14:-:-] (n=4) and *Salmonella* [6,7:-:-] (n=21). These isolates, from feedmills, hatcheries and farms, were selected to represent diverse antibiotic resistance patterns and geographical locations. They were serotyped and tested for antibiotic sensitivity against a panel of 16 antimicrobials using standard VLA protocols. PFGE was performed using the standardised PulseNet USA protocol (National Molecular Subtyping Network for Foodborne Disease Surveillance, CDC, Atlanta, Georgia) with *Xba*I and the resulting profiles analysed using BioNumerics software.

Although all the isolates in this study had a similar somatic antigen composition, the resultant PFGE profiles were highly polymorphic. There was no observable clustering of serotypes or structures but several *Salmonella* structure isolates showed a high degree of genotypic similarity to other known serotypes. Interestingly, several *S.* Virchow isolates of differing phagetypes had similar PFGE profiles and similar sensitivity patterns. Also, it was observed that no one farm showed a strong association with a specific X-type. With the exception of the *S.* Virchow V-X4 profile, no predominant X-type was identified within any other serotype. This group however, comprised diverse phagetypes, antimicrobial resistance patterns and geographical locations. Several *Salmonella* structure isolates from feedmills were found to be genotypically identical to isolates from hatcheries and farms. Several also had similar antimicrobial sensitivity patterns. This suggests a possible clonal propagation of particular isolates from the feedmills to the hatcheries and farms. In the case of *S.* Virchow, we also observed that phagetypes PT26 and PT4, PT2 and PT4 and PT53 and PT2 were genetically indistinguishable. Although the potential role of plasmids and phage conversion cannot be ignored, this finding addresses the limitations of many routine, traditional typing methods that rely primarily on defined phenotypic criteria.

With an increasing need for higher resolution and differentiation of isolates, this study clearly demonstrates the enhanced discriminatory ability of PFGE as a modern molecular tool for surveillance epidemiology and in the investigation of foodborne infection outbreaks.

P-A21

Molecular epidemiology of *Salmonella enterica* serovar Agona and identification of an diffuse outbreak caused by anise-fennel-caraway tea

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In 2002/2003 increased numbers of notified salmonellosis due to *S. enterica* serovar Agona were observed in Germany. In order to understand the recent spread of this serovar and to trace back the route of infection to its source, PFGE and phage typing were applied and considered as very helpful tools for an epidemiological subtyping of *S*. Agona isolates. A new phage typing scheme for *S*. Agona was developed and will be described. By using 14 bacteriophages, 52 phage types could be distinguished. Pulsed Field Gel Electrophoresis pattern(PFGE) allows to differentiates 52 different pattern, too. In combining both methods 94 clonal types were detected among the 165 *S.* Agona strains (originated from Germany, overseas countries such as USA, United Arabic Emirates, Turkey, India or Austria and Finland) indicating a great biological diversity of this serovar. The strains were isolated from humans (96), animals (26) as cattle, pig, chicken, turkey, camel, bird and dog, from food (23), feed (14) and environment (6). However, recent *S.* Agona isolates from infantile gastroenteritis in Germany and from an aniseed lot as well as from fennel anise caraway tea bags reveal a clonal identity (phage type 2, PFGE-type 1) indicating their epidemiological relatedness and a new source of infection. Moreover, it is suggested that strains of *S*. Agona will continue to be of public health concern and phage typing together with PFGE typing is proposed to be applied as reliable and rapid tools for their future monitoring.

P-A22

Rapid detection of *Salmonella* spp. in food by Real-Time-PCR

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According to the WHO *Salmonella* is the most prevalent pathogen causing foodborne infections with a relative frequency of more than 70 000 cases in Germany annually. In the US about 95% of the nontyphoidal *Salmonella* infections and 80% of infections with *Salmonella* Typhi are reported to be foodborne [1]. To ensure food-safety, methods that use the phenotypic or biochemical characteristics for detection have been established as DIN-methods. To accelerate *Salmonella* detection in food, PCR-based detection methods have been developped and improved in recent years. Among the different detection systems for *Salmonella*, Real-time PCR is one of the fastest systems whereby results can be obtained within less than two days, including enrichment. After enrichment of the food sample, DNA is extracted and can then be used for the Real-Time PCR. The results are analyzed interpreting the fluorescence data gained. In this study we used a formerly published system of oligonucleotide primers [2] which has been validated thoroughly and has also been accepted as a DIN method [3]. We combined it with a specific FAM-TAMRA-labelled probe. The advantage of the described method is the simultaneous detection and verification of the PCR-product within the same reaction by using a specific probe in addition to the specific primers. The assay is based upon amplification and detection of a segment of the invasion-associated invA gene [2].
The specificity of the probe was verified by testing DNA samples from more than 100 Salmonella strains, representing subspecies of *Salmonella enterica* as well as *Salmonella bongori* and 50 Non-*Salmonella* strains. To determine the sensitivity of the method chocolate samples were artificially contaminated with *Salmonella* Typhimurium before and after pre-enrichment. We spiked samples with 3,1-10,8 cfu/g before pre-enrichment. All artificially contaminated samples were tested positively for *Salmonella*. In addition, 4 chocholate samples (25g in 250ml pre-enrichment media) that had been incubated for 18h were spiked with 4 dilutions of 38, 114, 380, 1140, 3800 and 11400 cfu/ml of *Salmonella* Typhimurium respectively. All the spiked samples down to 38 cfu/ml showed positive results when analyzed using the described method. After establishing the PCR assay the method was used to analyze routine food samples. All results were compared with those of official methods[3]. The results received were in complete agreement with the results achieved by applying the official method. To prevent false-negative results caused by inhibitory effects a control of the amplification is needed for each sample. Alternative to an external amplification control it is possible to use a second target sequence as an internal amplification control. This heterologous sequence can be detected simultaneously with the tested *Salmonella* DNA by adding a predefined quantitiy of the corresponding template. We designed such a system using a sequence of Nicotiana tabacum and a HEX-TAMRA labeled probe whose fluorescence can be detected independent from the FAM signal generated from *Salmonella* DNA. The results demonstrate that with the applied Real-Time PCR method all present *Salmonella* strains can correctly be identified in a short amount of time with definite specificity and high sensitivity by showing clearly positive fluorescence signals.

[1] P. Mead et al, Emerging infectious diseases (1999) 5, No.5: 607-625 [2] K. Rahn et al., Mol. and Cell. Probes (1992) 6 :271-279 [3] Verfahren zum Nachweis von Salmonellen mit der Polymerase-Kettenreaktion (PCR), (1999) 11,DIN 10135 bzw. LMBG L 00.00-52, Ausgabe 2000-07 [4] Untersuchung von Lebensmitteln-Horizontales Verfahren für den Nachweis von Salmonellen, LMBG 00.00-20

P-A23

The sequence heterogenicities among 16S rRNA genes of *Salmonella* serovars and the effects on the specificity of the primers designed

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Previously, we have reported a 16S rDNA targeted polymerase chain reaction (PCR) method for the specific detection of *Salmonella* serovars [J. Appl. Bacteriol. 80 (1996) 659-666]. The target sites of its primers, i.e. 16SFI and 16S? , according to the data in GenBank, were found mismatched to the corresponding sequences of some *Salmonella* serovars, such as those of *S.* Houten*, S.* Chingola*, S.* Bareilly*,* and *S.* Weltevreden. Accordingly, a PCR method using a non-specific primer MINf combined with a primer modified from our 16SFI primer, i.e. the primer MINr, was developed and claimed with better detection specificity [Intl. J. Food Microbiol. 80 (2003) 67-75]. In this study, we show the sequence heterogenicity at the primer 16SFI targeting sites for some *Salmonella* serovars. Thus, the sequence used for designing of PCR primers might be just one of the several possible sequences. Such situation may lead to the misjudgment on evaluation of the specificity of the primers if one only based on the data in GenBank. Strains of the above described *Salmonella* serovars with mismatched sequences at primer annealing sites were reidentified and their PCR results were assured. Meanwhile, their 16SFI/16S? primer annealing sites were sequenced and the sequences were found highly homologous to those of 16SFI and 16SIII primers.

P-A24

Elucidation of the major subtypes for *Salmonella enterica* serovar Enteritidis by comparison of the Pulsed Field Gel Electrophoresis patterns of the poultry and human isolates from geographically far distant areas

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Since human infections by *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) have been increasing worldwide over the past years and epidemiological studies have implicated the consumption of meat, poultry, eggs and egg products, elucidation of the predominant subtypes for this *Salmonella* spp. is important. In this study, using pulsed field gel electrophoresis (PFGE) method, we analyzed 77 poultry isolates of *Salmonella* Enteritidis isolated from USA and compared these subtypes with those of the 63 human isolates obtained in Taiwan. The results showed that for these poultry isolates, when *Xba*I, *Spe*I and *Not*I were used for chromosomal DNA digestion followed by PFGE analysis, a total of 14, 12 and 13 PFGE patterns, respectively, were identified. Of them, 55 (71.4%), 63 (81.8%) and 62 (80.5%) of the 77 strains belong to a single pattern of VX1, VS2 and VN1, respectively. When PFGE patterns from *Xba*I, *Spe*I and *Not*I digestion were combined, it was found that 48 strains belong to a patterns combination of VX1VS2VN1. Thus, limited genetic diversity was found for these US strains and pattern VX1VS2VN1 was the major subtype. When PFGE patterns for these 77 US isolates were compared with those of the 63 human isolates previously reported for the Taiwan isolates, only two patterns combinations which count 62% of the US isolates and 73% of the Taiwan isolate were found co-shared by the Taiwan and US isolates. Since the US strains were isolated from different origin, ie, poultry, and from areas geographically far distant areas from Taiwan. The above described results may imply that strains of those two subtypes may be the most disseminated strains for *Salmonella* infection.

P-A25

Development of PCR primers from 16S-23S rRNA gene intergenic spacer (ITS) for the specific detection of *B. cereus* group strains

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Bacillus cereus is one of the important food pathogens and the *B. cereus* group strains, such as *B. cereus*, *B. thuringiensis*, *B. anthracis* and *B. mycoides*, shared many phenotypical characters and a high level of genetic similarity. Thus it is important to develop a method for the rapid detection of these *B. cereus* group strains. Based on the DNA-sequence in the 16S-23S intergenic spacer region, we designed PCR primers for the specific detection of *B. cereus* group strains. Using this PCR primers, all the 174 *B. cereus* group strains could be detected a single PCR product with M.wt equal to 145 bp was found for 171 of these 174 strains while product with M.wt equal to 374 bp was found for two *B. mycoides* strains and one *B. cereus* strain. All the non-*B. cereus* group strains including other *Bacillus* strains and non-*Bacillus* strains generated negative PCR results. As this PCR primer pair may be used for the detection of *B. cereus* cells in food samples.

P-A26

Outbreak of shiga toxin-producing *Escherichia coli* infection associated with mutton consumption in France

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An outbreak of shiga toxin-producing *Escherichia coli* (STEC) infections occurred following a wedding party, in June 2002. An investigation was prompted to identify the source and the vehicle of infection.

We conducted a retrospective cohort study of the wedding party attendees. We defined a case as a guest with an acute gastrointestinal illness with onset in the 10 days following the party. All guests were interviewed about foods eaten during the party. Leftovers (cooked mutton and raw offal) and processed foods from the same batches as served at the party were sampled.
Human, food and environmental samples were examined for Shigatoxin (*Stx)* and virulence traits by PCR. *Stx* positive samples were cultured for STEC. Cases with hemolytic-uremic syndrome (HUS) were tested for serum antibodies against 26 major serotypes.

Eleven cases were identified (10 adults and one child). Two adults developed HUS. A STEC O26 strain (*stx1, eaeA, ehxA*) was isolated from a case with diarrhoea and a STEC strain with R-148 molecular serotype (*stx2c*) from a HUS case. No serum antibodies were detected in the 2 cases of HUS. 82% of cases had eaten lightly roasted mutton and poultry pâté. Only the consumption of pâté tended to be associated with illness (RR 3,8 ; 95%CI 0,9-16,4).
Three STEC strains were isolated from the mutton and the offal (stx2c, R-148), and two from the pâté (stx2c, R-X and R-Y). The strains from the mutton were indistinguishable, using PFGE and molecular serotyping, from the human stx2c-strain, whereas the pâté isolates differed.

Whereas in total 4 different STEC strains were identified in patients and foods, the results of PFGE and molecular serotyping and analysis of food consumption patterns strongly suggest that this outbreak was due to the consumption of undercooked mutton contaminated with STEC R-148.

P-A27

Outbreak of multidrug-resistant *Salmonella* Newport due to the consumption of imported horse meat in France

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June 2th 2003, the National Reference Centre (NRC) for *Salmonella* notified an increased number of isolates of multidrug-resistant (MDR) *Salmonella enterica* serotype Newport in the North of France. An investigation was prompted to identify the source and the vehicle of infection.

A case was defined as a patient with acute illness and an isolate of MDR S. Newport between may and july 2003. Cases identified through the NRC were interviewed. The suspected food was traced back in order to identify a common supplier. All isolates were tested for the presence of bla_{CMY} gene by PCR.

From May to June 2003, 14 human cases of *S.* Newport resistant to beta-lactams (ampicillin, ticarcillin, piperacillin, 1st, 2nd and 3rd generation cephalosporins except cefepime and imipenem), streptomycin, sulfonamide, tetracycline and chloramphenicol were reported. Both sexes and all age groups (9 children, 5 adults) were affected. All patients presented diarrhea, bloody for seven patients (50%). Eleven patients were hospitalised. No death has been recorded.
All cases reported having eaten horse meat consumed as ground meat (11 cases) (consumed raw by at least 6 cases) or steak (3 cases). Cases had purchased their horse meat from butcheries (7 cases) and markets (7 cases) in different towns. Among the different suppliers of the retail outlets, one single wholesaler, located in the North of France, was shown to have supplied all fourteen outlets. The wholesaler purchased its horse meat abroad in 8 different countries in South and North America, Europe and Oceania.
Since the origin of the horse meat is not recorded after purchase by the wholesaler, it has been impossible to determine the exact origin of the contaminated meat.
All isolates were positive in a CMY-specific PCR assay. Sequencing of PCR products showed a beta-lactamase gene identical to *cmy-2*.
Descriptive epidemiology and trace-back investigations allowed to incriminate imported horse meat as the most likely source of this outbreak. This outbreak is the first documented outbreak of MDR *S.* Newport in Europe.
Salmonella surveillance system should closely monitor possible further spread of MRD *S.* Newport in Europe.

P-A28

An outbreak caused by *Streptococcus equi* subsp. *zooepidemicus* associated with consumption of fresh goat cheese

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Streptococcus equi ssp. *zooepidemicus* is a normal commensal of the mucosa of several animal species. It is commonly found as an opportunistic pathogen in respiratory tract and genitalia of horses. It can also be a cause of mastitis or genital and wound infections in cows, sheep and goats. It rarely causes infections in humans. However, small but serious outbreaks with high mortality, especially among elderly patients, have been described.

S. equi ssp. *zooepidemicus* was isolated from seven patients in Western Finland. All patients had consumed fresh goat cheese made of unpasteurized milk originating from a single farm. The cheese was produced in an approved small-scale cheese dairy on the farm. Samples of fresh cheeses made of unpasteurized milk were taken from retail stores. Environmental and water samples as well as raw milk samples from the storage tank were collected on the farm. Individual milk samples and vaginal swab samples were taken from all goats in lactation. All the samples were examined for *S. equi* subsp. *zooepidemicus*. In addition, pharyngeal swab samples were taken from two food handlers, one of which also took care of the goats and milking.

S. equi ssp. *zooepidemicus* was isolated from one production batch of fresh goat cheese made of unpasteurized milk, from one tank milk sample, from vaginal samples of one goat and from throat samples of both food handlers. All isolates were typed by pulsed field gel electrophoresis (PFGE) and ribotyping using Hind III and EcoRI as the restriction enzymes. Molecular typing suggested a common origin for the isolates.

The origin of this outbreak could be traced to fresh goat cheese made from unpasteurized milk on a single farm. It is likely that the milk was contaminated during milking by the goat that was a carrier of the causative agent. The food handlers carrying the organism in their throats might also have caused the contamination during the cheese making process. Whether the goat was a persistent or transient carrier of *S. equi* ssp. *zooepidemicus,* cannot be answered, because it was slaughtered to prevent further contamination of milk.

P-A29

Hemolytic uremic syndrome associated with extreme heat during summer 2003 in Switzerland

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Hemolytic uremic syndrome (HUS) is often caused by enterohemorrhagic *Escherichia coli* (EHEC) of various serotypes, predominantly O157:H7. It is a severe illness found mostly in small children. The main reservoir of EHEC is the intestinal tract of cattle and other animals, and humans become infected mainly through contaminated food but also through other vehicles and person-to-person transmission, due to the very low infectious dose. Although large outbreaks have been reported, sporadic cases seem to be responsible for the main burden of the disease. In Switzerland, around 20 cases are observed annually, one of which with a lethal outcome, on average. From late May to late July 2003, an unusual increase in cases was observed. Since there was an initial clustering, involving 6 cases in the eastern most canton (Grisons) an outbreak was suspected. Within this period, a total of 21 cases were observed with two more regions in central and western Switzerland being involved. The 21 cases concerned small children, aged between one and 13 years and an average of 3.4 years. Three more isolations of pathogen concerned adults with 60 to 64 years of age and were primarily considered epidemiologically unrelated. Samples and strains were collected and characterized at the National Centre for Enteropathogenic Bacteria (NENT) in Bern. Diagnostic procedures included (i) stool cultures on McConkey and McConkey-sorbitol media, (ii) detection of vero toxin (VT) from cultures and/or stool by a commercial ELISA kit, as well as (iii) detection of EHEC-typical virulence genes involved in attachment and toxin production by PCR and dot blot hybridization techniques. Epidemiological relationship was assessed by serotyping and pulsed field gel electrophoresis (PFGE).

From the 24 cases, a total of 10 strains could be recovered and characterized. Among these 10 strains, at least 6 different PFGE types and 7 different serovars were identified. Moreover, in one single patient, even two distinct strains were isolated. They belonged to different serovars and showed also clearly different PFGE banding patterns. With the total of this evidence, a single source responsible for the cases could clearly be ruled out, rather was it an unusual increase of sporadic cases. Epidemiological data gathered by means of a questionnaire suggested that potential risk factors associated with the disease were swimming outdoors or contact with water from private supplies (12 events) and living in or travelling to a rural area (13 events). Contact with sick family members or sick friends was also important (12 events) while contact with farm animals or pets (6 events) and consumption of raw milk or other raw and potentially risky food (5 events) seemed to be of minor significance.

Considering the fact that, in 2003, Switzerland experienced the hottest summer ever since reliable such recording was commenced in 1864, we suggest that the increase in HUS cases was related to higher counts of *E. coli* in the environment. Nine of the 21 pediatric patients were only one year old and were thus highly prone to ingestion of critical amounts of pathogen (i.e. through bathing water).

P-A30

Analysis of shigellosis outbreak in Latvia

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According to data from Infectology Center of Latvia and Public Health Agency there is an increase of registered acute infectious diarrheal diseases in Latvia in the period from the beginning of 1990s till 2003, in the same time Shigellosis cases have decreased.
There are 4groups of *Shigella* - group A (*S. dysenteriae*), group B (*S. flexneri*), group C (*S. boydii*), group D (*S. sonnei*). In Latvia *S. sonnei* and *S. flexneri* are isolated and only rarely *S. dysenteriae* and *S. boydii*.

Shigellosis in Latvia 1996 - 2003.

Year	1996	1997	1998	1999	2000	2001	2002	2003
Cases in total	360	177	325	439	409	1792	773	1388
S. sonnei	134	44	139	217	152	842	438	1145
S.flexneri	146	51	111	143	157	441	205	110

S. flexneri induced shigellosis patients more often undergo treatment in Infectology Center of Latvia due to severe course of illness.
In January of 2001 there was registered significant increase of shigellosis cases due to shigellosis outbreak in the military unit where 140 soldiers become ill and in the Eyesight social care and rehabilitation center where 64 persons were infected. At the same time considerable increase of shigellosis cases (153) in this district were registered.
By examining patients from these institutions as well as from home foci of infection, there was isolated *S. sonnei* II g that shows common source of infection.
When epidemiological investigation of food products was done, there was conclusion that source of infection was unpacked curd and sour cream produced by milk factory of this district.
Milk products of this factory for microbiological investigation were taken. In 19% *E. coli* from unpacked curd and sour cream was isolated. Serological investigation of employees of the milk factory was done and in 13 from them antibodies to *S. sonnei* was in high titre (1:800-1:3200) that demonstrates recent shigellosis infection.
125 soldiers – 35% out of 357 patients – were treated in the Infectology Center of Latvia. They were young men in the age 19-21 years. All of them have eaten unpacked curd and sour cream. Bacteriological analyses of feaces for shigellosis was done in all patients, but *S. sonnei* II g was isolated only in37% of cases. Likely it was associated with antibiotic use in 77% of cases before hospitalization. All patients were treated with ciprofloxacin. All patients were tested after treatment and microbiological investigation of feaces was negative for all.
Antibiotic resistance was analysed and it was the highest to trimethoprim-sulfamethoxazole 15/28 – 54%, to ampicillin1/28 - 4%, but no resistance was registered to ciprofloxacin.
Conclusions

- epidemiologically dangerous may be unpacked milk products which may be infected in the process of manufacturing
- low level of bacteriologically positive shigellosis cases in this outbreak confirms that this diagnose may be clinical
- due to high antibacterial resistance to trimethoprim- sulfamethoxazole, the drug of choice is ciprofloxacin.

P-A31

Food- and waterborne disease outbreaks in Germany: five years (1999-2003) in the State of Baden-Wuerttemberg

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Foodborne infections still play a major role in public health. The 7th report of the WHO Surveillance Programme for Control of Foodborne Infections reported for Germany only 933 outbreaks in a six year period (1993-1998) and the 8th report (1999-2000) reported 130 (1999) and 93 (2000) outbreaks for Germany on the basis of the non-mandatory information system. Yet many foodborne infections are likely to have gone unreported. The State Health Office (Landesgesundheitsamt Baden-Württemberg, LGA) started in 1999 an epidemiology surveillance program to register and investigate outbreaks within the State. An outbreak was defined as an incident in which 5 or more persons were involved.
Between 1999 and 2003, the Local Health Offices (LHO) reported 671 outbreaks to the LGA Laboratory, approximately as many outbreaks annually for the state as the WHO reports had listed for all of Germany. These outbreaks were registered and investigated with laboratory and epidemiological methods. Most frequent outbreak diagnoses were norovirus 42% (272) () and *Salmonella enterica*, 24% (169). Other pathogens found included *Campylobacter* (18), rotavirus (19), *Bacillus cereus* (5), *S. aureus* (4), adenovirus (3), EPEC (2), EHEC (3), *Clostridium perfringens* (2), *Shigella sonnei* (2), *Giardia lamblia* (2), *Cryptosporidium parvum* (2), *Cyclospora cayetanensis* (1), *Yersinia enterocolitica* (1), astrovirus (1), and Hepatitis A (1). A large outbreak of *Cryptosporidium parvum* among military recruits and an outbreak of *Cyclospora cayetanensis* were investigated. Two outbreaks of *Salmonella* (*S*. Oranienburg in chocolate and *Salmonella* Typhimurium in Helva) were parts of international outbreaks. Recorded Salmonella outbreaks were mainly linked to restaurant/hotel/catering (45%), schools/kindergarten/day-care facilities (27%) and private homes (19%). Whereas norovirus occurred predominantly in nursing homes (45%) and hospitals (25%).
The presented data show that foodborne pathogens still play a major role in the field of infectious diseases. Various factors have contributed to the change in the recognition of foodborne outbreaks in the last years: improvement of laboratory methods, implementation of epidemiological surveillance and investigation, as well as changes in consumer behavior and world wide trade and geographical distribution. Public health authorities and politicians have to face this challenge by researching and implementing further strategies to improve food safety on all levels.

P-A32

Outbreaks of foodborne infections in France, between 1987 and 2002: Impact of prevention and control measures.

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Introduction :
In France the monitoring of foodborne outbreaks is achieved by mandatory notification. It allows analysis of trends and contributes to the evaluation and implementation of sanitary control measures.

Method :
A foodborne outbreak is defined as the occurrence of at least two cases with a similar symptomatology, related to the consumption of a common food product; it must be reported to local health authorities (Ddass or DDSV). Demographic, clinical and microbiological information, as well as the results of veterinary and epidemiological surveys, are reported on each notification form. Data are centralised and analysed in the Institut de veille sanitaire (InVS, National Public Health Institute). This surveillance is supplemented by that provided by the National Reference Centre for *Salmonella* (NRC)

Results :
The number of foodborne disease outbreaks has been multiplied by 5 between 1987 (n = 129) and 2002 (n = 676). The main causative agents were *Salmonella* (68 %), *Staphylococcus aureus* (14 %) and *Clostridium perfringens* (9 %). Between 1998 and 2002, there has been a decrease in *Salmonella* related outbreaks (-52 %) caused by the decrease (-50 %) in the number of *Salmonella* Enteritidis related outbreaks, frequently linked to eggs or egg products. This trend is confirmed by the NRC for *Salmonella* data. The decline is due to the application of control measures in commercial egg laying farms. The decrease (-38 %) in the number of *C. perfringens* related outbreaks between 1987 (n = 21) and 2002 (n = 13) is related to the improvement of the food preparation process and storage in communities.

Conclusion :
It is most likely that improved notification has resulted in the important increase of the number of foodborne diseases outbreaks notified since 1987. The decline in *Salmonella* Enteritidis and *Clostridium perfringens* related outbreaks is related to the application and reinforcement of control and prevention measures, in the production and distribution as well as in collective catering services.

P-A33

Classification of reported food- and waterborne outbreaks by the quality of evidence

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In Finland, the local food control and health officials are responsible for investigating and reporting of food- and waterborne outbreaks. The National Food Agency, in co-operation with the National Veterinary and Food Research Institute and the National Health Institute, evaluates each municipal report in order to classify the outbreaks for an annual report. Criteria for the assessment of strength of evidence have been developed and reported outbreaks have been classified into four decreasing levels of evidence, (classes A-D). Classification is based on epidemiological evidence, laboratory results as well as contributing factors (Table 1). In 2001-2002, out of 96 food or waterborne outbreaks 24%, 19%, 27% and 30% were classified in A-D, respectively. In addition, 113 outbreaks which were not associated with food or drinking water were reported.

Table 1. Classification of outbreaks into four classes (A-D).

Class	Epidemiological investigation				Laboratory test results			Contri-buting factors
	Descriptive			Analytical				
	Cluster	Applicable symptoms	Other alternatives excluded	Cohort or case - control	Patient	Food/ drinking water	Food handler	Detected
A1	+	+	+	+	+	+	ND	ND
A2	+	+	+	+	+	ND	ND	+
A3	+	+	+	ND	+	+	ND	ND
A4	+	+	+	ND	ND	+	+	(ND)
A5	+	+	+	ND	ND[1]	+	ND[1]	(ND)
B1	+	+	+	+	ND	ND	ND	ND
B2	+	+	+	ND	+	ND	ND	+
B3	+	+	+	ND	ND[1]	+	ND[1]	ND
B4	+	+	ND	ND	+	+	ND	ND
C1	+	+	+	ND	ND	ND	ND	+
C2	+	+	+	ND	ND	ND	ND	ND
C3	+	+	ND	ND	+	ND	ND	+
C4	+	+	ND	ND	ND	+	ND	ND
D	+	+	ND	ND	ND	ND	ND	ND

+ = reported in the final report or a positive laboratory test result
ND = not done / not detected / not reported
[1]The symptoms suggest biogenic amines or bacterial toxins
[2]A positive test result of a food handler strengthens the evidence and may increase the level of classification. Positive test result is a requirement only in class A4.
[3]Essential contributing factors e. g. contaminated raw material, cross-contamination, insufficient cooling or heat treatment, inadequate temperature during storage or transport, too long storage time, inadequate hygiene practices as well as food handlers suffering from gastrointestinal symptoms.

P-A34

Risk factors for infections with enteropathogenic *Yersinia* spp. in Switzerland

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Objective: Identification of risk factors for sporadic human yersiniosis

Methods: The study was conducted between 1st January 2000 and 31st December 2003 with the participation of five medical diagnostic laboratories. All fecal isolates of *Yersinia* spp. in persons under 65 years of age were reported by telefax to the SFOPH on the day of isolation. The strains were collected for serotyping, determination of antimicrobial susceptibility and comparison with isolates from pigs and pork for molecular typing. Criteria for case definition were: 1. Swiss resident who had consulted a physician for gastrointestinal symptoms, 2. stool specimen sent to one of the participating laboratories, 3. isolation of *Yersinia* spp. in the fecal culture. For each case, the physician's consent to contact the patient was requested. Informed consent for participation was sought from patients who were reached. Participation in the study included completion and return of a questionnaire and the selection of two controls outside the patient's household. A control was defined as a person who reported having had no diarrheal illness within two weeks before completing the control questionnaire. Univariate and multivariate analyses were performed using R 1.6.2, library 'survival', function 'clogit'.

Results: Sixty-one cases (60 *Y. enterocolitica* [O3: 22, O5: 8, O9: 15, other: 15], 1 *Y. pseudotuberculosis*) and 107 controls were considered valid and eligible for analysis. Most of the cases (77%) lived in a rural environment and the majority of these (68%) with animal husbandry in close vicinity or on a farm. The results of the multivariate analysis revealed positive associations between sporadic yersiniosis and the following three factors:
- Contact with farm animals (OR 11.50; 95% CI 1.31-101.1)
- Presence of an underlying disease requiring permanent intake of medications (OR 6.16; 95% CI 1.56-24.4)
- Travel abroad (OR 4.47; 95% CI 1.65-12.2)

No association was found between yersiniosis and any of the inquired food items, including those generally suspected to be risk factors (e.g. raw pork).

Conclusions: Sporadic human infections with *Yersinia* in Switzerland are predominantly contracted in rural regions, either by direct contacts with domestic animals or indirectly by contact with their feces (manure). Cases with chronic underlying diseases demanding regular intake of medications and those who traveled abroad were subject to a higher risk of contracting the infection than their controls.

P-A35

Risk factors of sporadic STEC-associated illness in Bavaria - results of a case-control study

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At the Bavarian Health and Food Safety Authority (LGL) the Bavarian part of the nationwide case-control study was coordinated for the investigation of sporadic illness associated with Shiga toxin-producing *Escherichia coli* (STEC). The study was conducted in co-operation with the Robert Koch-Institut (RKI) and was embedded in the German network "Emerging Food-borne Pathogens in Germany", promoted by the Federal Ministry of Education and Research. The data acquisition took place between April 2001 and September 2003.
After case-notification, the local health authority was asked by the LGL to participate and conduct the interviews if appropriate. The case and one age-matched control were interviewed by telephone with the same standardized questionnaire that was used by the RKI for a parallel study conducted in other parts of Germany. The control person was selected from the same telephone district as the case person.
For Bavaria, 188 matched case and control pairs could be evaluated. The case persons´ median of age was 2.0 years (range: 1 month - 90 years), 91 (48.4 %) were female. Haemolytic uraemic syndrome, a life-threatening complication of STEC-infection, was reported for 18 (9.5 %) cases. The STEC serogroup isolated most frequently from the patients´ stools, was O157 (21%) followed by O26 (18%) and O103 (14%).
The results of the univariate analysis suggested different risk factors for STEC-associated illness for different age groups. Children under 3 years were at risk for STEC-infections if diarrhoea occurred in the family or a wooden cutting board was used for the preparation of meat, while a dog as a pet as well as certain nutrition factors were more frequent among controls. For children at the age of 3 to 9 years contact to ruminants and bathing in a lake were associated with an increased risk for the illness ($p < 0.1$). This indicates for an age-dependent different etiology, with a prominence of person-to-person transmission in infants and transmission by direct contact to ruminants in older children. The results of the multivariable modelling are presented on the congress.

P-A36

Microbiological risk analysis - The role of silage in the spread of *E. coli* O157 on Dutch Dairy farms

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The feed of animals can be a source and a vehicle for *E. coli* O157. This depends on the ability of the microorganism to survive during preparation or after recontamination respectively. In this study the possibility of grass silage to be a source or vehicle for *E. coli* O157 to infect cattle was investigated. This was done by monitoring the fate of a nalidixic acid resistant non -toxigenic strain of *E. coli* O157 during fermentation and after recontamination of grass silage. Also the influence of the quality (presence of fungus, high or low pH) of the silage on survival of *E. coli* O157 was recorded. Results demonstrated that in some cases the survival of the bacteria in silage is possible and that survival depends on the quality of the silage. Infected silage therefore can play an important role in the spread of *E. coli* O157 on Dutch dairy farms.

P-A37

Control strategy for *Salmonella* in Sweden – the role of animal feed

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The Swedish salmonella control programme emanated from two Salmonella epidemics in Sweden 1953 and 1954. The largest epidemic involved almost 9000 people, of which 90 died. To prevent similar outbreaks, intense research efforts were initiated with heat sterilization of feed in 1957.

An overall integrated strategy is based on breeding animals, feed and environment free from salmonella and to keep wild animals separated from livestock. The foremost goal of the Swedish salmonella control strategy is to prevent human infection. Since all salmonella serotypes are considered pathogenic to man, all findings of salmonella in animals, man, food of animal origin and feed must be notified. By monitoring critical control points, prevention of contamination takes place in all parts of the food chain. In the Swedish feed legislation it is stated that feed has to be free from salmonella. Every time salmonella is detected, necessary actions are taken.

The annual incidence of human salmonellosis in Sweden is 5-10/100 000 (1992-2002), 15% are considered of domestic origin. Less than 1% of all food producing animals and foodstuffs are infected with salmonella.

The main risk factors in feed production are considered to be raw materials of animal or plant origin, inappropriate heat treatment, condensation, recontamination after heating and insufficient cleaning of the premises and equipment. In the Swedish feed industry the annual production is about 2,5 million metric tons. There are 13 major feed mills (70% farmers cooperatives) and about 20 smaller ones.

In 1991 a HACCP approach for monitoring feed producers was implemented. The risk analysis lead to a strategy based on control of feed raw materials, process control in the feed industry, monitoring of finished feed and when outbreaks in animals occur, tracing the source and elimination of infection is carried out. Salmonella-positive raw materials of animal origin are destroyed or returned to supplier. Raw materials of plant origin are treated with acids (1-2%, > 48h) and monitored before use.

Monitoring of feed mills include sampling for salmonella at two (feed mills not producing poultry feed) or five (feed mills producing poultry feed) critical control points weekly. When salmonella is detected corrective actions are taken.

Summary: A continuously active salmonella control of feed, which is essential in an integrated control strategy, will prevent future human salmonella outbreaks.

P-A38

Concerns of human health in animal feeds

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Over the years, major transformation and intensification of livestock agriculture has led to increasing reliance on a wide range of manufactured animal feeds especially in developed countries. Rendering firms process meat trimmings, and other slaughter by-products into animal feeds. The firms combine these with plant materials to produce feed mixtures suited for specific animal groups. Contamination with biological, chemical and/or physical hazards during the milling process and even during the transportation is the matter of major concern.
Sources of contamination with these hazards can be identified at several stages in feed production, for example during production of raw materials and by-products, storage, distribution and feeding methods. In most cases of contamination, the animal is often affected without any overt consequences (animal functioning as “biological filter”). The effects, e.g. residues, can be unnoticeable in the animal, but in many cases the farm animal does not live long enough to manifest the adverse production performance or ill health. However, chemical or biological hazards can at times be accumulated in the animal and transmitted to human via products like milk, eggs and meat products. Depending on the types of contaminants, there is a risk of passing different hazards to human following consumption of different animal food products. This is due to the co-relationships between the quality and safety of feeds and that of animal products destined for human consumption. As a result of this, strong recommendations have been advocated for good quality and safe food chains.
In this review paper, issues pertaining to biological, chemical and physical hazards that are potential contaminants of animal feeds and can be transmitted to humans resulting in adverse effects will be addressed.

P-A39

Staphylococcus aureus isolates from raw materials and foodstuffs of animal origin differ in coagulase genotype

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The restriction fragment length polymorphism analysis of the coagulase gene of *Staphylococcus aureus* isolates from the food chain (333 isolates) revealed 23 different genotypes. Significant differences among the isolates (127 isolates) from the quarter milk samples from respective cattle farms (6 farms) were recorded; one genotype dominated in each locality (V, XIV, XV, XVI, XVII, XVIII). The follow-up samples and other pool milk samples (96 samples) were contaminated with 19 genotypes of *S. aureus* (96 isolates). The second dominant genotype was genotype III (25%), which was detected occasionally in quarter milk samples (nearly 3%). Raw meat samples (74 isolates), meat products (27 isolates) and swabs from the environment of the meat processing plants (9 isolates) were likewise most frequently contaminated with genotype III strains (44 strains). Finally, meat samples were furthermore contaminated with genotype IV strains (19 strains) and genotype VII strains (3 strains), which were identified in our previous study in the isolates from humans; however, they were not found in the isolates from milk in any case. Meat samples were also occasionally contaminated with the strains of the genotypes which were dominant for the strains from the quarter milk samples. Epidemiological consequentiality of the finding of *S. aureus* of a certain genotype was not explained, but it is likely that the sources of foodstuff contamination were the strains both from the environment (genotype III) and from animals which were probably ill (isolates with the genotype dominating in quarter milk samples).

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P-B01

The occurence of animal trichinellosis in the Czech Republic

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No case of human trichinellosis due to consumption of meat from industrialised pig-farms or meat of wildlife animals has been reported in the Czech Republic since 1954.
About 4 500 000 pigs are examined every year for detecting larvae of *Trichinella* in muscle tissues according to specific national regulations, which are currently undergoing a process of harmonization with appropriate EU legislation. The fact, that no *Trichinella* infection has been found in slaughtered pigs in the Czech Republic for more than 50 years, suggests that the infection pressure is very low. *Trichinella* testing is also required for wild boars destined for human consumption. In the past 3 years, six cases of wild boar trichinellosis were found in the Czech Republic and *Trichinella britovi*, the etiologic agent of sylvatic trichinellosis, was detected in one case.
Recently, foxes were also tested as wildlife indicator for trichinellosis. Foxes included in the investigation came from throughout the country and were obtained during a survey for rabies during 2001 to 2003. A total of 1160 foxes were examined using direct diagnostic techniques and muscle samples from tongue, masseters and hindlegs were tested by the standard artificial digestion method. Seven foxes were found positive corresponding to a prevalence of 0.6%. In two cases only, it was possible to obtain sufficient larval material for species identification and larvae were identified as *T. britovi*.
These results, compared with previous studies indicate that the prevalence of *Trichinella* infection in wildlife is increasing and wildlife animal trichinellosis forms a natural reservoir for possible infections in slaughter animals. Based on the outlined results, the implementation of the concept of *Trichinella*-free areas seems not feasible in the nearby future in the Czech Republic due to the impossibility to control trichinellosis in wildlife.

P-B02

Trichinellosis in pigs and wild boars in Poland

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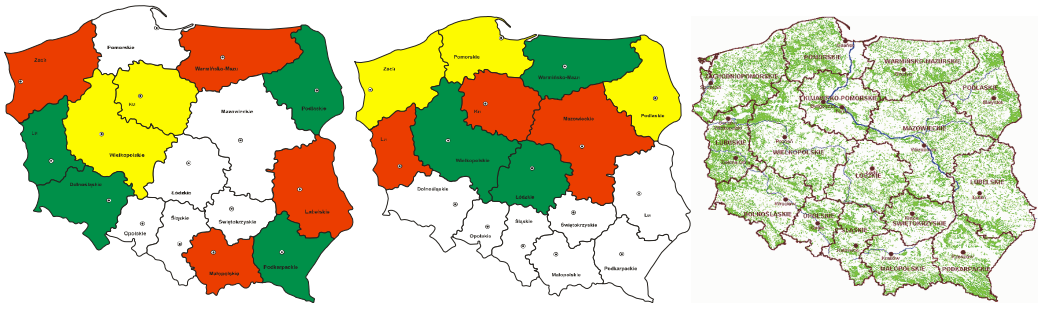
Trichinellosis, a disease caused by the nematode *Trichinella*, occurs in more than 100 animal species in areas with different geographical and ecological characteristics. The prevalence of the disease is difficult to evaluate. Ten species have been described within the genus *Trichinella*: *Trichinella spiralis*; *Trichinella nativa*; *Trichinella britovi*; *Trichinella pseudospiralis*; *Trichinella murrelli*; *Trichinella nelsoni*; *Trichinella papuae*; T6; T8; and T9. The geographical distribution of the different species is related to temperature and host behavior. As for now in Poland were detected two species of Trichinella. Trichinella spiralis sensu stricto and Trichinella britovi. In 2002 trichinella larvae were detected in 45 carcasses of farmed pigs and in 105 carcasses of wild boars. The geographical distribution of trichinella infected pigs in Poland shows fig. 1. and trichinella infected wild boars shows fig. 2. It is observed that trichnellosis occurs more often in region there are located large forest area fig. 3. The available data shows that the number of w of detected wild-boars carcasses with Trichinella spiralis larvae has increased. This is probably due to better diagnostic and knowledge of the laboratory stuff. The Reference Laboratory for Trichinellosis located in National Veterinary Research Institute has provided over 60 workshops on Trichinella diagnostic methods for official veterinary inspection service and laboratory stuff. According to Polish legislation digestive method is obligatory for examination of wild-boars carcasses.

Fig 1, 2 and 3.



P-B03

Characterization of *Staphylococcus aureus* strains isolated from raw milk samples of small ruminants

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Staphylococcus aureus is world-wide the most important pathogen in food poisoning and causes gastrointestinal symptoms like nausea, emesis, abdominal cramps and diarrhoea. Five classical staphylococcal enterotoxins SEA-SEE have been recognised and sporadic cases as well as outbreaks due to these enterotoxins are described (Balaban et al. 2000; McLauchlin et al. 2000; Asao et al. 2003). Recently, new staphylococcal enterotoxins were recovered: SEG, SHE, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ and SEU (Zhang et al. 1998; Orwin et al. 2001; Jarraud et al. 2001; Letertre et al. 2003; Orwin et al. 2003). However, their role in food poisoning has not yet been clarified.

S. aureus can gain access to milk either by direct excretion from udders with clinical and subclinical staphylococcal mastitis or by contamination. In a recent Swiss survey *S. aureus* was detected in 32% goat's and in 33% ewe's bulk-tank milk samples (Muehlherr et al. 2003) The median value was <1 log cfu/ml for goat's-milk samples, with a maximum of 2.20×10^4 cfu/ml, and <1 log cfu/ml for ewe's-milk samples respectively, with a maximum of 3.60×10^3 cfu/ml. Therefore, there is a clear need to have more characterization data of such strains. In contrast to strains isolated from bovine bulk-tank milk samples no data are available in literature for strains of small ruminants.

Two hundred and ninety three isolates of *Staphylococcus aureus* obtained from 127 bulk-tank milk samples of small ruminants collected throughout Switzerland were characterized by pheno- and genotypic traits.

Of the 293 isolates, 193 (65.9%) were egg yolk-negative and 15 (5.1%) were negative for clumping factor and/or protein A determined by a latex agglutinating test system. For 285 *S. aureus* isolates PCR amplification of the 3' end of the *coa* gene showed a single amplicon. Five different sized PCR products of 500, 580, 660, 740 and 820 bp were distinguished. In 191 isolates (n=293) toxin genes were detected: 123 isolates tested positive for *sec,* 31 isolates for *seg,* 28 isolates for *sea,* 26 isolates for *sej*, 24 isolates for *sei* and 4 isolates for *seb* and 4 isolates for *sed.* Furthermore, 126 isolates were positive for *tst*, the gene encoding the toxic shock syndrome toxin 1.

Coagulase gene restriction profile (CRP) analysis of the 145 isolates harbouring *sea* or *sec* genes revealed 6 different patterns using *Alu*I and 5 different patterns using *Hae*III. In summary, within these two groups, high genotypic uniformity within the different sized *coa* gene amplicons was proved.

This is the first study providing comprehensive characterization data of *S. aureus* strains originating from bulk-tank milk samples of small ruminants. Remarkably differences in phenotypic traits between *S. aureus* originating from small rumiants and bovine milk were found. Moreover, the high prevalence of toxin-producing *S. aureus* is an issue requiring consideration as it relates to food hygiene.

P-B04

Comparative studies to the survival of glycopeptide resistant enterococci (GRE) in fresh salami type sausage and dry salami

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Enterococci belong to the natural flora of the gastrointestinal tract in animal and in man. *Enterococcus* (*E*.) *faecalis* and *E. faecium* are at present the most important species and responsible for more than 80% (*E. faecalis*) and 10-20% (*E. faecium*) of enterococcal infections in humans. The quantity of infections caused by enterococci, in particular *E. faecalis* and *E. faecium*, has been increasing in the field of human medicine within the last years. Resistances to important antimicrobial agents especially glycopeptide resistant enterococci (GRE) have increased in this genus simultaneously. A link with the application of antimicrobial agents in livestock farming is supposed. Enterococci are usually considered as bacteria of low level pathogenity, which predominantly infect patients with prenounced predisposition. They are able to cause different infections, e.g. of the urinary tract and of the bile trays and are also responsible for severe lifethreatening diseases as bacteremia or endocarditis. Today, enterococci are accepted as important pathogens of nosocomial infections. Currently they are in second or third place of bacteria, that cause such infections and they are one of the most important of the Gram-positive bacteria.

The experimental design includes the preparation of a typical German dry salami and a typical German fresh salami type sausage with three different commercial available starter cultures. The selection of raw materials and ingredients were according to industrial standards. The maturation and storage of the sausages were carried out for a period of 2 weeks for fresh salami type sausage and 3 weeks for dry salami. The raw material was minced in usual grain size and filled into product typical skins before maturation in climatic chambers.
A select vancomycin-resistant *Enterococcus faecium* strain served as an indicator for the growth and decline of GRE at the maturation and storage of salami and fresh lean minced pork sausage. Different technological aspects i.e. acidification, drying, and colour stability and microbiological parameters (total viable counts, occurrence of Enterobacteriaceae, lactic acid bacteria) were investigated during ripening of the two sausage types.
The results indicate that use of the starter culture could limit the increase of the used vancomycin-resistant *Enterococcus faecium* strain during ripening and storage of both typical German dry salami and fresh salami type sausage. Other raw sausage typical parameters, as a decrease in pH and drying apparently did not have any influence on the growth of the selected strain in German dry salami and typical German fresh salami sausage.

P-B05

Similar genotypes of *Yersinia enterocolitica* 4/O:3 strains found from human and porcine sources in Southern Germany

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Yersinia enterocolitica is a highly heterogeneous species and has therefore been divided into various bioserotypes, only a few of which are associated with human disease. *Y. enterocolitica* belonging to bioserotype 4/O:3 is a common cause of human yersiniosis and present in region where pig reservoirs prevail. Yersiniosis has been a reportable disease in Germany since 2001. In 2002, its incidence was 9.1 reported cases per 100 000 inhabitants, with the number of cases in different regions varying from 4 to 27 per 100 000 inhabitants. Bioserotype 4/O:3 is the most frequently isolated type in humans in Germany, and the same bioserotype dominates in slaughter pigs in Southern Germany. While the epidemiology of this microbe remains unclear, *Y. enterocolitica* is considered to be an important foodborne pathogen, which is probably transmitted from pigs to humans via contaminated pork. To date we know that in Finland most of human *Y. enterocolitica* 4/O:3 strains were genetically indistinguishable from *Y. enterocolitica* 4/O:3 strains found in samples of pig origin, and that genotypes of *Y. enterocolitica* 4/O:3 strains isolated from pig tonsils in Southern Germany differed from the strains isolated from pig tonsils in Finland. This study was conducted to compare *Y. enterocolitica* 4/O:3 genotypes recovered from humans and porcine sources in Southern Germany.

A total of 150 *Y. enterocolitica* 4/O:3 strains isolated during 1999-2003 were studied. Of these strains, 50 were from humans and 150 from porcine sources. Human strains were isolated from faecal samples of patients with diarrhoea form Munich area. Porcine strains were recovered from slaughterhouses, butcher shops and meat factories in Southern Germany. All the strains were characterised with PFGE using *Not*I, *Apa*I and *Xho*I restriction enzymes.

In all, 45 different genotypes were obtained when 150 *Y. enterocolitica* 4/O:3 strains were characterised with *Not*I, *Apa*I and *Xho*I restriction enzymes. Of the 45 genotypes, 18 were found among 50 human strains and 40 genotypes among 100 porcine strains. A total of 44 (88%) human strains belonging to 13 genotypes were genetically indistinguishable from the porcine strains. The most common genotypes (GTI and GTII), representing 48% (24/50) of the strains in human infections, were found from pig tonsils, tongue, diaphragm, lungs, heart, liver and pork. Several human genotypes (GT I, II, VI, IX and XIII), representing 60% (30/50) of the human strains, were found from pork products, including minced meat, ham and bacon.
Most of human *Y. enterocolitica* 4/O:3 strains were indistinguishable from porcine strains when characterised with *Not*I, *Apa*I and *Xho*I restriction enzymes, supporting the hypothesis that pig is the main source of sporadic human *Y. enterocolitica* 4/O:3 infections in Southern Germany. Many human genotypes were found from pork products, demonstrating a possible transmission route from pigs to humans via contaminated pork products. The biodiversity among the porcine strains was higher than among human strains. A possible explanation may be that the strains belonging to these genotypes have a lower virulence, thus causing only mild infections and needing a higher infection dose.

P-B06

Detection and serotyping of enteropathogenic *Escherichia coli* (EPEC) in domestic Iranian soft cheese in Kerman

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Objective: To determine and evaluate the microbiological quality and detection and serotyping of enteropathogenic Escherichia coli (EPEC) in domestic Iranian soft cheese in Kerman.

Design: Random sampling, 60 samples of domestic soft cheese from different parts of the city in Kerman in 2002.

Material and methods: Samples were cultured and examined in sterile condition and in selective media (BHI,MC, EMB,TSB) and after incubated ad standards of microbiological methods for detection of E.coli, the IMVIC and serotyping of the isolated bacteria have been performed.

Results: *E. coli* has been isolated from 98.3% of the samples coli and 16.9% of the isolated E.coli were EPEC. They belonged to serogroup O114, O142, O119, O128, O26, O89, O129, O128. All of these types are pathogens. Specially O26 which causes hemorrhagic colitis.

Conclusion: It has been shown that the bacteriological quality of cheese sold in Kerman city market is not satisfactory, since the processing of this cheese is crude and far from standardized and the retail shops do not always display cheese samples in refrigerated compartments, but often only in salt water in open containers.The hygienic and microbiological control and examination of the samples in production plants and the distribution chain is recommended.

P-B07

Use of cultural detection methods by JOHNSON & MURANO and multiplex PCR by HARMON & WESLEY for the detection of *Arcobacter spp*. in fresh poultry and ground beef bought from Berlin retailers

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Formerly *Arcobacter spp*. were categorized as oxygen-tolerant *Campylobacter spp*., but, due to their thermo- and aerotolerance, they can be assigned to an own genus within the family of *Campylobacteriaceae*. Originally isolated from aborted bovine fetuses and aborted piglets, *Arcobacter spp*. have been associated with human enteritis and abdominal cramps since 1991 (KIEHLBAUCH et al.).
According to the current state of knowledge, a transmission is not only possible by drinking water but also by food. Hence, the aim of this study was to detect the incidence of *Arcobacter spp*. in fresh cooled broiler thighs and ground beef from Berlin retailers.

The cultural method used for detection was based on the selective media developed by JOHNSON & MURANO (JM) , the JM-enrichment broth (30 °C, 48 h, aerob) and the JM-plate (30 °C, 48 h, microaerob). For PCR verification the method by HARMON & WESLEY was applied. 25 g from each sample were mixed 1:1 with buffered pepton water from which 10 ml were transferred to the enrichment broth. After incubation, 1 ml was taken for PCR, and a three-phase streaking was applied on JM-plates (double charge). Since only few biochemical tests are available for confirmation, colonies appearing to be *Arcobacter*-positive were verified by PCR.

In the preliminary tests 20 out of 53 samples of broiler thighs (37,7 %) and 3 from 50 ground beef samples (6 %) were found to be *Arcobacter*-positiv. In the main experiment *Arcobacter*-positive results were achieved by 5 from 25 broiler samples taken from ecological husbandaries (20 %) and 13 from 25 broiler samples from conventional husbandaries (52 %). No one of the 25 samples of ground beef were found to be *Arcobacter*-positive in the main experiment.

This investigation shows a high contamination level with *Arcobacter spp.* in fresh poultry in Germany, or at least in Berlin, similar to the results of France, Belgian and USA studies.

P-B08

The occurrence and strain diversity of *Arcobacter* in animal feces

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Arcobacters are Gram-negative bacteria that can grow microaerobically and aerobically from 15 up to 42°C. *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii* are the animal and human related species. They have been isolated from humans with abdominal illness and septicemia and are associated with reproductive problems in farm animals. Arcobacters were found in feces from clinically healthy livestock animals and on food of animal origin. This food contamination is assumed to occur by fecal contamination during slaughter. Foods of animal origin are considered as the main source of *Arcobacter* infection in humans and, therefore, arcobacters are considered emerging foodborne pathogens.

Different isolation methods and molecular techniques are used for *Arcobacter* isolation from feces, although none of these techniques were validated for *Arcobacter* isolation from feces. In this study, a previously developed quantative and qualitative isolation method for poultry skin and meat was validated for *Arcobacter* isolation and enumeration from animal fecal samples. Furthermore, the prevalence and contamination level in feces from Belgian clinically healthy pigs and cows on different farms were determined.

During validation of the method, good repeatability, in-lab reproducibility and sensitivity were achieved and a small adaptation of the selective supplement improved the specificity of the method. The limit of detection of quantitative and qualitative analysis was 10^2 and 10^0cfu/g feces, respectively. All three animal-associated *Arcobacter* species could be isolated with a good suppression of the accompanying fecal flora.

Fecal samples were collected on four pig farms and three dairy farms. One gram of rectally collected fecal sample was examined using the validated isolation procedure. Isolates were identified at species level by a multiplex-PCR assay and further characterised below species level by a modified ERIC-PCR. Of the 294 pigs examined, 173 were negative for the presence of arcobacters, 66 pigs excreted less than 10^2 cfu/g feces and 55 animals had a bacterial load of 10^2 to 10^4 cfu/g feces. *A. butzleri* was the most frequently occurring species, but co-colonization was not uncommon as two and three *Arcobacter* species were found in 12.4% and 3.3% of the positive samples, respectively. The *Arcobacter* prevalence on the four unrelated pig farms ranged from 16% to 42% in porkers and from 59% to 85% in sows. A total of 478 isolates were obtained and revealed a large genotypic variation among the isolates. An animal in many cases excreted more than one genotype per species. Of the 276 cows examined, 4 had a bacterial load of more than 10^2 cfu/g feces and low levels were detected in 26 animals using enrichment. *A. cryaerophilus* was the most dominant species in cows. A co-colonization was found in 26% of the *Arcobacter* excreting animals. The prevalence on the three dairy farms ranged from 7.5% to 15%. Fingerprints of the 170 isolates showed a large heterogeneity and animals could be colonized with more than one genotype. The prevalence and colonization levels in bovine feces turned out to be far below those in porcine fecal samples. This is the first report of *Arcobacter* levels in feces of healthy animals.

In conclusion, the validated and optimized protocol is a reliable method for *Arcobacter* isolation from feces. The human and animal associated species are effectively isolated with a good inhibition of the fecal flora. The quantitative method provides an opportunity to obtain information on the bacterial load in feces. Arcobacters were detected in clinically healthy animals in levels up to 10^4 cfu/g feces. The prevalence in feces from livestock obtained using this protocol is higher than reported in other studies.

P-B09

Prevalence of antimicrobial residues in table eggs in Trinidad and Tobago

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The prevalence of antimicrobial (beta-lactam, macrolides, sulfa and tetracycline) residues in table eggs from supermarkets, shopping malls and layer farms in Trinidad was determined using the Charm II assay system. Prevalence was related to egg weight, storage temperature at sale outlets, use of medicated feeds and adherence to withdrawal periods on farms. Farms and shopping malls were sampled twice, one month apart, while supermarket outlets across Trinidad were sampled once. For the assay, 25 eggs constituted a composite sample from farm sources while for malls and supermarkets, 6 eggs constituted a composite sample. During each visit to the sale outlets egg, samples were collected from all the sources available. Overall, of a total of 184 composite egg samples, representing 1,978 eggs tested, 24 (13.0%) were positive for antimicrobial residues. Three (13.0%) of 23 farms, 5 (35.7%) of 14 malls and 16 (15.7%) of 102 supermarkets sold eggs contaminated with antimicrobial residues to the public. For composite egg samples, 3 (6.5%) of 46, 5 (15.0%) of 31 and 16 (15.6%) of 107 farms, malls and supermarkets respectively were positive for antimicrobial residues but the difference was not statistically significant ($p = 0.073$). Of a total of the 184 composite egg samples tested from all sources, 12 (6.5%), 7 (3.8%), 5 (2.7%) and 0 (0.0%) were positive for sulfa, macrolides, tetracycline and beta-lactam respectively and the difference was statistically significant ($p=0.011$). The differences in the prevalence of residues by weight of eggs, <60g (19.7%) versus >60g (10.7%), $p=0.125$; storage temperature, refrigerated temperature (12.2%) versus room/ambient temperature (16.0%), $p=0.412$; use of medicated feed (7.5%) versus non-use of medicated feed (0.0%), $p=0.718$; and adherence to withdrawal period following treatment (16.7%) versus non-adherence to withdrawal period (4.5%), $p=0.39$, were however not statistically significant. It was concluded that a prevalence of antimicrobial residues ranging from 13.0% to 35.7% across sources, with a predominance of sulfa may constitute a health hazard to the consumer and this is considered the first documentation of antimicrobial residues in table eggs in the country.

P-B10

Layout proposals for microtitre plates for routine antimicrobial susceptibility testing of bacterial pathogens from large food-producing animals and from mastitis cases

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Currently there is little if no standardisation in routine testing of animal pathogens from food producing animals for susceptibility against antimicrobial agents. The substances routinely tested in the various diagnostic laboratories differ significantly as do the testing methods applied. For effective therapy as well as for producing a reliable data base further standardisation is needed. Concerning the methodology, microbroth dilution was considered as the method of choice. In contrast to disk diffusion, microbroth dilution allows to determine the minimum inhibitory concentration which precisely indicates the degree of susceptibility of the tested bacterial strain against a specific antimicrobial agent or a combination of antimicrobial agents.

For a better standardisation of this method for use in routine diagnostics, two layouts for microtitre plates have been set up by the working group „Antibiotic resistance“ of the German Society for Veterinary Medicine. These plates should be used in microbroth dilution tests according to the NCCLS standard M31-A2. One of these layouts was designed for the testing of bacteria from cases of mastitis and the other for bacteria from infections in large food-producing animals. The choice of the antimicrobial agents and their concentrations to be included in these layouts were based on (1) the bacteria frequently associated with the respective diseases/animals, (2) the antimicrobial agents licensed for therapeutic use in these diseases/animals, (3) the currently available breakpoints, and (4) cross-resistances between the antimicrobial agents so far known to occur in the respective bacteria. Thus, the layout for pathogens from large food-producing animals includes 19 different antimicrobial agents or combinations of antimicrobial agents in 3 – 9 concentrations, whereas the layout for mastitis pathogens comprises 11 antimicrobial agents or combinations of antimicrobial agents in 3 – 7 concentrations. Due to the smaller number of bacteria involved in mastitis and the smaller number of antimicrobial agents licensed for the treatment of mastitis, the layout for testing mastitis pathogens was designed to test two different pathogens on the same microtiter plate.

Especially for food producing animals the application of approved and effective antimicrobial agents is crucial to produce safe food for human consumption. Unwanted side-effects as resistances in commensals or foodborne pathogens can otherwise be promoted. The standardisation of methods and plate layouts should facilitate the choice of effective substances for antibiotic therapy.

P-B11

Antimicrobial resistance of *Staphylococcus* spp. isolates from food chain – incidence analysis

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Staphylococci are the important causative agent of infections in hospitals and the community. Following the introduction of antimicrobials, staphylococci rapidly developed resistance. It is highly likely that food chain constitutes a reservoir for disseminating antibiotic-resistance into the community. *Staphylococcus aureus* (SAU) strains and coagulase-negative staphylococci (CoNS) contaminated 22.7% and 66.5% of 1382 raw milk samples, respectively. SAU was not found in pasteurized milk (22 samples) and only one sample (soft fresh cheese) of dairy products (213 samples) was contaminated. However, 36.4% and 51.2% of pasteurized milk and dairy products samples, respectively, were found to be contaminated with CoNS. SAU and CoNS were also isolated from raw beef meat (23.9% and 58.5% of positive samples, respectively). Samples of unheated fermented dry salami were contaminated significantly more frequently ($P<0.01$) with SAU (46.7%) than heat treated meat products (7.7%). However, both types of meat products were found again to be highly contaminated with CoNS (84.4% and 89.2%, respectively). From investigated samples, 455 isolates of SAU and 1978 isolates of CoNS were collected and examined for resistance to 12 antibiotics. Isolates of SAU and CoNS were most frequently resistant to penicillin (61.3% and 42.2%, respectively), streptomycin (50.9% and 18.3%, respectively), tetracycline (14.1% and 15.1%, respectively) and erythromycin (10.8% and 10.6%, respectively). Prevalence of resistance of SAU and CoNS strains to other agents was as follows: clindamycin (1.3% and 7.7%, respectively), oxacillin (0.2% and 4.2%, respectively), and neomycin (2.6% and 1.7%, respectively). Low frequency of resistance to gentamicin, cephalothin, vancomycin, ampicillin-sulbactam, and norfloxacin was observed. Multiresistance (resistance to 3 or more antibiotics) was found in 11.9% of SAU and in 9.2% of CoNS. Most investigated isolates of SAU and also CoNS were resistant to one or more antibiotics tested. Potential vectors of antibiotic resistance are not only SAU strains, but also CoNS, frequently isolated from samples of both commodities. Similar resistance patterns (penicillin, streptomycin, tetracycline, and erythromycin) dominated in staphylococcal isolates both from raw material (milk and meat) and final products. Our results suggest that milk and meat may act as potential sources of antibiotic-resistant *Staphylococcus* spp. posing a threat to consumers.

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P-B12

Detection of vancomycin-resistant Enterococci in Styrian livestock production

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Enterococci are important pathogens with the ability to develop resistance to most antimicrobial agents, for example by intake of genetic resistance determinants that can be passed on to other pathogenic bacteria such as listeria, streptococci and staphylococci. Enterococci can thus be the central agents in the transfer of drug resistance between bacteria species. Glycopeptide resistance in particular is of great importance in this context, since glycopeptides are often so-called “drugs of last resort”, the last effective therapy option against human enterococci infections.

The goal of the project was to identify the existence of glycopeptide resistance determinants in enterococci isolated from livestock. For this purpose, 172 enterococci strains were isolated from faeces of 74 cattle, 58 pigs, and 40 broilers and tested for vancomycin resistance. Vancomycin-resistant enterococci (VRE) were detected by multiplex PCR (1, 2) at species level and their genetic resistance determinants (*vanA, vanB, vanC1, vanC2, vanC3*) were identified.

The preliminary results show that the majority of enterococci isolated from broiler faeces were vancomycin resistant (67.5%) with a predominance of the “high level” resistance gene *vanA*. Fewer cases of vancomycin resistance (24.5% and 34.5%) were found in enterococci isolated from cattle and pig faeces and primarily concerned “low level” resistance genes (*van C1, vanC2, vanC3*). The results show the importance of livestock production, in particular poultry production, as a reservoir of VRE.

P-B13

Prevalence of gentamicin high-level resistant enterococci isolated from cattle, pig, poultry and food

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Enterococcal strains isolated from materials of animal origin and food were examined with regard to their resistance behaviour against gentamicin. The minimal inhibition concentration (MIC) was determined by the microdilution method (Sensititre®-system) and particularly for high level resistant strains with the Etest®. Four genes responsible for this kind of resistance (*aac(6')-Ie-aph(2")-Ia, aph(2")-Ib, aph(2")-Ic, aph(2")-Id*) were investigated by PCR. All gentamicin high level resistant strains (n = 9 with MIC values =500µg/ml) only possessed the first mentioned gene. The remainig three genes were neither detected for these strains nor for strains with MIC values from 32 - 256µg/ml against gentamicin. The majority of the examined strains showed MIC values from 1µg/ml to <64µg/ml (n=754 of a total of 862 strains).

The nine mentioned high level resistant strains were subsequently well-investigated by molecular biological methods. Their species affiliation was determined by both microbiological and biochemical test schemes and by PCR. Due to the results the strains were classified as *E. gallinarum* and *E. faecalis.* Dependent on their species they possessed the same resistance patterns against 16 selected antimicrobial agents. Fingerprint examinations using PFGE showed comparable fragment patterns for all *E. faecalis* strains independently from material (minced meat from cattle, pig, ham from pork) and from the region. However only one high level resistant *E. gallinarum* strain from poultry showed an evaluable fragment pattern. For all other high level resistant *E. gallinarum* strains a fragment pattern could neither begenerated by the restriction enzyme *Sma*I nor by *Apa*I. The results of this study revealed a minor frequency of gentamicin high level resistant strains.

P-B14

Phage types, antimicrobial resistances and presence of class 1 integrons in non-typhoid human *Salmonellae* isolated in Hungary in 2002-2003

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To get information on the spread of the different phage and resistance types and as a preliminary characterization of the occurrence of class 1 integrons, a collection of 1585 Salmonella enterica subsp. enterica strains was set up. The strains were isolated from human salmonellosis cases during 2002-2003 and represented the five serotypes (*S*. Enteritidis, *S*. Typhimurium, *S*. Infantis, *S*. Hadar and *S*. Virchow) most commonly isolated in Hungary. All the strains were tested for phage type and antibiotic susceptibility. Class 1 integron content of the strains resistant to at least one antibiotic was tested by PCR using the conserved integron primers and the obtained PCR products were sequenced.

Phage typing of the *S*. Enteritidis strains has been carried out by the method of Ward et al. and that of the S. Typhimurium strains by the method of Anderson et al. Strains of the other serotypes were phage typed by using a set of Hungarian phages. Antibiotic susceptibility was tested by a standard disk diffusion method. The antibiotics used were: ampicillin (A), cefotaxime (Cef), chloramphenicol (C), tetracycline (T), streptomycin (Sm), gentamicin (Gm), kanamycin (Km), nalidixic acid (N), ciprofloxacin (Cip) and sumetrolim (Sum). Selected strains were screened for production of extended-beta-lactamases (ESBLs) by E-test.

The vast majority (77%) of the examined 337 *S*. Enteritidis strains represented only one phage type: the PT4. No other PT could be highlighted as a frequently found one. Interestingly, almost all the PT4 strains were found to be resistant to one antibiotic: namely the nalidixic acid. Multi-resistance was rare among the strains (10.1%) and only 4 strains possessed a 1.0 kb integron with aadA2 gene content.

Out of the examined 1058 S. Typhimurium strains 36% belong to the DT104 and 21% to the DT104-related U302 phage-type. The other more frequent phage types were: RDNC, PT14, PT30, PT1 and PT99. In accordance with the literature most of the DT104 and U302 strains could be characterised by the well-known penta-resistance and harboured the 1.0 and 1.2 kb integrons characteristic for the DT104 strains. 10 strains were resistant to cefotaxim. One of them was found to be the first Hungarian SHV-5 type ESBL-producing isolate.

From 99 S. Infantis strains 93 were found to be multi-resistant. All but 4 of these strains possessed an appr. 1.0 kb integron having an aadA1 gene. All but one of the 60 *S*. Hadar strains were multi-resistant having the same resistance type (Sm, T, N) as the multi-resistant *S*. Infantis strains, but the integron-harbouring was not characteristic, only two strains had an appr. 1.6 kb integron with dhfr and aadA cassettes. All but one of the 17 *S*. Virchow strains were multi-resistant and had different patterns of 3 different integrons: those two that were found in the case of the *S*. Infantis and *S*. Hadar strains and an appr. 750 bp long one, with a dhfrV gene.

This study would like to give information about the current phage and resistance types among the most commonly isolated human *Salmonellae* in Hungary and call the attention on the threatening spread of the multi-resistance among *S*. Infantis, *S*. Hadar and *S*. Virchow, which might be the consequence of the ability of these serotypes to capture such mobile DNA elements like the integrons.

P-B15

Ribotype and pulsed field gel electrophoresis analysis of multidrug resistant *Salmonella* Newport

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The increased use of antimicrobials in human and veterinary medicine, and agriculture, has created enormous pressure for the selection of antimicrobial resistance among bacterial pathogens. Recent foodborne outbreaks of *Salmonella enterica* serotype Newport infection appears to be related to the recent emergence of multiple antimicrobial resistant strains of *S*. Newport. These strains are resistant to multiple antimicrobials, including third generation cephalosporins such as ceftriaxone, an antimicrobial agent commonly used to treat serious *Salmonella* infections in children.

The objective of this study was to use the techniques of ribotyping, pulsed field gel electrophoresis, plasmid profiles and integron analysis to investigate the degree of DNA banding polymorphism exhibited by strains of antimicrobial resistant *S.* Newport.

Antimicrobial resistance of the *S.* Newport isolates was determined using the Sensititre automated antimicrobial susceptibility system. Ribotyping was accomplished using the Dupont Qualicon Riboprinter. Pulsed field gel electrophoresis was performed according to the directions of the Centers for Disease Control and Prevention (CDC). Plasmid and integron analysis were performed according to previously described methods.

PFGE and ribotype profiles were used to compare the genetic relatedness of the *S*. Newport isolates. Both methods clearly resolved the *S*. Newport isolates into eight distinct clusters. Plasmid analysis revealed that most isolates contained plasmids ranging in size from 2.0 - 23.0 kb. Class 1 integrons were observed in many of the isolates, and several isolates contained more than one integron. Restriction fragment length polymorphism (RFLP) indicated that integrons of the same size were identical.

These results demonstrate the high degree of DNA banding pattern polymorphism found in some strains of antimicrobial resistant *S*. Newport, and illustrates the presence of complex genetic structures contained within the isolates, which may be involved in the rapid spread of antibiotic resistance among *S*. Newport strains.

P-B16

Stability of retron reverse transcriptase in multidrug resistant *Salmonella enterica* serovar Typhimurium

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Since 1985, multidrug resistance in strains of *Salmonella enterica* serovar Typhimurium is on increase. The resistance is connected with presence of Class I integron. Integron is a genetic element which is able to acquire promotorless gene cassettes. Because of missing promoters, reverse transcription catalyzed by bacterial retron reverse transcriptases, was speculated to contribute to the acquisition of new gene cassettes. Surprisingly, immediately downstream from the multidrug genomic island coding for resistance to ampicillin, tetracycline, chloramphenicol, streptomycin and sulfonamides, gene encoding retron reverse trascriptase was identified. Therefore, aim of the study was to investigate possible interaction between the integron and retron elements. Using PCR we detected presence or absence of the retron element in 175 strains of *Salmonella enterica* serovar Typhimurium. We have found that in 17 strains the retron element was absent. Interestingly, all these 17 strains were multidrug resistant and integron positive. We therefore considered that the excision was due to the presence of the integron. We were further interested in mechanisms by which the excision happened and whether this happened in all the strains by the same mode. By PCR we first determined genes which remained present in the retron-free strains. Next, a specific PCR amplifying over the missing DNA sequence was designed and the resulting PCR product was sequenced using Abi Prism 310 Genetic Analyser (Applied Biosystems). Obtained sequence was compared with the GenBank entries using BLAST algorithm available at www.ncbi.nlm.nih.gov. After the excision, insertion sequence IS6100 localized upstream from the retron element, and *yieF* gene localized downstream from the retron, remained intact. Subsequent sequence analysis showed that the deletion event occurred precisely on the left boundary of insertion sequence IS6100, which is the part of integron, and inside *yie*E gene, upstream from *yieF*. Deleted DNA sequence was 8164 bp in length and covered not only the gene for retron reverse transcriptase but also other 6 neighboring genes including the partially deleted *yieE*. The deletion event must be a well-controlled process as the excision site was 100% identical in 6 selected sequenced strains. Genomic organization of the retron locus in multidrug resistant *Salmonella enterica* serovar Typhimurium is *thd*F - SGI1 - retron – *yid*Y. Detailed sequence analysis of the genomic structure of *thd*F-*yid*Y locus showed that this locus is a hot spot for recombination. It is free of any insertion in *S. enterica* serovar Enteritidis. The *thd*F-*yid*Y intergenic region in *Salmonella enterica* serovar Enteritidis contains 2 stem loop structures reminding transcription terminators. These might be target for the recombination as in *E. coli* K12 *tna* operone is inserted in this region and multiple insertions in this region could be observed also in *Shigella flexneri* and *Klebsiela pneumoniae.*

P-B17

Rapid detection of *Campylobacter jejuni* and *Campylobacter coli* in food by PCR

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Campylobacter (*C*.) *jejuni* and *C. coli* are the most frequently isolated bacterial pathogens from human gastroenteritis worldwide. Farm animals, especially poultry, cattle and pigs as well as pets like dogs and cats or wild living birds are considered as reservoirs. The pathogens are normally transferred by contaminated food, primarily by poultry meat and offals as well as raw milk and contaminated water. The working group "molecular-biological methods in microbiology" developed a PCR method for the detection of *C. jejuni* and *C. coli* and published it as a preliminary §35 LMBG-method (Article 35 of the German Federal Foodstuffs Act; Bundesgesundheitsblatt, Gesundheitsforsch., Gesundheitsschutz 2000 43 : 816-824).
A total of 251 samples of poultry meat and/or offals were examined by the PCR method as well as the Vidas screening for *Campylobacter* at the Bavarian Health and Food Safety Authority. Positive findings were confirmed culturally and with a Latex agglutination test.

Of 251 samples *Campylobacter spp*. could not be detected in 140 samples, whereas 101 samples yielded positive results with both test methods. Six samples only showed positive results with the VIDAS method. In these samples *Campylobacter* could be culturally conformed. Four of the examined samples only showed positive results with the PCR-method. In these samples *Campylobacter* could not be culturally conformed.
96% of all exmined samples showed concurrent results by the PCR and the VIDAS method.

P-B18

Isolation and PFGE typing of Finnish *Campylobacter jejuni* strains from cattle, poultry and organic hens

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Campylobacter spp., especially *C. jejuni*, is recognized as the most common bacterial cause of gastroenteritis in many developed countries, including Finland. Risk factors associated with campylobacteriosis include eating and handling raw or undercooked poultry products, drinking raw milk or untreated surface water, and contact with pet animals. The aim of this study was to isolate *Campylobacter* spp. and compare the pulsed-field gel electrophoresis (PFGE) profiles of *C. jejuni* isolates from different food production animals, i.e. cattle, poultry and organic hens in Finland.

We examined 511 fecal samples from slaughter-aged cattle, 409 samples of retail poultry products (345 of which were chicken products, 58 turkey, and 6 mixed products), and 347 fecal samples from organic hens. The fecal samples from cattle were collected from the middle of April to the end of August from 13 different slaughterhouses in Finland. The samples were plated on CAT selective agar medium by direct culture, and after enrichment of the samples. Retail poultry products were purchased from the beginning of June to the end of August from Helsinki area, and analyzed on CCDA by direct culture from peptone diluent and after enrichment in Bolton broth. The fecal samples from organic hens were collected from the end of August to the beginning of October from 20 organic farms in Finland, and analyzed on CCDA by direct culture and after enrichment in Bolton broth. Finally, 73 *C. jejuni* isolates from cattle, 62 from retail poultry (4 of which were isolated from turkey products) and 61 isolates from organic hens were further typed by PFGE using *Kpn*I restriction enzyme.

As a result, 130 (25%) of the 511 fecal samples from cattle were found to be campylobacter-positive. *C. jejuni* was identified in 64% of the positive samples. Three samples were found to contain *C. jejuni* by enrichment culture and, in addition, a hippurate-negative *Campylobacter* isolate by direct culture. A total of 72 (18%) of the poultry products (19% of chicken and 7% of turkey products) were found to be campylobacter-positive. Only 2 (3%) of the chicken isolates were identified as *C. coli* (97% of chicken and 100% of turkey isolates were identified as *C. jejuni*). Typical seasonality was observed for the poultry products with higher numbers of positive samples obtained in July and August. No similar seasonality was observed for the cattle fecal samples. *C. jejuni* was isolated from 18 (90%) of the organic hen farms.

A total of 81 different PFGE types were obtained from the 196 isolates studied. The cattle isolates resulted in 33 PFGE types, chicken 29, turkey 4 and organic hen isolates in 26 PFGE types. Only one PFGE type was found to be common for all four sources. Several isolates (one to six) from the 18 positive organic hen farms were typed, resulting in more than one (two to three) profile from 11 (61%) of these farms. *C. jejuni* isolates from cattle, obtained by direct culture and enrichment of the same sample, gave two different PFGE types in four cases out of 14 (29%).

This is the first study to report PFGE typing results for Finnish *C. jejuni* strains isolated from slaughter-aged cattle and organic hens. Long-term surveillance and typing studies are useful to resolve the epidemiology and ecology of *C. jejuni*.

P-B19

Typing of *Campylobacter jejuni* strains by using PFGE method

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Campylobacter jejuni and *C. coli* are among the commonest causes of acute bacterial enteritic diseases in humans. *Campylobacter* spp. is commonly found in the current intestinal flora of a number of domestic as well as wild animals. Regularly reported sources of *Campylobacter* spp. infections in humans include insufficiently cooked poultry and cross-contamination of cooked food or of water. Relatively frequent are findings of *C. jejuni*, *C. coli* and other *Campylobacter* spp. in animals and in the environment, as well as in human infections. *Campylobacter sp.* findings are very frequent in the environment of chickens slaughters and chicken farms. During examination of the slaughtered poultry, there are detected very different findings of *Campylobacter sp.* In some farms there was repeatedly found 80 - 100% incidence of *Campylobacter sp.*, while other farms have incidence about 30 - 40%.
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**.** Chromosomal DNA was isolated from *Campylobacter* isolates cultivated on Blood agar Base no.2. Lysis of harvested and washed bacteria (in phosphate-buffered saline – PBS) was performed in 1% agarose blocks with 1mg/ml proteinase K in ESP buffer (0,5M EDTA, 1% sarcosyl) 15 min at 54^{o}C. After washing in dest. H_2O and three washing steps in Tris-EDTA, the lysed agarose bolcks were equilibrated in restriction enzyme reaction buffer, and the consequent DNA digestion with *Sma*I was performed for 5 h at 30^{o}C. For PFGE, a Bio-Rad CHEF-DR III apparatus was used with pulses increasing from 5 to 10 sec for 4 h, from 10 to 40 sec for 14 h and from 50 to 60 sec for 4 h at 200 V and 9^{o}C.
Results and discussion. Isolates of *Campylobacter jejuni* derived from various poultry flocks were typed by the molecular-typing methods. The question of whether these isolates represented different clones or had a common clonal ancestry was addressed by PCR/RFLP and pulsed-field gel electrophoresis (PFGE) of chromosomal DNA. 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. *Afa*I, *Mbo*I and *Hae*III 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 *Sma*I. All human isolates gave different genotypes regardless of flagellin PCR subtypes. 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 *Sma*I-defined subtypes. The PFGE and PCR profiles suggest that *Campylobacter jejuni* strains originating from diverse sources were present in poultry and human samples. The results indicate that combination of at least two genotyping methods may be necessary for epidemiological studies of *C. jejuni* infection.

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P-B20

Campylobacter jejuni isolated from raw milk contaminated by *Campylobacter* mastitis

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Introduction

Campylobacter jejuni (*C*.) *and Campylobacter coli* belong to the most important foodborne pathogens of diarrhoeal infections [NOTERMANS, 1992]. Especially contaminated unpasteurized raw milk is a potential cause of diarrhoeal infections of adults and children. *Campylobacter* is most likely to arise in milk because of faecal contamination but on rare occasions outbreaks have been traced to *C.* mastitis which has caused *Campylobacter* to be directly excreted into the milk [ORR, 1995, HUTCHINSON, 1985]. Two outbreaks of campylobacteriosis in March 2002 and August 2003 both caused by contaminated unpasteurized raw milk from the same farm are reported. The isolates were identified as *C. jejuni,* in both cases the contamination of the raw milk caused by a cow with subclinically *C.* mastitis in only one quarter.

Materials und Methods

The *C*. strains from raw milk were isolated after enrichment in Preston bouillon on CCDA agar (Charcoal Cefoperazone Desoxycholate Agar) and Karmali agar. After isolation and biochemical confirmation all isolates were proved as *C. jejuni.* By using the polymerase chain reaction (PCR) the Flagellin coding gene *fla*A with the primer „pg3"- „pg50" [450 Bp, OYOFO, 1992] and *fla*A/ *fla*B with the primer „CF02", „CF03" and „CF02" [WEGMÜLLER, 1993] was examined.

Results and Discussion

In both cases the isolates of the unpasteurized raw milk were identified as *C. jejuni.* The *fla*A gene was found in all *C.*- isolates of the milk samples. Further the milk and the faeces of all cows from the farm were examined systematically. It could be clearly demonstrated that the contamination of the raw milk in both cases was caused by one cow with subclinically *C.* mastitis. At the same time the *C.* were isolated from the raw milk at retail level, the human incidence of *C.* infections in the district close to the involved farm rose significant.

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P-B21

Detection and identification of Campylobacter-like bacteria from poultry samples as *Helicobacter pullorum*

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The rRNA superfamily VI comprises three rRNA homology groups: the first group contains the genus Campylobacter and the second group comprises the genus Arcobacter. The third one is more diverse and contains the genus Helicobacter beside some others which are less well defined.
Beside *Campylobacter jejuni* and *Campylobacter coli*, *Helicobacter pullorum* is also seen as an emerging foodborne pathogen causing acute enteritis in humans. Earlier reports have shown the presence of *Helicobacter pullorum* in poultry. Consequently, poultry products are suspected as source for human infections.
The differentiation between the above mentioned organisms is difficult, particularly when employing biochemical tests as a sole criteria. In addition, biochemical testing is somewhat more laborious and time consuming.
Nine unclassified Campylobacter-like strains previously isolated from chicken faecal samples were examined using phenotypic identification methods. All strains were gram-negative, slightly curved, slender rodshaped and motile under phase contrast microscopy. They were oxidase and catalase positive, indoxylacetate-hydrolysis and hippurate-hydrolysis negative. Sensitivity to cephalothine and nalidixic-acid differed between the strains. Analytical Profile Index identification kits developed for Campylobacters (API Campy) failed to identify these bacteria, or identified them as *Helicobacter fennelliae* or *Arcobacter cryoaerophilus*.
In order to achieve some more criteria for differentiation of the pathogens the 16S rRNA of the isolates was sequenced. To extend the investigation, some reference strains of *Helicobacter pullorum* obtained from the American Type Culture Collection (ATCC) were included. The nucleotide sequences from the field isolates and the reference strains were aligned to identify conserved and variable regions of the gene. The respective sequences of *Campylobacter* spp. and *Arcobacter* spp. were included to demonstrate the differences.
Primers were constructed based on the variable parts of the gene primers to develop a PCR suitable to detect specifically *Helicobacter pullorum*. Furthermore, a multiplex PCR was set up in order to differentiate thermophilic Campylobacter, *Arcobacter butzerli* and *Helicobacter pullorum*. The efficacy of the method was demonstrated by investigating the Campylobacter-like isolates, proven to be *Helicobacter pullorum*.
In conclusion, the present investigation is useful to develop new molecular diagnostic tools for detection and differentiation of various members of the RNA superfamily VI, as demonstrated for a sensitive and specific multiplex PCR.

P-C01

Growth potential and inactivation kinetics of *Listeria monocytogenes* in spreadable raw sausage

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Spreadable raw sausages are considered to be high-risk products because their production does not include heat treatment or other antimicrobial measures and therefore, if contaminated, they possibly permit multiplication of *L. monocytogenes.*

To examine this possible multiplication a study on the behaviour and the inactivation kinetics of *L. monocytogenes* during ripening and storage of fine spreadable raw sausage ("Teewurst") was carried out. For that purpose batter was inoculated with a pool of *L. monocytogenes* at levels of 3.0 - 7.1 x 10^4 cfu per gram.
Sausages were produced in accordance with standard recipes. In parallel, batches were produced with the addition of 15 g sodium lactate per kg of sausage meat. Moreover, the influence of an elevated ripening temperature (26 °C) was evaluated. The storage temperatures were either 7 °C or 21 °C. Non-inoculated batches served as controls.
Sequential microbiological assays and determination of pH and a_w values were performed during 28 days.

The results show that *L. monocytogenes* does not multiply within 28 days in spreadable raw sausages when procedures common in practice are followed. Spreadable raw sausages ("Teewurst") must therefore defined as microbiologically stable.
Moreover, higher ripening and storage temperatures do not present a microbiological risk; in contrast, they lead to a more marked decrease of the *Listeria monocytogenes* amounts during prolonged ripening in batches with sodium lactate.

P-C02

Synthesis and activity of extracellular lipases and proteases of important spoilage microorganisms at chilling temperatures

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Extracellular bacterial and endogenous lipases and proteases are known as important causes food spoilage. The optimal efficacy of protein- and fat-hydrolysing enzymes is described between 30 – 65 °C. Temperature reductions resulted – as expected- in a lower enzyme synthesis and activity. In general, enzymatic activities are obvious at a low temperature range even if bacterial growth has already stopped. Detailed investigations (supported by DFG and EU) on the effect of heated and unheated endogenous and bacterial lipases and proteases were carried out. Further, the activity and synthesis of bacterial enzymes of 15 spoilage microorganisms were tested in the range of –2 up to 10 °C in combination with different pH-values and water activities. For detection of lipases the Merck-Reflektoquant-Test, for detection of proteases a modified agar-diffusion-assay using gelatine was used. Results confirm a slowing down of both enzyme activity and synthesis at chilling temperatures but the remaining activities even when enzymes were heat treated before should not be underestimated and explain the high spoilage potential (e.g. synthesis of *Pseudomonas* lipases at 6 °C; proteolytic activity at -2 °C). First enzyme activities could be observed after 3 days and hydrolyses obtained continuously up to the end of the investigated period of 27 days. Bacterial enzymes are much more resistant to heat compared to endogenous enzymes. Data were already partially verified and validated in food (chicken, vegetable, milk).

P-C03

Assessing *Salmonella* risk in imported pork and pork products as a part of the whole food chain evaluation

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A hierarchical Bayesian model called Import Prevalence Inference Model (IPIM) was built in order to assess the true *Salmonella* prevalence in imported pork and pork products. The work was a part of the risk assessment on *Salmonella* in pork production in Finland, and it referred to 1999.

IPIM was based on reported testing results from the main exporting countries. The true prevalence for each country was modelled by describing the uncertainty due to variability in the true prevalence between exporting countries. The parameters were estimated from the country specific data, and predictions for those countries with no data were then based on this estimated population model. It was considered sufficient for predicting the prevalence in countries without data, as long as the profile of the exporting countries remains the same as in 1999.

Because of the national *Salmonella* control programme (FSCP), Finland has permission to require additional *Salmonella* guarantees showing negative test results before importing fresh meat, with some exceptions. The *Salmonella* examinations required to prove test negative were also included as data, although the number of such additional tests was based on an estimate. The sensitivity of the microbiological test was evaluated as an expert opinion based on a few references.

Sweden, as a country with low *Salmonella* prevalence and a similar control programme as the FSCP, need not fulfil the testing procedure requirements concerning pork export to Finland. Because there were no data available on a Swedish *Salmonella* risk assessment on pork, in this assessment Swedish pork prevalence was estimated on the basis of the Finnish results.

According to the model the true prevalence in imported fresh meat varied among exporting countries from mean 0.18%, 95% probability interval [0.12%, 0.25%], to mean 5.65%, 95% probability interval [3.93%, 7.70%]. The true prevalence in imported pork products varied from mean 0.01%, 95% probability interval [0%, 0.11%], to mean 0.59%, 95% probability interval [0%, 4.0%].

P-C04

Post mortem findings in a big slaughterhouse of quail (*Coturnix coturnix japonica*)

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Among the species of game bred for human consumption, those of quail (*Coturnix coturnix japonica*) are the most appreciated in Europe and as a matter of fact they are the most demanded. In the northern province of Vicenza, there is a long tradition of breeding this kind of game, and so also of slaughtering. For this reason, in the same province, 10 years ago has been authorized one of the biggest slaughterhouse of Italy, able to slaughter more than 10 million quails/years. Therefore, the meat inspection performed into this establishment assumes an important role from the epidemiological point of view. To the slaughterhouse that we are checking, arrive many quails coming from 13 farms spread out in the provinces of Verona and Vicenza; an average quantity of 11 millions of quails are slaughtered there yearly and at present it represents one of the leader enterprises of this field in Italy. In this poster are reported and statistically evaluated the findings collected between September 2001 and December 2003 during our regular *post mortem* inspection activity in the above mentioned slaughterhouse, conforming the terms provided for by the Council Directive 91/495. As far as the classification of pathologies or causes of partial or total rejection of carcasses are concerned, we do refer to the chap. VIII and IX of the Council Directive 92/116, as the previous directive (91/495) clearly does. Dealing with animals submitted to partial gutting**,** the sanitary *post mortem* inspection has been carried out on samples, on a total number of 22.346.644 quails. 4.982.000 carcasses have been checked by direct sensorial evaluation and at least 1.200.000 of them have been completely checked, assuring in this way the inspection of 5% of the manufactured products. Totally 565.405 carcasses of quails (2.53% of the slaughtered animals) have been rejected. The most important causes of total rejection have been: animals which arrived dead or not accepted to the slaughtering (20%), sternal bursitis (22%), insufficient bleeding (14%), starvation (13%), dirtiness of the carcass (13%), diffused haematoma (12%), mechanic injuries *post mortem* (5%).

P-C05

Risk analysis and public health safety of food products: A new EU approach

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The new proposal for a European Parliament and Council regulation laying down specific rules for the organisations of official controls on products of animal origin intended for human consumption (COM(2002) 377-C5-0340-2002/0141 (COD)), in line with the opinion of the Standing Committee on the Food Chain and Animal Health, embodies the new European approach for official control system, which is based on the application of the principles of **Risk analysis**. This Regulation is also a necessary supplement to and condition for the success of Regulation n. 178/2002/EC laying down the general principles and requirement of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. The European Food Safety Authority as independent and transparent consultation body, is in charge with a scientific and technical assistance with a view to identify and evaluate new and emergent risks associated to food (**Risk assessment).** Under the new approach, the type and organization of official controls put a special emphasis on European Risk Analysis, with the formulation of science-based measures that are key elements in **Risk management.** In the new scenario the strategy for an effective food safety policy foresees four main actors: the Commission, Member States, Food Authority and Consumers involved in a communication process (**Risk communication)** where all relevant social, economic, political factors are taken in a due consideration. The official veterinary controls (audits and inspection activities) of the Regulation COD/2002/0141, according to the concept of "from stable to table ", cover the following issues: food chain information, ante-mortem inspection, post-mortem inspection, specific risk material and laboratory testing and animal welfare. To underline food safety what can reasonably be achieved in terms of elimination, reduction and prevention of any risk associated to food consumption, should be analysed in relation to risk that is risk analysis with the three components: risk assessment, risk management and risk communication. A food production chain using science-based and transparent pre-harvest and post-harvest food safety programs is much more likely to satisfy consumers food safety concerns than present meat inspection procedures and end-point sampling. The following activities are essential elements for effective food safety programs: 1) science-based inspection: frequency and intensity of official controls are dependant on an assessment of health risk represented by the type of animal and process (efficacy and efficiency of "tailored controls"); 2) up-to-date active disease surveillance and information systems with alert field veterinarians and public health officials able to detect animal and food-borne illnesses; 3) fully participating and cooperating animal and food industries with an effective information flow among all actors of the food chain. Surveillance and information systems, along with GMP and HACCP and import/export regulations all require a basic understanding of risk analysis elements: risk assessment , risk management and risk communication.

1. Proposal for a European Parliament and Council regulation laying down specific rules for the organisations of official controls on products of animal origin intended for human consumption (COM(2002) 377-C5-0340-2002/0141 (COD)
2. Regulation n. 178/2002/EC of European Parliament and Council of January 28, 2002.
3. Opinion of the Scientific Committee on Veterinary matters relating to public health on revision of meat inspection procedures. February 24, 2000. European Commission, Health&Consumer Protection Directorate

P-C06

"Risk Analysis" and "Hazard Analysis"

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Risk Analysis:

Risk Assessment > Risk Estimate
- Hazard Identification
- Hazard Characterisation
- Exposure Assessment
- Risk Characterization

Risk Management
- Laws
- Regulations
- Standards
- GHP
- HACCP > **Hazard Analysis**
- Consumer behaviour

Risk Communication

Quantitative Hazard Analysis for a definite product by Fellner / Riedl:

Partial stage a: Identification and listing of all potential hazards associated with the food under consideration and assigning the hazards according to their respective hazard category

Partial stage b:
Delete all hazards that are not targeted by the HACCP-objective

Partial stage c:
Delete all hazards that are not relevant in terms of the considered endproduct

Partial stage d:
Assort all important hazard characteristics, the possible health effects and all factors that are able to influence the respective hazard

Partial stage e:
Quantitative estimate to what degree the raw materials and ingredients of the product can be afflicted with the respective hazard

Partial stage f:
Estimation of the development of the hazard quantity during processing

Partial stage g:
Determination of a quantitative limit for the acceptable hazard quantity or for the justifiable risk

Partial stage h:
Delete all hazards that are not relevant in terms of the considered situation of the production

Result: All hazards associated with the considered food are identified, the general characteristics and specific possibilities of interference of the hazards are known and for each hazard a quantitative limit for the acceptable hazard quantity or for the justifiable risk is determined.

P-C07

Public perceptions and expectations on food safety issues

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Background In the last years the media played an important role in risk communication and the formation of public views on an issue. People have greater access to information through vehicles such as Internet and 24-hour news, for example. Drama seems to be the mainstay of media coverage. This is a style of communication, which rarely comes close to true risk communication. Media tend to highlight existing concerns, uncertainties and conflicts and present all sources on a rather equal footing. It has been suggested that media coverage is driven by rarity, novelty, commercial viability and, unfortunately, very little by risk evaluation. Often these effects, which originate from routine media coverage, can lead to what is termed by some as a "media event". That's what happened in Europe later on episodes of bovine spongiform encephalopathy or BSE, genetically modified organisms (GMOs) in the UK and dioxin-contaminated animals feedstuffs in Belgium that magnified loss of trust in public institutions. Thereupon the public is becoming increasingly aware of food nutrition and is demanding more information.
The aim of this study was to develop communications strategies that may help the public to understand the real complexities of the phenomenon and to acquire an upright knowledge on food risks and with this object in view to modify behaviours that could incise on public health. A field study in a Health Service District has examined attitudes, habits and opinions on food safety and food habits in order to develop an evaluation model on perception of risks and participated analysis of safety food themes.

Methods A representative sample (*N*= 366) quoted by age (young people, adults and old people) and residence areas (urban, hilly and flat country) filled in a questionnaire on the following issues: 1) food safety, 2) knowledge about risk's factors and its prevention, 3) information and trust in the purchases and eating habits, 4) knowledge and trust in quality and information.

Findings The multivariate analysis shows that citizens do not consider themselves absolutely informed about how facing food risks and so they delegate responsibilities to the official controls of production process and to the producers, convinced, but unfortunately wrong, that their home is the safest place where they can eat. Significant difference emerged between young people and other participants. Results are interpreted as supporting at the elaboration likelihood model of persuasion.

P-E01

The effect of logistic slaughtering and/or decontamination on the contamination of broiler chicken meat with *Campylobacter* – a model based approach

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The contamination of chicken meat with *Campylobacter* spp. is unacceptably high. Despite intensified hygiene measures in primary production, the prevalence of infection of broiler flocks in the Netherlands has not decreased in recent years and it is not likely that this situation will change in the near future. To reduce the health risk to consumers additional measures in the production chain are necessary. One possible intervention is logistic slaughter. Testing all broiler flocks and slaughtering all positively tested flocks either at the end of the day or at another location is one method to reduce cross-contamination in the slaughterhouse. Additionally, positively tested flocks can be treated with bactericidal agents. This reports presents estimates of the effectiveness of logistic slaughtering and bactericidal treatment, alone or in combination, based on mathematical modelling.

The prevention of cross-contamination in the slaughterhouse was found to depend strongly on the accuracy with which infected flocks are detected. Due to the long delay between sampling of broiler flocks at the farm and delivery of these flocks to the slaughterhouse, only one-third of all infected flocks are currently tested positive. As a consequence, the expected effect of logistic slaughter on the prevalence of *Campylobacter* spp. on chicken carcasses is limited. Furthermore, the number of campylobacters on a cross-contaminated carcass was found to be considerably lower than on a carcass from a flock that was contaminated upon arrival at the slaughterhouse. Since the probability of human infection is directly related to the number of campylobacters that consumers are exposed to, this implies that prevention of cross-contamination in the slaughterhouse will have little effect on public health. Public health risks could, however, be considerably reduced through a combination of logistic slaughter and germicidal treatment after slaughter of carcasses from positively tested flocks. This combined strategy is only effective when a highly sensitive test is used with minimum delay between sampling and processing. Germicidal treatment of all flocks circumvents the need for testing, thereby reducing costs and complexity. However, product quality may be negatively influenced and the treatment itself incurs costs. Optimising the combination of logistic slaughter and germicidal treatment will be the subject of further studies.

Data to calculate the model results were only partly available. Therefore, a combination of observational data and expert judgement was used. Despite the associated uncertainty, the above mentioned conclusions can be considered as very robust.

P-E02

Effect of organic acid addition on the shelf life and the microbiological quality of Moroccan Merguez sausages

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Merguez is a raw sausage made from beef and seasoned with condiments. It is widely popular meat product in North Africa where it is consumed grilled or fried in sandwiches, or used as an ingredient in traditional dishes. The shelf life of the product is short and does not exceed two days at refrigeration temperature. In the present study, the effect of organic acid addition on the shelf life and on the hygienic quality of Merguez sausages was studied. A commercial organic acid; Acetolac^{TM} consisting of a mixture of sodium lactate (90%) and sodium acetate (10%) was added at different levels (0, 5, 10, 15 or 20 g/kg) to the butter mixture during sausage making. Microbiological counts (total aerobic count, coliforms, fecal streptococci, and *Staphylococcus aureus*) and chemical parameters (pH, total volatile basic nitrogen; TVB) were monitored during storage at approximately 8°C for 15 days. The results showed that addition of the organic acids reduced significantly the microbiological counts during the first three days of storage and such effect increased with the concentration of the organic acids. Thereafter, a steady increase in the colony forming units of all the microbial groups studied was recorded with the exception of *S. aureus* counts that remained practically constant with a concomitant pH and TBV increase. As for the shelf life, control samples (without added Acetolac^{TM}) spoiled at day 5 of storage as judged by growth of molds and surface mucoidness Such perceivable alteration was delayed at least 5 days in samples with added organic acids. Addition of Acetolac at levels ranging between 10 and 20 g/kg of butter mixture appears to be optimal to enhance the hygienic quality Merguez sausages and extend its shelf.

P-E03

Shelf-life of equine meat stored in modified atmosphere with low concentrations of carbon monoxide

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In Italy, as also in France, equine meat is widely appreciated by consumers, even if the *per capita* consumption of this kind of meat (2,2 kg/year in average) is largely lower than that of pork meat (25 kg/year) and of bovine meat (22 kg/year). Horse meat is mostly consumed as fresh cuts and only a few part is used for fresh and seasoned meat products (salami and cooked ham). In the last 20 years, the quality of horse meat has greatly increased; actually, in Italy mainly young horses are slaughtered (< 2 years old) another part of meat is imported from USA or South America as vacuum packaged pieces which are then sliced or ground for retail markets. One of the most important needs for meat factories is to guarantee a good shelf-life to sliced or ground fresh horse meat, but from this point of view the equine meat is amazing more sensible to the oxidation of myoglobin as the bovine one, causing a rapid darkening of the product. The packaging in modified atmosphere (MAP) is a reliable tool to preserve the freshness of horse meats, but the results obtained till now with atmospheres at high oxygen concentrations (> 80% O_2) or with quite 100% CO_2 are not completely satisfying particularly for the maintenance of a brilliant red colour.
Knowing that the carbon monoxide has a very good stabilization effect on the myoglobin, we have performed a storage test to evaluate the effectiveness of low percentages (0,5% and 1,0%), of carbon monoxide in fresh horse meat stored in MAP with CO_2 and N_2. 100 samples of fresh sliced horse meat were prepared from a uniform batch of meat cuts, and stored at <4°C, 50 with a 0,5% of CO and 50 with 1,0% CO, till the 7th day of storage. The samples were then submitted to microbiological and sensory analyses at 1st, 3rd and 7th day of storage. From the microbiological point of view, total viable count (TVC), total and faecal coliforms and lactic acid bacteria were determined. With a specific test panel the colour, the aroma and the taste of the meat were evaluated. From the results obtained, it can be concluded that the packaging in MAP of fresh horse meat with low concentrations of carbon monoxide can stabilize the colour of meat sliced in a very good brilliant red. The CO has instead no effect on the microbial flora of meat, therefore the use of low concentrations of carbon monoxide could be a very good mean to make more stable the colour of fresh equine meat sold in MAP, with good performances from the sensorial point of view.

P-E04

Prevention of the development of *Bacillus cereus* on germinated wheat grain as an ingredient of special bread

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On their search for new special bread with high nutritional physiology, the bread industry has developed formulas for dough containing germinated wheat grain. The increased amount of vitamins, essential amino acids and other compounds, still present after the baking process, promote the nutritional value of these special breads.
From nature wheat grain is contaminated with a wide variety of microorganisms, including spore forming bacteria as *Bacillus cereus* (*B. cereus*). Bakery products have been implicated in several outbreaks of food-borne illnesses. The two toxins produced by *B. cereus* can cause either diarrhea (diarrhea toxin), or vomiting (emetic toxin). The emetic toxin, also called Cereulide, is mostly found in cereal-based foods like bread-products. Toxin production is associated with increased levels ($\geq 10^6$ cfu/g) of *Bacillus cereus*. Because of its heat-stability the emetic toxin cannot be inactivated through normal thermal treatment. Previous examinations showed that during the germination process of the wheat grain, lasting 48 h at 27°C, there can be a four to five fold increase of *B. cereus* (vegetative cells and spores) within 24 hours, from contamination levels below detection limit (10^2 cfu/g) up to 10^6 cfu/g. With the growth of *B. cereus* at these favorable conditions there is a high risk of toxin production. The acceptable maximum value (10^5 cfu/g, Swiss Guidelines: Hygieneverordnung, HyV, 2002) is passed over.
The objective of this work was to elaborate possible methods to reduce the health risk of germinated wheat grain by *Bacillus cereus* and their toxins. Examinations showed that there was no acceptable treatment of the grains preceeding germination: Despite previous treatment (peeling, irridation) there was only a small delay in the development of *B. cereus* reaching concentrations of 10^6 to 10^7 cfu/g at the end of the germination phase. On the other hand the inactivation of *B. cereus* following germination process through chemical (lactic acid, acetic acid) or physical (irridation) treatment showed either insufficient results or uncertainty about inactivation of possibly present toxin.
It is well known that bacterial spore germination can be inhibited by lowering the pH to 4.5 or below. Examinations showed that it was possible to retard or even suppress the growth of *Bacillus cereus* by adding acids (lactic acid or acetic acid) during the germination process of the wheat grain. The challenge consisted of suppressing the development of *B. cereus* without retarding or affecting the grain germination. With high concentrations of acids (0.1% acetic acid) suppression of *B. cereus* could be achieved, but the grain didn't germinate. Low concentrations of acetic acid (0.01%) didn't show any effect on *B. cereus*, but wheat germination was similar to the standard. Best results were achieved when adding 0.05% acetic acid each time water was supplemented during the germination process. Concentrations of *B. cereus* were within the range of the detection limit, while germination of the wheat grain was hardly altered showing a shorter germ bud. Microbial flora was dominated by the presence of yeasts and lactic acid bacteria, which also resulted in faster fermentation process when adding the germinated grain to bread dough. Sensorically seen, bread containing treated germinated grain could be differentiated with high significance from bread with untreated grain, showing preference for the first one.

P-E05

Use of medium-chain-fatty-acids (MCFA's) against foodborne infections

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Foodborne infections are caused by a.o. *Salmonella, Campylobacter*, *Listeria. Campylobacter* is actually among world's most common human enteropathogens, causing campylobacteriosis. Therefore, campylobacteriosis is now the major zoonotic cause of human inflammatory intestinal infection, followed by salmonellosis and listeriosis.

The problem with increasing or continued high incidence of human foodborne infections cannot only be solved on the basis of present knowledge. Maintaining increased hygiene standards have had an impact on salmonellosis but not on campylobacteriosis. Existing knowledge will certainly not be able to solve these problems, since it is obvious there is lack of understanding the complex mechanisms how the zoonotic bacteria invade and infect.
Outbreaks of campylobacteriosis are frequently traced to contaminated milk or water, whereas the most common cause of sporadic cases is eating undercooked meat, e.g. poultry. Contaminated chickens are, by far, the principal vehicles of infection. Also salmonellosis is mainly related to poultry consumption.

Current methods of hygiene and biosecurity used are improvement of the biosecurity in the hatchery, a competitive exclusion technology or using chlorinated water. But they are insufficient to control or eliminate food-born infections from the poultry food chain. Another strategy concerns preventive dosing of antibiotics (growth promoters) to the animals. However, concerns over potential health risk of antibiotic residues and resistant strains of pathogenic bacteria from animal sources have increased over the years and there are increasing pressures on the regulatory bodies to ban the use of these growth promoters. Therefore, a total ban of antibiotics is foreseen for end 2005. Finally, another alternative approach for control of food-born pathogens can be active immunization of the birds. However, at the moment there is limited information about the function of the chicken immune system. Although some international research institutes are dealing with this topic, a real break-trough of this technique is for far future.

Therefore, new alternatives for the control of foodborne pathogens – *in casu Salmonella* and *Campylobacter* sp. – are urgently needed. In this context, specific medium-chain-fatty-acids (MCFA's) are a valuable alternative, since they can be used as novel and innovative agents against foodborne infection, in order to control a.o. *Salmonella* and *Campylobacter* contamination and growth. Supplying specific MCFA's, their salts or derivatives thereof or mixtures thereof to *Salmonella* or *Campylobacter*, inhibit their further growth. Growth of both strains is surprisingly inhibited and the respective micro-organisms are killed by the MCFA's. The fatty acids that can be used to control these types of food-born pathogens include both fatty acids with an even and an odd number of carbon atoms, for example C_6 (caproic acid, hexanoic acid), C_7 (heptanoic acid), C_8 (caprylic acid, octanoic acid), C_9 (pelargonic acid, nonanoic acid) and C_{10} (capric acid, decanoic acid). Applicable doses MCFA's are low and varies between 1250 ppm and 50 ppm depending the type of food-born pathogen. The MCFA's can a.o. be supplied to the animal via its feed or drinking water, resulting in reduced foodborne zoonosis from animal to people.

P-E06

Thermal inactivation of *Salmonella* spp. and other microorganisms during chocolate making

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Enteritic salmonellosis and campylobacteriosis in humans are mainly caused by the consumption of contaminated food and are the most common “food poisoning” in developed countries. Especially meat and eggs are the source of these microorganisms and might be infectious if food is consumed raw or undercooked. Although chocolate is a microbial stable product, chocolate has been described as a vehicle for *Salmonella* spp. (*S.* spp.). Because of the low water-activity and high fat content of chocolate *S.* spp. shows an increased heat resistance against the process of chocolate-making. In addition it was found, that in the case of consuming contaminated chocolate, the infectious dose is rather low although usually a high infectious dose (>10^6 cells/g) is necessary to cause diarrhea.
Some studies carried out that *S.* spp. survive long term storage of over 12. Additional studies examined the phenomena of increased heat resistance of *S.* spp. due to low water but less was investigated if *S.* spp. survived during conching of chocolate making, which is attended to high temperature and long duration. The aim of the presented study was to evaluate the thermal inactivation of *S.* spp. and other microorganisms like *Escherichia coli* during conching (final refinement of chocolate) in different masses of chocolate at different temperatures (50°C, 60°C, 70°C, 80°C, 90°C). After thermal inactivation survivor cells of *S.* spp. should be estimated with a suitable quantitative method. Evaluation of different quantitative methods for the detection of *S.* spp in chocolate yielded satisfactory results for the MPN-method. This method was then used for experiments showing thermal inactivation of *S.* spp.. Results of thermal inactivation showed D-values from $D_{50°C}$= 245 min to $D_{60°C}$= 306 min in cocoa butter and $D_{50°C}$= 999 min to $D_{90°C}$= 26 min in cocoa liquor. At 90°C the D-values for cocoa liquor and dark chocolate were rather similar. The highest D-value was found for dark chocolate: $D_{50°C}$= 1570 min. z-values were found to be z= 20°C in cocoa mass and z= 14°C in dark chocolate. Those are higher than the z-values for spores (z= 10°C). *Escherichia coli* could already be inactivated at temperatures below 70°C in milk chocolate.
This study shows that the conching process does not warrant the inactivation of *Salmonella* spp. in different chocolate masses. This is because of the high fat content and low water-activity of chocolate as described before, but might be probably due to the added emulsifier. To ensure that products made out of chocolate are free from *S.* spp. from the beginning of the chocolate-making process, it is necessary to investigate a HACCP-concept. At the beginning of chocolate making it would be possible to sterilize cocoa beans at an SLSA-plant (debacterisation plant by Buehler AG, Switzerland), representing a CCP.

P-E07

Survival of *Escherichia coli* O 157 in hamburgers during microwave heating

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The object of the study were three *E. coli* strains: *O 157 - B 032, LMG 8223, IVK 805,* isolated in Europe from humans suffering from food poisoning. Inoculum was prepared each time from the above strains (18-24 h culturing in a TSB – Tryptone Soya Broth), and used for infecting breaded and unbreaded beef hamburgers. The initial bacterial count was 10^6 cfu/g of the product. The hamburgers were placed in a microwave oven (Dialog cook, Moulinex) and heated at 480 and 760 Watt for 0.5, 1.0, 1.5, 2.0 and 2.5 minutes. Then they were homogenized in a stomacher for one minute and their samples were cultured onto an agar medium Eosin Methylene Blue Agar (Modified) – mEMB. After 48-hour incubation at 37^{o}C the number of *E. coli O 157* colonies was counted on two plates, and the average values per gram of the product were determined. Two series of experiments were performed with each bacterial strain, applying all planned parameters. The same procedure was followed in the case of uncontaminated, control hamburgers.

It was found that inactivation of cells of *E coli* O157 strains depended on bacterial strain, type of hamburgers, power level (wattage) of a microwave oven, and heating time. In both breaded and unbreaded hamburgers none of the strains analyzed survived 1-minute microwave heating, regardless of the power level of a microwave oven. A different situation was observed during half-minute heating, which reduced the bacterial count in breaded hamburgers by one log cycle - B 032 strain and by two log cycles – LMG 8223 and IVK 805 strains. In unbreaded hamburgers the degree of bacterial count reduction was lower, by 0 and 1 log cycle respectively.

P-E08

Pulsed electric field application for inactivation of *L. monocytogenes* in raw milk

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Preservation of liquid food by high intensity pulsed electric fields (PEF) is an interesting, mild alternative to traditional techniques like thermal pasteurization, but high costs of operation inhibited an industrial application so far. This work is focussed on improving treatment efficiency by a combination of PEF and synergetic effects by mild heat to enhance PEF lethality and ensure product safety while maintaining product quality.
The exposure of biological cells to an external electrical field induces an electroporation of phospholipid bilayers and pore formation, leading to cell death. The inactivation of *Listeria monocytogenes* in raw milk was investigated with an electrical field strength of 23 kV/cm dependent on specific energy input and initial treatment temperature. Raw milk was obtained by the German Federal Institute for Risk Assessment (BfR) with a natural fat content and as skim milk and treated after homogenization. The presence of fat globules might have, due to electric field perturbation a protective effect on the microbes.
Based on the underlying mechanism of action and making use of highly synergetic effects of mild heat in the range of 45 - 55°C a gentle processing concept for pasteurization of raw milk by PEF was developed. The need to preheat the media before treatment provides a possibility to recover the dissipated electrical energy, leading to a drastic reduction in costs of operation.

P-E09

Microbiological and organoleptic investigations of organic and conventional meat and meat products

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The target of this study was to examine the quality of organic meat and meat products in comparison with conventional products. A total of 400 pork samples, 200 each organic and conventional products, 124 organic and 124 conventional fermented sausages were analysed microbiologically (total aerobic plate count, *Enterobacteriaceae*-count, lactic acid bacteria-count, count of coagulase-positive *Staphylococcus*, presence of *Salmonella, Listeria monocytogenes, Campylobacter spp., Yersinia enterocolitica*). Further 85 organic meat products (18 fermented sausages, 25 scalding sausages, 20 boiling sausages, 3 cured meat products, 19 preserves) were tested microbiologically, chemically (fat, water, muscle protein, sodium nitrite) and organolepticly.

All pork samples and fermented sausages were free of *Campylobacter spp*., *Salmonella* and *Yersinia enterocolitica*. *Listeria monocytogenes* was found in organic and conventional pork samples and in fermented sausages (<10^2 CFU/g). In 20 % of the organic and in 13 % of the conventional pork samples a total aerobic plate count of >10^7 CFU/g was observed. In 10 % of the organic but only in 0.5 % of the conventional pork samples the *Enterobacteriaceae*-count was very high (>10^5 CFU/g).

In the fermented sausages the following results were determinated: organic products - lactic acid bacteria-count 10^6 to >10^9 CFU/g, *Enterobacteriaceae*-count <10^2 to 10^5 CFU/g, presence of coagulase-positive *Staphylococcus* in 22 samples; conventional products - lactic acid bacteria-count 10^5 to 10^9 CFU/g, *Enterobacteriaceae*-count <10^2 to 10^3 CFU/g, presence of coagulase-positive *Staphylococcus* in 2 samples (<1,5x 10^3).

In the organic scalding sausages a high *Enterobacteriaceae*-count and lactic acid bacteria-count (one sample) and a high total aerobic plate count (five samples) were detected.

In the organic boiling sausages, preserves and cured meat products the microbiological status was normal. Food borne pathogens were not detected. Protein-values were in agreement with the minimum values according to the „Leitsätze zum Deutschen Lebensmittelbuch“ (2003). The organoleptic results show most deficiencies in smell and taste (65.9 %).

In conclusion the prevalence of food borne pathogens in organic meat and meat products seems not to be higher than in conventional products. In the organic meat and meat products problems concerning the normal microflora were observed more often. Most of the problems in organic pork and meat products are caused by technological and hygienic deficits of the production process.

P-F01

Microbiological quality of selected food products in the Mazovia Voivodship (Poland)

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Food products routinely collected by the Voivodship Sanitary-Epidemiological Station in Warsaw from the market chain of the Mazovia Voidvodship as well as the products tested by the independent commercial laboratory upon the customers` request (most often within their internal quality systems, e.g.: HACCP) were investigated in this studies.
The following selected groups of products were examined in the period of 2000 – 2003year:

1. meat and meat products
2. dairy products (except of butter)
3. confectionery products
4. ready to eat products
5.

Microbiological tests of the above-mentioned groups of products covered full range of analyses as defined by Polish regulations.
The results of the studies have been limited solely to the presence of pathogen bacteria, i.e. *Staphylococcus aureus, Salmonella sp.*, and others (*Escherichia coli, Listeria monocytogenes, Bacillus cereus*), less frequently detected in the analysed products.
In the period 2000 – 2003 the rate of the questioned samples of meat and meat products due to the presence or excessive number of pathogens (according to the limits defined by the Polish regulations) averaged 3.8%., with the most commonly encountered pathogen being *S. aureus*.
Within the analysed groups of products dairy products (excluding butter) exhibited the lowest level of questioned samples (0.2%) because of the presence of *S. aureus* and *L. monocytogenes.*
The four- year studies showed that the confectionery products have had the highest average number of the samples questioned for the some extent due to the presence of *S. aureus* and *Salmonella sp.* and less often due to the presence of *B. cereus*.
Ready to eat products were tested in the highest amount., among the questioned samples the presence of *S. aureus* averaged 1.0%.
Analysis of the results obtained during the four-year period indicated the positive downward trend in the amount of the questioned samples from 4.0% in 2000 to 0.6% and 0.9% in 2002 and 2003, respectively.
Furthermore, *S. aureus* appeared to be the most often detectable pathogen in the analysed food samples.

P-F02

Observations regarding the presence of somatic cells in milk within the county of Salaj

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The significance and importance of SCC (Somatic Cell Count) in milk are generally accepted but as far as their number and especially their accepted limit go for the physiologic milk, a controversy is created. Consequently, next to the implications concerning human health we find legal implications, as the accepted limit differs between countries (max.400000SCC/ml-EU, max.750000SCC/ml-USA). In healthy cows, SCC is 100000/ml but a SCC of 200000/ml shows that a small number of the cows in the group get udder infections. The growth of the SCC is directly related to the milk production (at a SCC of 400000/ml, the loss reaches approx. 1360gr/cow/day). Also, the specific milk consumption for obtaining 1kg of cheese products is greater the higher the SCC is, and the quality of the cheese and its term of preservation are dramatically affected by a high SCC. As a result, several detection methods for SCC, and with it, for the state of health of the udder were perfected.
In the framework of the performed study, raw milk samples were tested from cows collected from a certain area of the Salaj county. The tests were conducted with a Somacount device, manufactured by Bentley-USA. The device operates on the following principle: the somatic cell DNA is marked with ethidium bromide, thus becoming fluorescent, and then they are passed through a LASER beam where they cast a luminescent emission. This is then conveyed by a system of mirrors and processed into electric pulses, which represent the SCC. The results are displayed and they can be printed. All the samples with a SCC higher than allowed were both bacteriologically tested and through CMT. From the samples with a unacceptable SCC, pathogen bacteria (*Staphylococcus, Streptococcus, E. coli*) were isolated and antibiograms were performed.
As illustrated in the enclosed chart, from the total of 243 tested samples, 85 samples (34.98%) contained under 100000SCC, 87 samples (35.80%) contained between 101000-400000SCC and a number of 71 samples (29.51%) were over the 400000SCC EU limit. The fact that a number of 25 samples (10.29%) were over 1000000SCC is to be noted. According to the calculations, the average for the SCC was of 437877/ml. The rate of CMT positive samples from those with a unacceptable SCC was extremely low, statistically insignificant (8.4%).
Conclusions:
- The presence and the SCC in milk represents an extremely important matter under the incidence of public health, animal health as well as legally.
- From the 243 milk samples, a number of 85 samples (34.96%) contained under 100000 SCC/ml and 87 samples (35.80%) contained between 101000-400000 SCC/ml. So 70.78% of the samples were accordingly to the EU legislation, the rest of 29.22% surpassing the allowed limit.
- The average of the SCC was of 437877/ml, gently higher than the data in the literature (412000SCC/ml-Lombardia, 316000/ml-SUA), but the heterogeneity of the cow groups, the differences in the system of maintenance, milking, feeding and hygiene within the given area do not allow an objective comparison.
- The correlation between the results of the exam for SCC and the results of the CMT is not significant, being statistically irrelevant.

Ask authors for bibliography

P-F03

Study of microbiological flora of "sweet rice" served in buffets

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The distribution of the meals in system of free service ("buffet") it is a usual procedure in services of distribution of meals.
However, this system of distribution of meals can present some risk factors for the safety of the consumers, such as: Foods or culinary preparations potentially dangerous, Inadequate exposition temperatures, High time of exposition, cross contaminations, effective protection of the meals relatively to the external contaminations, equipments of distribution inadequate and/or without control of temperature.
So, it's important, to study the behaviour of some foods, when placed for distribution in systems of "buffets." The risks associated to the dangers biological resultants of this type of alimentary distribution, represent a serious problem and a hazard for the consumers' health.
The intention, of this work, is to obtain information, with an experimental study in laboratory, of the microbiologic behaviour of a representative food of Portuguese traditional dessert, as it is the case of the "Sweet Rice". The choice of this alimentary product is justified, for the fact of being practically constant its presence in "buffets", to be of great acceptance for the consumers and representing a product of international projection, as image of the Portuguese traditional dessert. However, this dessert may be considered potentially dangerous, because the ingredients that compose it (cook rice, pasteurised milk, raw eggs and decorated with cinnamon powder).
Methods: Several samples selected in production places and own distribution were analysed (hotels and restaurants), as well as a producer and distributor of these dessert to mass catering and schools.
These samples were analysed in 4 different stages: after the production, after 8 hours to exposition temperatures in buffets, after 48 hours and 96 hours in cold storage.
In the Laboratory we have tested the samples regarding *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium s*ulphite reducers, *Enterococcus*, total coliforms, faecal coliforms and total colony counts.
Results and conclusions: The results that we have, to the moment, they are quite stimulating and although presenting significant percentage in some samples of the presence of spores of *Clostridium s*ulphite reducers (14.3%), this dessert type doesn't demonstrate to present risks for the consumers' health, butt we intend to evaluated the danger in conditions of exposition to favourable temperatures for the development of micro organisms that cause food poisoning.

P-F04

Cuisine “chefs” and autocontrole knowledge

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The implementation of systems that may increase the food safety in catering levels are of extreme importance, since this sector is a crucial link of the farm to fork chain. Nevertheless, these systems are frequently settled without an adequate diffusion of the enterprise’s Politics of Quality and Food Safety to the responsible staff of the production sectors.
With the purpose of evaluating the grade of familiarization, knowledge and satisfaction with the food safety implemented systems a survey was conducted with 25 questions.
We noticed that in most part of the units we got a high number of positive answers in: plague control (95%); cleaning and disinfections programmes (98%); GMP (90%); knowledge concerning high risk food (95%); temperature records (98%); trust level conveyed by the system (95%).
On the other hand, having in consideration the number of answers, we realized that several aspects need to be improved; certified suppliers, training planning, absence of training inside of the enterprise, lay out design and cross contaminations, knowledge and participation in the HACCP system and record of remedial measures.

Food safety and disposable gloves in food service workers

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As retail sale is the most common way that food is distributed to the consumer, hygiene and food safety in retail is critical to consumer health protection. When unpackaged meat products, fish and cheese are sold, personal hygiene is an important issue. Hand hygiene, central in the medical sector, is also crucial in retail. Beside foodborne pathogens, indictor bacteria and spoiling organisms play an important role in potential contamination. The use of disposable gloves at deli counters, selling open foodstuffs, is one of the most common and recommended procedures. The effectiveness of glove-use is questionable, however, not only from a hygienic standpoint, but also from the standpoint of occupational safety.
We conducted a cross-sectional study to assess the hygienic conditions in stores and supermarkets and to measure the bacterial contamination with pathogens and indicator bacteria of both bare hands and gloved hands during the sale of food. Technical personnel of the Association of Retail Trade Employers (Berufsgenossenschaft Einzelhandel; BGE) tested supermarkets and retail stores during their routine controls. Standardized questionnaires for stores and personnel as well as RODAC contact plates (RCP) for microbiological examination were used to obtain data. The questionnaires included personal data concerning professional education in the sector of retail trade, work experience, use of disposable gloves, frequency of glove-changing, skin problems on hands as well as skin and allergic diseases. The data obtained from the stores included questions about the type of store/market, food stuff sold, “disposable glove-use-policy”, the HACCP concept and “skin-protection-plans”. Data from 68 supermarkets and retail stores were obtained and analyzed. 260 questionnaires were filled out (192 personnel, 68 stores) and 410 RCP were examined. Results of RCP, comparing bare hands and gloves during work showed no statistical significant difference in the number of CFU, *E. coli*, fecal streptococci and enterobacteriacea per dm^2. 97.8% of bare hands, 96.4% of the gloved hands and 45% of unused gloves out of the dispenser box showed more than 400 CFU /dm^2. Enterobacteriaceae, fecal streptococci and *E. coli* were found on bare hands versus gloved hands in 54.7 versus 54.5, 38 versus 41.8 and 2.2 versus 0 percent. One third of the employees (33.3%) always used gloves, one third used them occasionally (35.4%) and one third never (31.3%). Nearly half of the employees(46.5%) only changed the gloves after more than 5 customer-contacts, a further 21.9% after 3-4 customers, 28.1% after 2-4 customers and only 3.5% after each customer. Vinyl (35.8%,) powdered Latex, non-powdered Latex (22,6%), Nitril (17%) and Polyetyhlen (5.7%) gloves were used. 57 of 65 stores had a HACCP concept (87.7%), but only 46.2 % covered hand safety with this plans. 21.4 % of the employees reported “skin problems on hands”. People who always used gloves reported significantly more frequent skin problems on hands (OR 3.27 CI 1.54-6.96, p=0.01) than persons not using gloves.
Disposable gloves can be criticized by several reasons: they represent a financial cost, they cause difficulties in the handling of foodstuffs, and most importantly they have a detrimental effect on the health of the employees. The results presented here show that the current use of gloves in food retail trade has no hygienic advantage over bare hands. We conclude, therefore, that only select use of gloves, rather than general use, should be recommended.

P-F06

Sporicidal effectivity of disinfectants for food processing areas - in vitro tests and experiments under practice-oriented conditions

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Routine epidemiological examinations of gastrointestinal symptomatologies frequently occurring in the Bundeswehr (German military service) provide valuable data on the prevalence of diseases caused by *Bacillus cereus.* In the course of microbiological hygiene status inspections conducted in food service facilities of the Bundeswehr, *Bacillus cereus (B. cereus)* is often isolated from surfaces and utensils. In these facilities, amphoteric detergents are generally used for disinfection. In vitro examinations as well as test procedures conducted under practice-oriented conditions (germ carrier tests) to demonstrate the sporicidal effectiveness were therefore aimed at providing a comparison between the normally used product (Tego 2000®) and a peracetic acid-based disinfectant (Wofasteril® E400 / alcapur®). Tego 2000® did not have any sporicidal effect on a *B. cereus* test strain, whereas Wofasteril® E400 / alcapur® showed a clear sporicidal effect both during the suspension test and the germ carrier test. Within the scope of efforts to prevent diseases caused by *B. cereus* in mass catering, these results offer an excellent approach to minimize risks.

P-F07

Field trials to evaluate the sporicidal effectivity of amphoteric and peracetic acid-based disinfectants for food processing areas in mass catering

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Commercial disinfectants used in food processing areas have not been evaluated for their sporicidal effect. Since there are no standards for such evaluations, the preparation normally used in the Bundeswehr (German military service), Tego 2000® (amphoteric detergent), and a peracetic acid-based disinfection procedure (Wofasteril® E400 / alcapur®) were examined in a comparative test which was carried out in three stages. In the suspension and germ carrier tests, Tego 2000® did not have any sporicidal effect on a *B. cereus* test strain. However, a marked sporicidal activity could be determined for Wofasteril® E400 / alcapur®. To evaluate these results, an experiment was conducted under practice conditions as a field trial in seven food service facilities. This experiment revealed that the effect of the disinfectants varies significantly. Taking the actual original prevalence of *B. cereus* into account, the sporicidal effect of Wofasteril® E400 / alcapur® was 6.25 times higher than that of Tego 2000®. The suspension and germ carrier tests developed to determine the sporicidal effect of disinfectants thus proved to be easily transferable to practice conditions. Such tests should be integrated into standards to evaluate the sporicidal activity of disinfectants used in food processing areas. The establishment of such standards is being vigorously demanded.

P-G01

Authentication of fish species by isoelectric focusing and two-dimensional electrophoresis: application to freshwater fish commercially labelled “perch”

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According the United Nation’s FAO the average consumption of fish per inhabitant is rapidly increasing, and predicted to multiply 7 fold in the next decade. The growing consumer demand together with the opening of new international markets have boosted the global trade of fisheries products and the variety of fish species that regularly arrive on the worldwide markets. The seafood safety will be therefore a major challenge for veterinary inspectors. A serious risk for human health is the mislabelling of seafood products. For example it is indispensable to unambiguously identify species that may contain toxins or can cause allergic reactions, or during an official block of the import of a particular species due to sanitary reasons. In addition, difficulties in identification can encourage fraudulent practices such as replacing species with different commercial values. Morphological criteria are commonly used for seafood identification, but in doubtful cases or when the fish are sold without clear morphology (i.e. filleted) biochemical analyses are fundamental to ensure accuracy (1-2).

In the present paper isoelectric focusing (IEF) and two-dimensional electrophoresis (2-DE) were applied to identify four fish species commercially sold under the generic label of “perch”, which arrive on the fish markets already filleted in view of their numerous bones. The four species are: 1) the European perch (*Perca fluviatilis*); 2) the Nile perch (*Lates niloticus*); 3) the European pikeperch (*Stizostedion lucioperca*); 4) the Sunshine bass (*Morone chrysops x saxatilis*). These fish have significantly different commercial values, the European perch being the most appreciated by consumers and commanding the highest prices. Hygienical and sanitary problems arise from the treatment of Nile perch, which is filleted in the country of origin (Egypt and Central Africa countries) and then exported to Europe. Many cases of sanitary blocks of import of products coming from Africa, occurred in recent years.

Each species showed characteristic IEF pattern and 2-DE map, with differences making for easy discrimination. The IEF patterns of the water-soluble proteins extracted from the white muscle of the four species were species-specific, and, when analysed with suitable software and compared with standard patterns archived in a data-base, permitted a quick and correct identification. Interestingly, none of the IEF bands was common to the four species. The 2-DE maps showed numerous spots, distributed mainly in the acidic part of the IPG pH gradient. Numerous spots are species-specific, although some were similar in the four species. These similarities allowed to assign some spots to specific proteins, an thus allowed a first insight into the proteome of these poorly characterized species.

In conclusion, the simple and quick native IEF analysis of the water-soluble proteins extracted from the white muscle serves to distinguish the four species. Thus, the method results a suitable tool for the authority whose task is to guarantee quality control in order to reduce or even avoid mislabelling and frauds. The more sophisticated, expensive and time-consuming 2-DE, may have major application to deepen the knowledge of fish proteome. Moreover, the proteomic approach has been recently shown to be suitable to identify makers of health status of an organism, contamination levels and post-mortem changes (2).

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P-G02

Bacteriological monitoring of carcass contamination according to 2001/471/EC: A baseline proposal for the non-destructive technique

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The EC Decision 2001/471/EC sets out the requirement to achieve and ensure standardized EU-wide microbial performance standards for monitoring of slaughtering hygiene (Anonymous, 2001). Swiss abattoirs with EU approval must also satisfy the EU legislation. For microbiological monitoring of carcasses, this decision defines baselines for three categories (acceptable, critical and unacceptable) into which the results obtained (average daily $\log_{10}$ value) must be classified when destructive sampling techniques are applied. Such baselines have so far not been specified for the nondestructive wet-dry double-swab technique also permitted in the EC Decision. However, this decision requires their evaluation when the wet-dry double-swab technique is applied. For reasons of practicability, abattoirs often prefer nondestructive techniques. So there is a great need for applicable baselines.

The aim of this study was (i) to set up characteristic time trend graphs of daily average $\log_{10}$ values for different abattoirs and slaughtered animal species (cattle, pig); (ii) to calculate “warning” and “action” boundaries for abattoir-specific quality control charts to assess successive results by statistical process control methods; and (iii) on the basis of the data from all examined abattoirs, arranged by animal species, to suggest possible baselines for the wet-dry double-swab technique applicable to all abattoirs in order to classify the results into the required categories. Therefore, 800 cattle carcasses and 650 pig carcasses were examined by wet-dry double-swab technique according to the directives of the EU Decision in the year 2003 at four (pig carcasses) and five (cattle carcasses) EU-approved abattoirs in Switzerland.

This study showed that: (i) the wet-dry double-swab technique is suitable for the long-term monitoring of carcasses and allows to determine impressive differences between the examined abattoirs; and (ii) data obtained from carcasses by nondestructive sampling techniques allow to determine possible baselines for classifying the results of microbiological examinations (daily average $\log_{10}$ values; total microbial count, *Enterobacteriaceae*) into the categories of “acceptable”, “critical” and “unacceptable” when slaughtering cattle and pigs. However, such values must always be seen merely as baselines in the context of standardized hygiene monitoring. It is of critical importance for abattoir to implement a monitoring system based on abattoir-specific data of the kind permitted by quality control charts, for instance.

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