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“Public health relevance of foodborne outbreak investigation in Austria with special emphasis on *Listeria monocytogenes*”

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LIST OF ABBREVIATIONS

AGES	Austrian Agency for Health and Food Safety
CI	confidence interval
DNA	deoxyribonucleic acid
DT	definitive type
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EFSA	European Food Safety Authority
EMS	epidemiological reporting system
EU	European Union
EWRS	Early warning and response system
FBO	Foodborne outbreak
FMH	Federal Ministry of Health
FWD	Food- and waterborne diseases
HACCP	Hazard Analysis and Critical Control Points
HUS	Haemolytic-uraemic syndrome
L.	*Listeria*
LMSVG	Austrian Food Safety and Consumer Protection Law
MS	Member States of the European Union
PFGE	pulse-field gel electrophoresis
PT	phage type
S.	*Salmonella*
sv	serovar
TESSy	The European Surveillance System
VTEC	verotoxigenic *Escherichia coli*
WHO	World Health Organization

INTRODUCTION

Foodborne diseases and outbreaks are crucial contributors to morbidity and mortality worldwide [FLINT et al., 2005]. Many different factors may lead to foodborne infections like contaminated food, poor personal hygiene, inadequate cooking, improper holding times and temperatures, and cross contamination [U. S. FOOD AND DRUG ADMINISTRATION, 2009].

For the prevention of foodborne infections it is of utmost importance to know the pathogens, their reservoirs and possible infection ways (i.e. the incriminated food item that brings the infectious agent to the susceptible host). Such knowledge can prompt reasonable and purposive interventions with the aim of eliminating the pathogen, to disrupt the chain of infection in order to stop the outbreak and to prevent this kind of infections in the future.

Combating zoonotic pathogens in animals is difficult, as animals may harbour great numbers of pathogens without getting a disease. The successful combat of these zoonotic agents is an important goal as consumers expect proper food without any (microbial) contamination and the food industry is eager to produce high quality products.

In Austria, a reduction of human cases can be observed for many zoonotic agents. This is due to the successful control of zoonoses in the animal production, among others by maintaining the official status free of certain epizootics and the excellent cooperation between veterinarians, farmers and the food industry in combating foodborne zoonoses. [MUCH et al., 2013a]

A key aspect stated in the World Health Organisation surveillance program report for control of foodborne infections and intoxications in Europe is that “foodborne illness is almost 100 % preventable” [WORLD HEALTH ORGANISATION, 2003]. In order to achieve that position knowledge of the infection sources and transmission routes is essential. Thorough investigation of foodborne outbreaks is a critical part of this pro-

cess. Therefore the epidemiological and microbiological investigation of foodborne outbreaks is necessary and a fruitful collaboration of patients, health authorities, food inspectors, laboratories, veterinarians, manufacturers and many more is required. The persuasion and the willingness of the treating physician to submit patient samples for microbiological testing is of equal importance, since without regular laboratory confirmation of an infectious disease, the implementation of adequate public health measures to prevent additional disease outbreaks cannot be achieved. [MUCH et al., 2009]

In this thesis the public health relevance of foodborne outbreak investigation in Austria is demonstrated and answers for the important question: “Why are we investigating foodborne outbreaks?” will be given.

LITERATURE (OVERVIEW)

1 FOODBORNE OUTBREAKS

1.1 Definitions

Foodborne outbreak

'A foodborne outbreak (FBO) is an incidence, observed under given circumstances, of two or more human cases of the same disease and/or infection, or a situation in which the observed number of human cases exceeds the expected number and where the cases are linked, or are probably linked, to the same food source'. [ANONYMOUS, 2003]

Zoonosis

'Zoonosis means any disease and/or infection which is naturally transmissible directly or indirectly between animals and humans'. [ANONYMOUS, 2003]

Zoonotic agent

'Zoonotic agent means any virus, bacterium, fungus, parasite or other biological entity which is likely to cause a zoonosis'. [ANONYMOUS, 2003]

1.2 Introduction to foodborne outbreak investigation

Zoonoses are infectious' diseases transmissible from animals to humans. Transmission may occur by direct contact with infected animals, consumption of contaminated foods, particularly those of animal origin, or indirect contact, such as environmental contamination. Infants, the elderly, pregnant women, and individuals with compromised immune system are known to be the most vulnerable to acquiring zoonoses. [MUCH et al., 2012]

Combating those pathogens in animals is difficult, as animals may harbour great numbers of pathogens without getting a disease. However, humans can get ill very quickly

as long as they come in contact with incriminated products or excrements of these animals. The combat of these zoonotic agents is a goal as consumers expect proper food without any (microbial) contamination and the food industry is eager to produce high quality products. Nevertheless, elimination with 100 % safety also means to kill other microorganisms which are helpful, e.g. ripening cultures for soft cheeses or raw meat sausages or microorganisms that are needed to mature certain foods and ensure the unique taste of some delicatessen. Therefore it is important for consumers and food handlers to be cautious with the right storing and handling of possibly contaminated food and to act carefully during the preparation of meals – the safe handling of food is important at any step of the food chain. [AMBROŽIČ et al., 2010]

Despite thorough control of food production processes, retail outlets and restaurants contaminated foodstuff occur with the possible consequence of foodborne outbreaks. When consumers get infected after consuming contaminated food the clarification of the cause of these infections should be the priority for the responsible authorities. [MUCH et al., 2012]

1.3 Notification of an infectious disease in Austria

The consulted medical doctor and the medical laboratory have to share the diagnosis of a notifiable infectious disease with the competent district health offices. Here the data are entered in the national epidemiological reporting system (EMS) which was established in January 2009 in order to have a “real-time” overview. This surveillance system for infectious diseases works as an electronic web-based system and data excerpts can be performed easily on a regular basis. Also the national reference laboratories have to send a notification to the district health offices as soon as a notifiable disease is detected in an isolate. Via EMS the responsible authorities get an overview about the situation and if necessary they set actions to clarify the background of the infections and to prevent further spreading of the pathogen. Although there is a legal obligation for an investigation of the source of an infection, most of the disease histories remain unknown. [MUCH et al., 2011b]

The following figure shows the information flow and involved parties for reporting a notifiable disease in Austria.

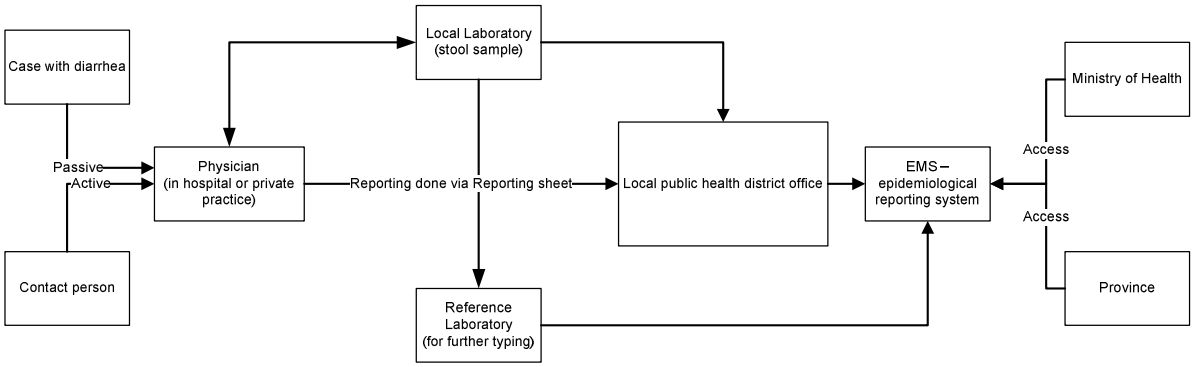

FIGURE 1: Information flow for reporting a notifiable disease in Austria

The recorded cases in the EMS are summarized and published monthly in the national bulletin of notifiable diseases on the homepage of the Ministry of Health: http://bmg.gv.at/home/Schwerpunkte/Krankheiten/Uebertragbare_Krankheiten/Statistiken/Monatliche_Statistik_meldepflichtiger_uebertragbarer_Infektionskrankheiten_2013. At the beginning of the following year, the preliminary case numbers are validated, confirmed and afterwards published as the 'annual data of notifiable communicable infectious disease': http://bmg.gv.at/home/Schwerpunkte/Krankheiten/Uebertragbare_Krankheiten/Statistiken/Jahresstatistiken_meldepflichtiger_Infektionskrankheiten_seit_dem_Jahr_2000. These data only contain month and province of a notified case, no information is given concerning age and sex of the patient, or results of the differentiation of the causative agent (species or serotype etc.).

1.4 Characteristics of foodborne outbreaks

In case of a foodborne outbreak, it is important to find the source and the cause of the infection, to stop the spreading, to prevent further cases and to regain consumers' confidence. In order to do so, the concerned patients have to be identified and epide-

miological and microbiological investigations conducted, if possible [PICHLER et al., 2009a]. An outbreak includes all cases of the same disease that have an epidemiological relationship, such as the same exposure as a confirmed case (eating foods from the same source). All cases that cannot be counted as outbreak cases are classified as sporadic cases [MUCH et al., 2009a]. For single diseased cases it is quite difficult to find the source of the infection in the wide range of consumed food in the days before an infection.

But if we find groups of cases, cumulated according to agents, time and place we have an outbreak and it is much easier to identify the cause during an outbreak situation through thorough investigation of common characteristics and associations between the individual cases.

By the means of detailed and systematic search not only the vehicle of an infection (the incriminated food item that brings the infectious agent to the susceptible host) but also of the reservoir where the pathogen normally lives can be identified. Such knowledge can prompt reasonable and purposive interventions with the aim of eliminating the pathogen, to disrupt the chain of infection in order to stop the outbreak and to prevent this kind of infections in the future.

1.5 Detection of a possible outbreak

When zoonotic agents are isolated from samples from human, animal or foodstuff origin, the respective laboratories are obligated to send them to the responsible national reference laboratory or national reference centre. In these institutions the isolates are subtyped (including serotyping and DNA fingerprinting or pulse-field gel electrophoresis (PFGE)) in order to find isolates with a common pattern and to elucidate the way of infection throughout the food chain. So if a larger number of isolates than expected in a given period and area appears, responsible professionals working in the national reference centres are alerted and get in contact with regional health officials, as there is already a cluster of cases. When an investigation shows that patients of a cluster have something in common to explain why they all have the respective disease,

the group of illnesses is called an outbreak. In the EMS these cases are marked with a special outbreak ID.

Possible outbreaks are not only detected by public health surveillance. Also informal reports occur when members of a community call the local health department to report a group of suspected food-related illnesses. This might happen if several people got sick after eating at a group dinner or family gathering.

In addition information is given through the media when there are short reports in local newspapers or on national television about big numbers of gastroenteritis or vomiting in schools or seniors homes with the consequence of temporary closing down of these public institutions.

1.6 Legal responsibilities within Austria – outbreak investigation and control

If there is a foodborne outbreak or even just the suspicion of an outbreak the public health officers of the affected district administrations have to initiate investigations to determine the extent of the disease and to find the source of infections according to the regulations in the Epidemic Act 1950 [ANONYMOUS, 1950]. Additionally, the Zoonoses Act 2005 obliges the involved authorities for an outbreak investigation with adequate microbiological and epidemiological analyses and that every investigation must provide a short report summarizing its findings and the measures implemented to correct the situation [ANONYMOUS, 2005]. The authorities have the possibility to consult experts like epidemiologists from the AGES (Austrian Agency for Health and Food Safety) in order to perform this investigation. [PICHLER et al., 2009a]

In the case of an outbreak affecting more than one province the so called „Bundeszoonosenkommission" (located in the Ministry of Health) have to be informed. An updated list of ongoing outbreak investigations can be accessed by authorized persons in an online database for reporting foodborne outbreaks affecting more than one province.

An annual summary of foodborne outbreaks in Austria is available for the public on the homepage of the Ministry of Health: http://www.bmg.gv.at/cms/home/attachments/0/7/5/CH1400/CMS1379490681062/jb_lmbka_2012_final_revm.pdf

1.7 Reporting of foodborne outbreaks

The Austrian Zoonoses Act 2005 commits the AGES to collect the foodborne outbreak data annually and to send them to the EFSA (European Food Safety Authority). According to the EFSA 'Manual for reporting of food-borne outbreaks in accordance with Directive 2003/99/EC from the year 2012' the European nomenclature for reporting was directly adopted into the structure of the EMS. The most important terms are listed below.

1.7.1 Terms for reporting foodborne outbreaks

Household versus general outbreak

Outbreaks are classified either household outbreaks, when people from only one household are concerned or general outbreaks, when consumers from several households are involved (e.g. in a restaurant or senior homes). Every year, the majority are household outbreaks as it rarely works that disease cases of different household outbreaks are connected to one general outbreak by identifying a common food vehicle as the source of infection. In 2012, Austria reported 81 % of all foodborne outbreaks as household outbreaks.

Outbreak with strong or weak evidence

Based on the strength of evidence implicating a particular suspected food vehicle, outbreaks are distinguished in those with strong or weak evidence. For outbreaks with weak evidence aggregated data is sufficient. Only numbers of outbreaks, cases, hospitalisations and deaths are reported. Outbreaks with strong evidence have to be reported in detail, either by an analytical epidemiological study with statistical significant

association or by convincing descriptive evidence like a microbiological proof of the pathogen in humans, in food or in the environment of the food. Additionally to the numbers of cases, hospitalisations, deaths and the information concerning the pathogens, the nature of the evidence implicating the food vehicle, and the factors in food preparation and handling that contributed to the outbreak have to be reported. [EUROPEAN FOOD SAFETY AUTHORITY, 2013]

In 2012, Austria reported three out of 122 outbreaks (2.5 %) with strong evidence to EFSA: *S*. Enteritidis PT8, *S*. Stanley, and VTEC O113:H8, respectively. [MUCH et al., 2013a]

Food vehicle

Food contaminated with bacteria, viruses or toxins. Poultry meat, pig meat, egg and egg products, fish and fishery products, milk and dairy products, crustaceans or buffet meals are most often identified as food vehicles. [EUROPEAN FOOD SAFETY AUTHORITY, 2013]

Setting

Place of exposure to the food vehicle. I.e. the location where the food was consumed or where the final stages of preparation of the food vehicle took place (e.g. café/ restaurant, take-away outlet, canteen, residential institution, domestic kitchen). [EUROPEAN FOOD SAFETY AUTHORITY, 2013]

Contributory factors

One or more circumstances that led to a foodborne outbreak; e.g. wrong behaviour in contact with sensitive food, infected food handler, contaminated raw material, cross contamination, inappropriate storing (time, temperature), and insufficient heat treatment. [EUROPEAN FOOD SAFETY AUTHORITY, 2013]

1.8 Historical overview of foodborne outbreaks in Austria

The following table 1 gives an overview about the situation in Austria in the last seven years, the numbers of outbreaks for the most common pathogens (including the bacteria *Listeria monocytogenes*) and the numbers of people involved in these foodborne outbreaks.

Year	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
Number of FBOs	609	438	368	351	193	232	122
- caused by *Salmonella*	452	305	223	208	98	100	53
- caused by *Campylobacter*	137	108	118	120	82	116	61
- caused by *L. monocytogenes*	0	0	1	1	1	1	2
Number of diseased	2,530	1,715	1,376	1,330	838	789	561
- Number of hospitalized	493	286	338	223	155	179	97
- Number of deaths	3	1	0	6	2	0	0

TABLE 1: Foodborne outbreaks in Austria from 2006 – 2012
FBO = foodborne outbreak, *L.* = *Listeria* [MUCH et al., 2011a; 2013]

1.9 Incidence of foodborne outbreaks in Austria, 2012

In 2012, 122 foodborne outbreaks were notified and 561 persons were involved. For the second time outbreaks caused by *Campylobacter* have a greater number (n = 61) than those caused by *Salmonella* (n = 53). Additional outbreaks were due to verotoxin-producing *E. coli* (2-times), *Listeria monocytogenes* (2-times), Norovirus (2-times), hepatitis A virus and *Entamoeba* spp. one time, respectively. [MUCH et al., 2013b]
Generally, there is a decline in the reported numbers of outbreaks until 2010, mainly caused by lower numbers of affected people, but also through greater effort of the responsible authorities to do proper outbreak investigation and to merge apparently disconnected small household outbreaks to greater general outbreaks via identification of a common vehicle of infection. From 2010 to 2011 there is a 20 % increase in the numbers of outbreaks; a possible explanation is the increased number of *Campylobacter* infections in Austria in general. From 2011 to 2012 there is again a decrease of 47 % in all documented foodborne outbreaks in Austria. Outbreaks due to *Salmonella* were re-

duced from 452 to 53 within seven years; that reflects the great success of the *Salmonella* reducing program in laying hen flocks. [MUCH et al. 2011, 2010]

According to the numbers of notified cases in the EMS (as at 28.04.2013) 7,661 persons were infected due to foodborne pathogens in Austria in 2012. But only 561 cases (7 % of all cases of foodborne infections; in 2010: 9 %; in 2009: 13 %) can account for the 122 outbreaks, the remaining 7,100 are apparently sporadic single cases.

Since an outbreak investigation may be able to combine independently reported cases or individual outbreaks into one epidemiologically linked outbreak, this reduces the number of individual outbreaks and sporadic cases respectively [MUCH et al., 2009b]. A more intense and precisely conducted outbreak investigation could considerably decrease this proportion. [MUCH et al., 2013b]

Another important aspect is that the thorough disease notification structure in Austria may also explain why single outbreak numbers are higher for Austria than for other countries included in the European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Foodborne Outbreaks. [MUCH et al., 2009b]

1.10 Prevention and Control on an European level

In order to prevent zoonoses and foodborne outbreaks, it is important to identify which animals and foodstuffs are the main sources of infections. The European Centre for Disease Prevention and Control (ECDC) collects data of human zoonoses cases in Europe annually. Together, ECDC and EFSA analyze the information submitted by 30 European countries (presently including 27 EU Member States) on the occurrence of zoonoses and foodborne outbreaks. The results of the analyses of the data are published in the annual European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Foodborne Outbreaks. The latest report from 2011 is available on the EFSA homepage:

http://www.efsa.europa.eu/de/efsajournal/pub/3129.htm.

1.10.1 Comparison between Austria and the EU in 2011

The increase in cross-border trade and therefore internationalization of food supply sources as well as the increase of global tourism and migration sets the epidemiological investigation of foodborne outbreaks to an EU-wide duty. But it is important to note that reporting systems for foodborne outbreaks and investigation systems at national level are not harmonised among member states. Therefore, the differences in numbers and types of reported outbreaks, as well as in the causative agents may not necessarily reflect the level of food safety among member states; rather they may indicate differences in the sensitivity of the national systems in identifying and investigating foodborne outbreaks. [MUCH et al., 2013a]

In 2011, a total of 5,648 foodborne outbreaks, including both weak and strong evidence outbreaks, were reported by 25 member states. Overall, 69,533 human cases, 7,125 hospitalisations and 93 deaths were reported. The evidence supporting the link between human cases and food vehicles was strong in 701 outbreaks.

Like in Austria, the decline in the number of *Salmonella* outbreaks within the EU continued, from 1,888 outbreaks in 2008 to 1,501 outbreaks (26.6 % of all outbreaks) in 2011. Bacterial toxins, Campylobacter and viruses accounted for 12.9 %, 10.6 % and 9.3 % of the outbreaks, respectively. No causative agent was identified for more than 2,000 outbreaks in the EU (36 %). [EUROPEAN FOOD SAFETY AUTHORITY and EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL, 2012]

1.10.2 Early warning systems

Early Warning and Response System

On the European level the Network for the Surveillance and Control of Communicable Diseases has been established by the European Parliament with Council Decision 2119/98/EC. The aim of the Network is to promote cooperation and coordination between the Member States to improve prevention and control of communicable diseases and to protect public health. The Network includes an Early Warning and Response System (EWRS) and has contact points nominated by the national Ministries of Health

on duty 24 hours a day and seven days a week. Their duty is to inform each other about incidents related to infectious diseases potentially affecting other EU-member countries.

The head office for public medical services at the Federal Ministry of Health in Austria serves as the EWRS contact point, thus overseeing and ensuring the relevance of the information fed into the system. In order to ensure comparable reports of the different countries case definitions for the different diseases were developed.

In response to the installation of the EWRS on the EU level, a national warning system was created. It features a mailing list including all sanitary authorities of the provinces, reference centres and other relevant organisations. [LADURNER et al., 2010]

In 2005, the European Centre for Disease Prevention and Control (ECDC) was established. It is an independent agency of the EU with the mission to strengthen Europe's defences against infectious diseases. The objective is to operate surveillance networks and to identify, assess and communicate current and emerging threats to human health from communicable diseases. Data from all Member States and three countries (Iceland, Liechtenstein and Norway) from the European Economic Area (EEA) are collected and entered in ECDC's database system, known as The European Surveillance System (TESSy). In the Annual Epidemiological Report epidemiological overviews of all diseases are provided (http://www.ecdc.europa.eu/en/publications/surveillance_reports/annual_epidemiological_report/Pages/epi_index.aspx).

ECDC works in partnership with national health protection bodies across Europe to strengthen and develop continent-wide disease surveillance and early warning systems.

Rapid Alert System for Food and Feed

In the European Union, a rapid alert system for food and feed (RASFF) was put in place to provide food and feed control authorities with an effective tool to exchange information about measures taken responding to serious risks detected in relation to food or feed. This exchange of information helps member states to act more rapidly and to

respond to a health threat caused by food or feed in a coordinated manner. During the last decade, RASFF messages with information concerning listeria have increased by the factor of three. While in 2012, 90 notifications concerned *L. monocytogenes* (2011: 107, 2010: 108, 2009: 85, 2008: 51, 2007: 37, 2006: 26, 2005: 117, 2004: 122, 2003: 58, 2002: 51, 2001: 36), only 33 alerts were issued in the year 2000. This increase seems to reflect increased awareness about listeria contamination as a potential public health risk. In 2012, fish, meat, and milk products accounted for 66 % (n=59) of the 90 RASFF notifications. [Oral communication with Franz Allerberger, data not published yet]

Information of the public

As with the amendment of the Austrian Food Safety and Consumer Protection Law (LMSVG) [ANONYMOUS, 2006] in 2010, the information of the public concerning unsafe products or food was improved [ANONYMOUS, 2010]. Already if there is a reasonable suspicion (due to laboratory results, risk assessment of the AGES or RASSF alerts) that harmful products or food are on the market and could negatively affect people, the Austrian Minister of Health has to inform the public. The sole responsibility of the producer or trader is no longer valid, but already set actions by them have to be taken in account.

1.11 Factors causing food-related infections

Many different factors may lead to foodborne infections and outbreaks; high risk factors for foodborne disease are food from unsafe sources, poor personal hygiene, inadequate cooking, improper holding times and temperatures, and cross contamination [U. S. FOOD AND DRUG ADMINISTRATION, 2009].

Generally there are three lines of defense against a foodborne infection. The first is primary production, with the aim of a preferably small amount of pathogens in raw material and an improvement of the hygiene. The second utilizes food processing technologies like pasteurization, salting, irradiation, but also the implementation of a sound HACCP-System (Hazard Analysis and Critical Control Points), mandatory in the

European Union since 2006 [JEVŠNIK et al., 2008]. And the third line of defense concerns the education and training of all food handlers in restaurants and catering services, but also home-based food handlers in order to reach a greater awareness for food safety and hygiene (e.g. prevention of cross contamination on chopping boards, adequate knowledge of safe temperatures, hand washing techniques, and the use of a cool bag for the transport of sensitive food after shopping). [SCOTT, 2003; AMMON and BRÄUNIG, 2002]

Lack of food safety knowledge can result in inadequate food preparation and inappropriate hygiene behavior and often causing a transmission of foodborne pathogens to the consumers [DEBESS et al., 2009]. Shapiro *et al*. identified that a lack of food handlers' knowledge of proper cooking and storage temperatures could lead to multiplication of bacterial pathogens in food, with resultant outbreaks of illness [SHAPIRO et al., 1999]. Therefore it is of utmost importance to educate and train food handlers properly. [WORLD HEALTH ORGANISATION, 2006]

In 2006, the WHO launched a health promotion campaign named *„Five keys to safer food"* [WORLD HEALTH ORGANISATION, 2006]. Through observing these rules for a safe food preparation in restaurants and private homes, risk factors for food-related infections can be minimized.
The 5 recommended keys to safer food are:

"Keep clean!"
"Separate raw and cooked!"
"Cook thoroughly!"
"Keep food at save temperatures!"
"Use safe water and raw materials!"

To prevent restaurant-associated foodborne disease, it is important to determine gaps in food safety knowledge of food handlers in order to guide effective educational and behavioral interventions [PICHLER et al., 2014]. In a recent study for evaluating the

levels of knowledge on food safety and hygiene among food handlers from restaurants and different catering businesses in Vienna, Austria, Pichler *et al*. identified substantial knowledge gaps concerning the correct temperatures for cooking, holding, and storing foods (see *original article V*). The authors recommend targeted educational material to be created with special focus on time and temperature for cooking, holding, and storing foods to increase the knowledge of food handling staff and to keep up with safe food handling practices. [PICHLER et al., 2014]

1.12 Documented outbreaks in Austria and their contributing factors

1.12.1 Infected food handler – a norovirus outbreak

During October 2008, the Austrian Agency for Health and Food Safety (AGES) investigated a cluster of gastroenteritis at a military base in Vienna. The objective was to find the causative agent, source, and mode of transmission of the outbreak. Descriptive epidemiology identified 63 cases, which fell ill between October 8 and October 10, from 6 out of 7 companies located at the base. A case control study was performed on 53 cases and 133 controls. Questionnaires were created based on the menu served in the dining hall at the military base to identify potential risky meals and food items. A total of 99 stool samples from 56 cases and 43 controls were examined for norovirus using PT-PCR; 47 cases tested positive. Of 24 kitchen staff members, all of them asymptomatic, two carried norovirus. None of 126 stool specimens tested yielded bacterial pathogens or staphylococcal enterotoxin. Two meals were associated with illness caused by norovirus: breakfast and lunch served in the dining hall on Tuesday October 7 (odds ratios of 5.4 (95 % CI 2.0-14.5) and 17.2 (95 % CI 2.3-129.0), respectively ($p<0.001$)). The analytical epidemiological study made allows proving a point-source foodborne genesis. The asymptomatically infected food handlers were the most likely source of this foodborne outbreak. Interventions to prevent occurrence of similar outbreaks in the future must include securing adequate hand washing facilities in the kitchen area, i.e. contact free faucets, and basic hygiene training of the frequently changing kitchen staff, with special focus on hand washing. [MUCH et al., 2009b]

This was the first documented foodborne outbreak in an Austrian military base due to norovirus. Only by the means of an analytical epidemiological investigation it was possible to distinguish whether the way of infection was food-related or caused by person-to-person spread. The occurrence of person-to-person transmission (secondary cases) was not provable. This foodborne outbreak was restricted to only one location so the contamination of the meals happened in that single venue and not in the big military catering facility working with a cook & chill technique and preparing meals for several military locations in Vienna. Food samples were collected by health authorities two days after exposition; no norovirus was detected in these samples. Sampling and testing of food where no epidemiological link to the outbreak is given, should be critically questioned and gives no profound validity whether the outbreak was food-related or not.

1.12.2 Inadequate cleaning and thermal disinfection – two consecutive *Salmonella* Typhimurium outbreaks

A *Salmonella* Typhimurium outbreak happened from 25 May – 7 June 2009 and 122 soldiers who consumed food in the military base A contracted from salmonellosis. Despite of extensive investigations in a clearly closed setting, the vehicle of infection was not identified. From 31 August – 2 September 2009 again an outbreak with the same *Salmonella* definitive type (*S*. Typhimurium DT193) happened and 61 soldiers got infected during a corporate field exercise formed by persons from military base A + B.
A descriptive-epidemiological investigation, a cohort study of the first outbreak, and a broad microbiological investigation were carried out. However, the microbiological testings (such as eggs from the local egg producer) were all *Salmonella*-negative and the vehicle of infection was not identified. The most probable link between the two outbreaks was the use of mobile cooking units and heating boxes from the military base A, which underwent inadequate cleaning and thermal disinfection due to a failure of the steamer used. Furthermore, the dish washing facility was in a very poor hygienic

condition and a consequent resoiling of cooking utensils from the floor was very likely. [PICHLER et al., 2010]

The above mentioned outbreaks show clearly the serious consequences if catering businesses do not maintain good hygiene practices. Many companies produce big numbers of meals daily and already little failure in kitchen hygiene and food safety (e.g. cross-contamination, insufficient heat treatment) can result in foodborne outbreaks with many involved parties.

1.13 Public health relevance of foodborne diseases

Foodborne diseases and outbreaks are crucial contributors to morbidity and mortality worldwide [FLINT et al., 2005]. The whole extent is hard to comprehend as only a small number of infections are obviously due to food [FLINT et al., 2005]. In estimating the burden of disease within a population it is important to recognize that many people do not visit a physician when they become ill with gastrointestinal disease and that not all physicians send in stool samples for microbiological verification. [MUCH et al., 2009b]

For the United States Mead *et al.* estimated 13.8 million foodborne infections by known pathogens and 62 million of unknown etiology [MEAD et al., 1999], and Scallan *et al.* estimated 9.4 million cases of food-related diseases caused by 31 major pathogens [SCALLAN et al., 2011b] and 38.4 million by unspecified pathogens [SCALLAN et al., 2011a] every year. The diversity of these numbers demonstrates the complexity of such estimations.

The total health-related cost of foodborne disease in the United States is equivalent to $ 51.0 billion in the basic cost-of-illness model (including values for medical care, productivity losses and mortality) and $ 77.7 billion in the enhanced model, including added pain and suffering [SCHARFF, 2012]. No published data for Austria and the European Union are available concerning socio-economic costs of foodborne infections.

Globally, the incurred costs and dimensions of foodborne diseases are still unknown but they seem to be substantial. [KUCHENMÜLLER et al., 2009]

In 2002, Ammon and Bräunig published in the German 'Bericht der Gesundheitserstattung des Bundes' that foodborne infections and intoxications raised enormously in the last 25 years. This is due to the improved laboratory diagnosis but also due to new ways of infections caused by changed environmental conditions (worldwide trade of goods, animals, altered food consumption behaviors etc.) [AMMON and BRÄUNIG, 2002]. An increased mass production can influence the outbreak scenario - from being small and confined to a community or region, outbreaks change to large ones, affecting hundreds of people and spread cross-border [ALLERBERGER, 2007].

In recent years, new pathogens appeared. Verocytotoxic *E. coli* (VTEC) strains are a pathogenic variant of the otherwise harmless intestinal inhabitant *E. coli*; VTEC can cause bloody diarrhoea and, in rare cases, the life-threatening Haemolytic-uraemic syndrome (HUS). In spring 2011, a new variant of VTEC, *E. coli* O104:H4 caused an outbreak in Germany also affecting other European countries with more than 3,800 cases and 53 fatalities. This strain developed from a human pathogen, an enteroaggregative *E. coli*, acquired different pathogenic genes, e.g. to produce proteins to adhere to human enterocytes, shows resistance to several critically important antibiotics and has the ability to produce verotoxin 2; those latter factors highly increased its virulence. [FRANK et al., 2011]

Also multiresistant bacteria – organisms resistant to antimicrobial agents from more than three different classes of substances usually effective against the same bacterial species – represent an additional risk to humans. Examples of multiresistant organisms are methicillin-resistant Staphylococcus aureus (MRSA), extended-spectrum ß-lactamase (ESBL)-producing enterobacteria, carbapenemase-producing enterobacteria, or *Salmonella* Typhimurium DT104. [MUCH et al., 2013a]

2 *LISTERIA MONOCYTOGENES*

Listeria monocytogenes can cause a foodborne outbreak, but outbreaks are very rarely documented. Information concerning occurrence, reservoir, mode of transmission, symptoms and therapy, and advice for prevention is given in this chapter 2. Additionally, Austrian data from humans (compared to the EU MS in 2011), animals and foodstuff from the year 2012 are presented.

2.1 Zoonotic agent

The zoonotic agent *Listeria (L.) monocytogenes* is a short, non-spore-forming, rod-shaped bacterium; human infections are usually caused by serotypes 1/2a, 1/2b, 1/2c and 4b. [ALLERBERGER and WAGNER, 2010]

2.2 Occurrence

The pathogen is widely distributed in the environment, from sewage water to soil and plants. Food products of animal origin, such as raw milk, soft cheese, pâté, smoked fish and raw meat, can become contaminated during the production process (e.g. during milking or slaughter). The bacterium is commonly found in food-processing plants, where it is potentially becoming a 'domestic pathogen' that is difficult to be eliminated by routine cleaning and disinfection practices. Studies have shown that it can grow and exist in biofilm, enabling attachment to stainless steel surfaces in food production systems [IWAMOTO et al., 2008]. Unlike most other zoonotic bacteria, *L. monocytogenes* can multiply in low-temperature environments, such as refrigerators. It's unusual viability at temperatures between -1.5 and 45 °C, in high salt concentrations (up to 10 – 14 %) and low or high pH values (4.3 – 9.1) are a meaningful challenge for the food industry. [SCHODER and WAGNER, 2012]

2.3 Reservoir

Ruminant animals (especially cattle, sheep and goats) and contaminated production facilities (mainly machines for slicing and vacuum-packing).

2.4 Mode of transmission

Consumption of contaminated foods of animal or vegetable origin is the main transmission route. Transmission among humans has rarely been observed (nosocomial infection of neonates). Infection through direct contact with carrier animals is rarely documented.

2.5 Listeriosis

Listeriosis is an infectious disease caused by *L. monocytogenes.*

2.5.1 Incubation period

Within a foodborne infection the incubation period is 3 – 70 days, median incubation is three weeks.

2.5.2 Symptoms

In healthy adults, the infection with *L. monocytogenes* is mild, although some may develop diarrhoea. However, in immuno-compromised individuals, such as neonates, the elderly and patients with chronic diseases the infection may spread to the nervous system. Symptoms for invasive listeriosis include: intense headache, high fever, muscle aches and sometimes nausea, diarrhoea, stiff neck, confusion, and loss of balance. Severe cases may develop meningitis or may result in death.
In pregnant women, the infection is mostly asymptomatic, however, infection can lead to miscarriage, stillbirth, premature delivery, or infection of the newborn. Infected infants often develop meningitis. [PICHLER et al., 2009a]

2.5.3 Diagnosis

Listeriosis is confirmed by culturing the infectious agent from blood, cerebrospinal fluid, pus or stool.

2.5.4 Therapy

Treatment is done with antibiotics. Despite specific therapy, up to 30 % of clinical cases of listeriosis end fatally.

2.6 Preventive measures

The compliance with common kitchen hygiene rules is important to avoid infections with *L. monocytogenes*.

Rules to minimize the risk of foodborne infections include:

- Thoroughly cooking of meat and fish dishes
- Boiling of raw milk before consumption
- No consumption of raw minced meat
- Highly sensitive food like soft cheeses or smoked fish should be stored separately in the refrigerator and not consumed after the expiration date
- Regular hand washing (before and after preparation of meals) is an important basic measure of protection against pathogens
- Fruits, vegetables and salads should be washed properly before eating
- The preparation of meat and raw vegetables should be done with separate kitchen equipment or at separate times
- Work surfaces should always be cleaned thoroughly after use
- During storage in the refrigerator, freshly cooked meals should be covered to avoid later contamination [PICHLER et al., 2009a]

2.7 Data from Austria, 2012

2.7.1 Humans

Listeriosis notification in Austria

Generally, the reporting system for listeriosis is compulsory, comprehensive (covers the whole population of a region), passive, and case-based (each individual case of the

disease under surveillance is reported separately to the national level). Data are reported from laboratories, physicians and hospitals nationwide. [PICHLER et al., 2009a] A main task of the national reference centre for *Listeria* (also binational Austrian-German Consiliar Laboratory for Listeria) is to elucidate possible outbreaks through serotyping and DNA fingerprinting of all of the isolates and to collect epidemiological data of the patients in order to set targeted preventive measures [KASPER et al., 2009]. But the large part of invasive listeriosis cases are sporadic ones and it is rarely possible to find a common food vehicle with the same genetic pattern in the patient's and food's isolate.

In 2012, 34 cases of invasive listeriosis were investigated and serotyped in the national reference centre for Listeria within the AGES. Congenital listeriosis was reported in four of the 34 cases (the foetus, stillborn, new-born or the mother count as one case). The incidence is only 0.4/100,000 population and therefore Listeriosis is considered to be a rare infectious disease in Austria. In 2012, the case fatality ratio was 12 % (4 of 34 patients died). Figure 2 shows microbiologically confirmed cases of invasive listeriosis and deaths in Austria between 2000 and 2012.

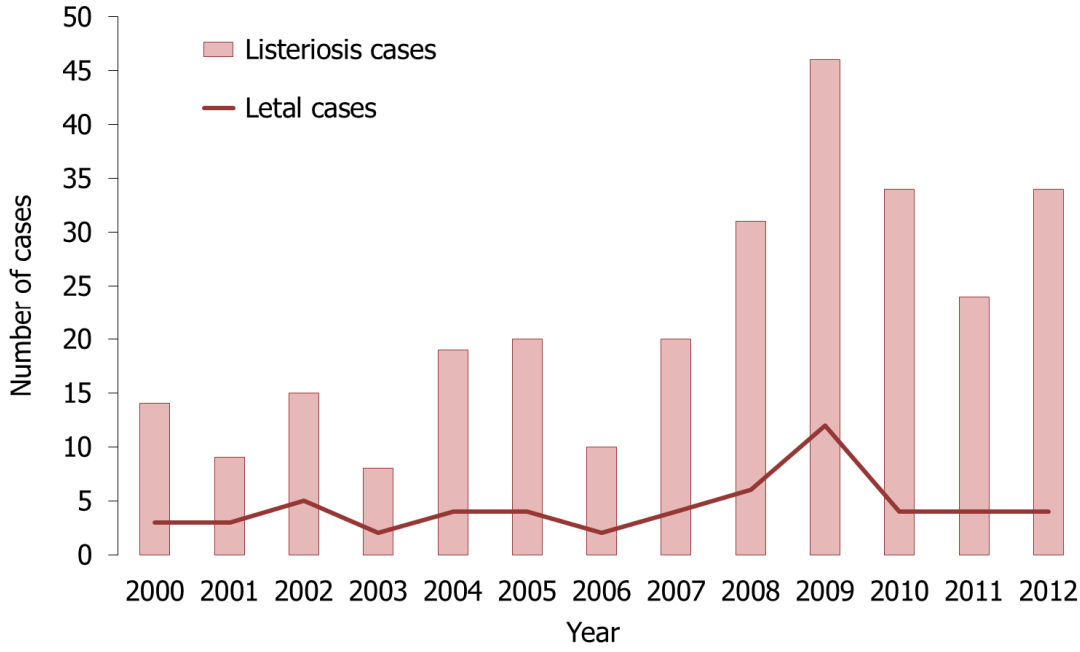

FIGURE 2: Microbiologically confirmed cases of invasive listeriosis and fatal outcomes in Austria between 2000 and 2012
[MUCH et al., 2013a](Reprinted with permission)

Comparison between Austria and the European Member States in 2011

In 2011, the incidence of notified human listeriosis cases in Austria was 0.31/100,000 population and similar to the EU average of 0.32/100,000 population. The incidences for listeriosis in the single Member States varies EU-wide from 0.04/100,000 population in Romania to 0.88/100,000 population in Denmark. [EUROPEAN FOOD SAFETY AUTHORITY and EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL, 2012]

2.7.2 Food

The revision and sampling plans of the Federal Ministry of Health (FMH) specify the number of food enterprises (restaurants, dairies, retail outlets, manufacturers etc.) and food products that must be tested in each province in a given year. During these inspections samples are taken for investigation and the food production processes are assessed.

In 2012, *L. monocytogenes* was detected in 25 grams of the following food categories: In two out of 1,181 samples of milk, milk products or cheeses; in nine out of 168 samples of fish products and in two out of 72 smoked fish samples. In 570 samples of meat and meat products from different animal species (raw or cooked), the pathogen was found in 31 samples; out of those the bacteria was most prevalent in 12 out of 148 samples of cooked, ready to eat pig meat samples. Six out of 176 samples of fermented sausages were contaminated with *L. monocytogenes*. In 3/313 sampled other food stuffs like salads, sauces, dressings, and vegetables *L. monocytogenes* was detected. Out of all *L. monocytogenes* positive food samples, in only four samples (two samples of smoked fish and two samples of cooked, ready to eat pig meat) the pathogen was present in 10-100 colony-forming units (cfu); none of the samples exceeded 100 cfu/g. Figure 3 shows data from food of animal origin tested for *Listeria monocytogenes* (including positive samples) in Austria in the year 2012. [MUCH et al., 2013a]

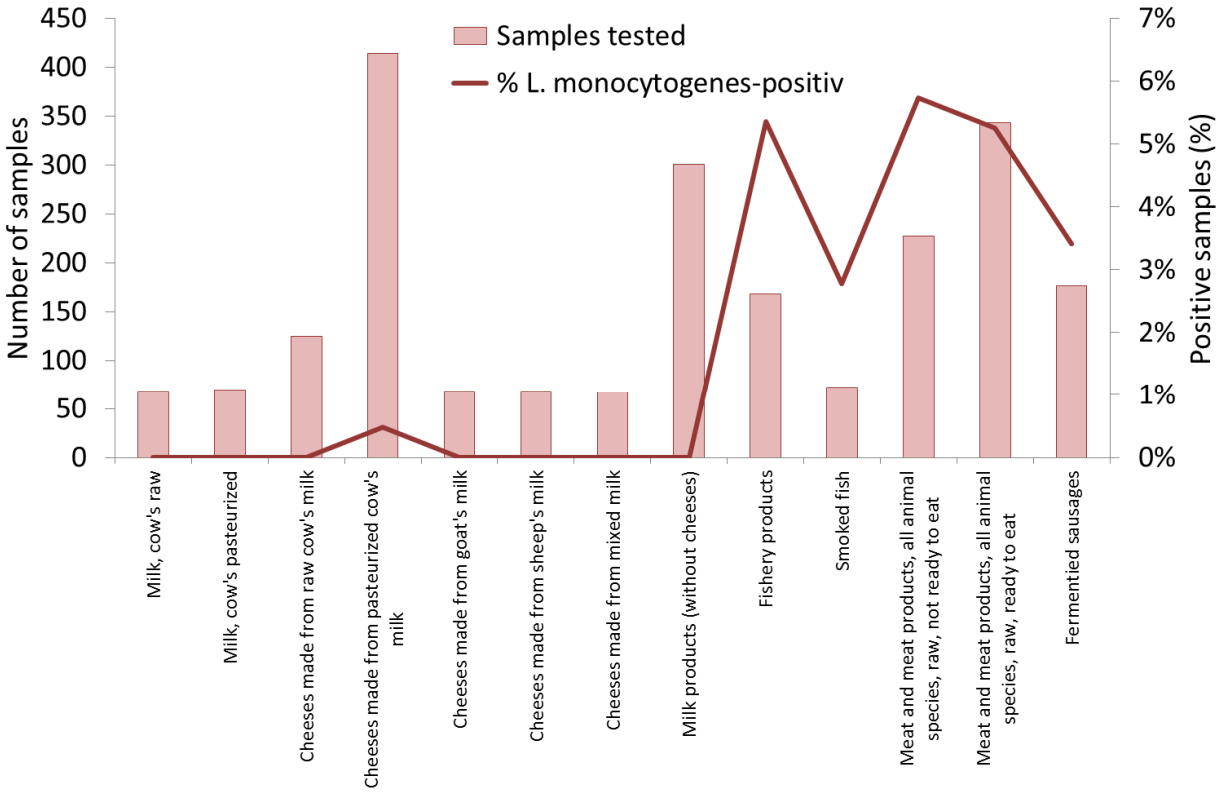

FIGURE 3: Food of animal origin tested for *L. monocytogenes* in Austria, 2012
[MUCH et al., 2013a] (Reprinted with permission)

2.7.3 Animals

Listeria monocytogenes is mainly transported into the food via the production process in the plants and not via animals. Therefore a surveillance of the animal population for *Listeria* is not worthwhile.

3 EPIDEMIOLOGY OF COMMUNICABLE DISEASES

Chapter 3 deals with the topic epidemiology of communicable diseases and the different steps of a foodborne outbreak investigation are illustrated. In addition, unusual ways of outbreak investigations for rare pathogens are shown by two different outbreaks caused by *Listeria monocytogenes*.

3.1 Definitions

Epidemiology

Epidemiology is the study of the distribution and determinants of disease, in order to control diseases and other health problems in the population. Surveillance and descriptive studies are performed to study distribution; analytical studies are used for determinants. [WHO, 2013]

Epidemiology is the cornerstone of public health, and supports health policy decisions and evidence-based medicine by identifying risk factors for disease and targets for preventive medicine.

Communicable disease (synonym: infectious disease)

'An illness due to a specific infectious agent or its toxic products that arises through transmission of that agent or its products from an infected person, animal or inanimate source to a susceptible host; either directly or indirectly through an intermediate plant or animal host, through a vector, or through contact with the inanimate environment' [HEYMANN, 2008].

3.2 Outbreak investigation: how does it work in theory and in practice?

3.2.1 Steps in a foodborne outbreak investigation

Doing outbreak investigation is the chance to learn something new about the organism or the disease. An outbreak response should be systematic and based on epidemiological evidence. A foodborne outbreak investigation goes through several steps. Here, the ten steps proposed by the Centers for Disease Control and Prevention (CDC) are

described in the given order, but in reality investigations are dynamic and several steps are taken concurrently. [HEYMANN, 2008]

10 Steps in a foodborne outbreak investigation: (according to CENTERS FOR DISEASE CONTROL AND PREVENTION, 2013 and DWORKIN, 2010)

1. Confirm the outbreak and verify the diagnosis
2. Create a preliminary case definition
3. Count cases
4. Perform descriptive epidemiology
5. Perform supplemental laboratory or environmental investigation
6. Develop hypothesis
7. Test hypothesis – analytical epidemiological studies
8. Controlling an outbreak
9. Communicate findings and prevention information
10. Maintain surveillance

In the following paragraphs the different steps in a foodborne outbreak investigation are explained in detail and highlighted with comments from my personal experience of performing outbreak investigation with special emphasis on two outbreaks caused by *L. monocytogenes* serovar (sv) 1/2a and 4b.

1. Confirm the outbreak and verify the diagnosis

There are several ways in being informed about an unexpected high number of cases of a disease or syndrome (as already mentioned in the chapter of foodborne outbreaks) and now it is important to verify, that an outbreak is occurring and that it is not only a cluster of cases with no common link. A useful method of verifying that an outbreak exists is to examine public health surveillance data, in case it is a reportable disease. It can quickly be determined whether the suspicion of a high number of case reports of e.g. *Listeria monocytogenes* can be confirmed by comparing the report to a median number of reported cases during a similar time period historically. Additionally, it is necessary to confirm the diagnosis by forwarding the isolates to the national reference centre (as the information about the cluster is not originated there). Here it

will be determined whether the outbreak is definitely four isolates of the pathogen *Listeria* and which serotype is involved.
Furthermore it should be determined whether the outbreak is waterborne, foodborne, or caused by person-to-person spread or via vectors, where this outbreak happened and if it is still ongoing either in the community or in closed settings like public institutions (senior homes, kindergartens, hospitals, etc.).

2. Create a preliminary case definition

Once an outbreak is really occurring and the diagnosis is confirmed, a tentative case definition is needed to determine the extent of the outbreak in a systematic way. The case definition should include features of the illness and typical symptoms, the pathogen (including serotype) and the time range when the illnesses occurred. Special attention should be paid to sensitivity and specificity. As additional information is gained, there may be the need to modify the preliminary case definition to make it more specific and useful for analysis. During the investigation of the *L. monocytogenes* sv 4b outbreak cases were defined as follows: 'a confirmed case is a person who had dinner at the wine tavern on September 6, 2008 and developed diarrhea or fever within the following seven days, with an isolate of *L. monocytogenes* from stool, blood or cerebrospinal fluid (CSF)' (see *original article IV*).

3. Count cases

After a case definition has been determined, the work of identifying as many cases as possible starts. The gained information should be entered in a 'line list', where the first reports are summarized on paper or excel sheets. Important to mention is that primary cases are defined as cases that were exposed to the implicated source and secondary cases usually arise from their contact with an infectious primary case. E.g. subjects eating *Campylobacter*-contaminated chicken develop gastroenteritis are called primary cases. They will shed the organism in their stools, and if they do not practice good hand hygiene after using the toilet, they may transfer the organism to a family member, for example during food preparation at home. These new cases of campylobac-

teriosis may never have been to the implicated restaurant and are secondary cases. When later performing epidemiologic analysis it is important to exclude the secondary cases from the analysis of risk factors in order to find the primary source of the outbreak.

4. Perform descriptive epidemiology

When the number of cases of the initial 'line list' has become numerous they must be transferred in a special computer data base. The descriptive epidemiologic analysis gives information about person, place and time; frequencies of variables like age, gender, symptoms, clinical manifestation and exposure information can be elucidated. Cases can be examined for their geographical distribution (see *original article I*), and the results may lead to a hypothesis regarding a common exposure site. When having identified the onset of illness an epidemic curve can be designed and it shows clearly the distribution of cases over time and whether it is a point-source outbreak like the *L. monocytogenes* sv 4b outbreak in 2008 (see *original publication IV*). Or it is a continuous source outbreak – temporary epidemic curve from 12/2009 shown in figure 4 – as originated from the *L. monocytogenes* sv 1/2a outbreak in 2009/10 [FRETZ et al., 2010b], investigated by the Competence Centre for Infectious Disease of AGES and demonstrated detailed in *original article II* and *III* and in *review II* adjacently.

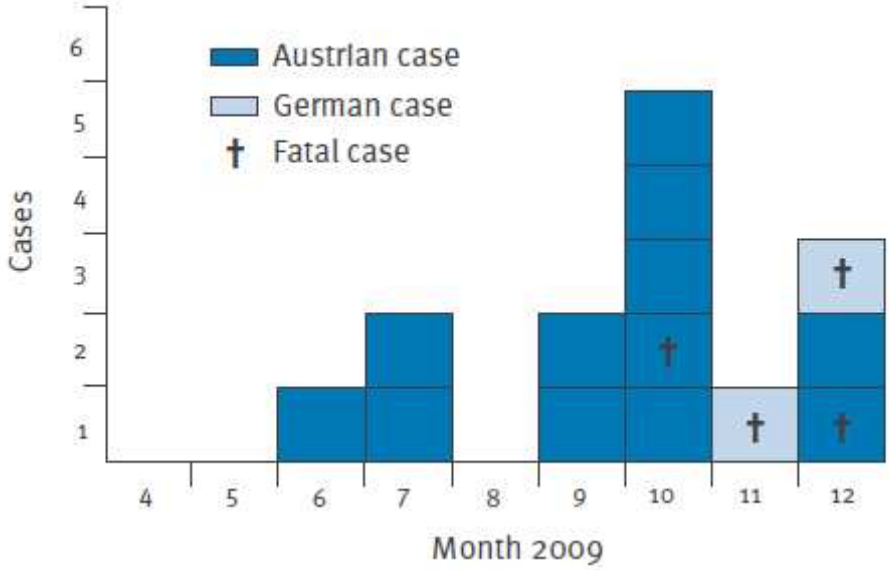

FIGURE 4: Interim epidemic curve of the *L. monocytogenes* sv 1/2a outbreak in December 2009. Outbreak cases of listeriosis by onset of illness, Austria and Germany, 2009 (n=14)

[FRETZ et al., 2010a] (Reprinted with permission)

Several statistical software packages are available for analyzing outbreak data, but one of the more commonly used and freely accessible epidemiological software is Epi Info™, available for download from the CDC homepage at http://www.cdc.gov/epiinfo/. Epi Info™ is easy to apply also for investigators with limited epidemiologic and analytical skills and has many convenient functions without the need to write programming codes.

5. Perform supplemental laboratory or environmental investigation

Depending on the type of outbreak environmental investigation may help bringing light into the so far unclear situation. In case of a foodborne outbreak investigation where a food establishment is implicated by some or all of the cases, a restaurant inspection by the local health authority is a routine response. During the *L. monocytogenes* sv 4b outbreak also the outbreak investigation team was onsite and staff working in the wine tavern was interviewed to find out the ingredients used, the steps followed in food preparation, and the applied temperatures for preparing and holding

food. Also samples from the incriminated food (hog head, self-made sausages, etc.) and microbiological paddle tests from the environment (refrigerated warehouse, washing machine, wooden chopping boards, etc.) were taken for laboratory testing. The inspection could reveal useful clues that support the interpretation of the epidemiologic data such as misbehavior of the food handlers during food preparation or working while being ill. Also actual plumbing problems or inadequate kitchen hygiene and practices (e.g. insufficiently cleaned chopping boards or storing sensitive foods uncovered on the kitchen floor) are elucidated during an inspection. Health practices and training of the kitchen stuff may be checked as well as past inspection reports to see if there has been a history of problems. If an onsite inspection reveals the need of immediate closing down of a restaurant one should not wait until epidemiological analysis is finished. Although, each decision has to be made individually; the financial consequences and loss of credibility of the business owner should be weighed carefully against the public health implications of delaying such an action.

6. Develop hypothesis

The generation of a hypothesis is often a very early step in the outbreak investigation; particularly experienced investigators have a good 'gut feeling' to assume what have happened and what is the likely source of the outbreak. So knowing the pathogen and exploring the descriptive data of the first cases at the beginning of the *L. monocytogenes* sv 1/2a outbreak already lead to the 'quick and dirty' hypothesis that if it is an outbreak due to consumed food, this food will be a ripened 'stinky' one which male persons, especially older pensioners, love to eat but women not. Our experienced team leader said that from the beginning and we kept the possibility of an intense-smelling smear (or raw-milk) cheese in our minds when starting with the interviews. In general, a hypothesis should be developed including the aspects of source of infection, mode of transmission, risks of infection, the vehicle and the reservoir.

When exposure to a food is assumed, the investigators must consider the large number of foods that may be the source or vehicle of infection and narrow the list to the foods that the infected people actually consumed before they got sick, and then fur-

ther narrow it to the specific foods that many of the ill people remember eating. Health officials interview the infected subjects in order to find out where and what they consumed in the days or weeks before they got sick. These interviews are called 'hypothesis-generating interviews'. The time period they ask about depends on the pathogen's incubation period. This varies for different pathogens. If several cases have occurred at a restaurant, in a public institution or a catered event, interviews will focus on the menu items prepared, served, or sold there. Generating hypothesis also depends on the quality of the interviews of the diseased persons as their memories may not be properly released. The time from the start of a disease and the recognition that this person was part of an outbreak is typically 2 – 3 weeks. Since they hardly remember their eating pattern infected subjects may be interviewed multiple times as new ideas arise about possible sources. It can also be helpful to visit someone's home and check the foods in their refrigerator, or to get the permission to review the information from their shopper cards or collected grocery bills, as it was done during the *L. monocytogenes* sv 1/2a outbreak.

7. Test hypothesis – analytical epidemiological studies

A sound hypothesis should be tested with additional analysis; the two most common study methods are *cohort* or *case control*. A *cohort study* is mainly applied when the entire population exposed is clearly defined, like in a family gathering, a wedding party or a closed group of people at a restaurant, as we had to deal with during the outbreak of *L. monocytogenes* sv 4b in 2008. The results of this analytical epidemiological investigation can be found in chapter 4, *original article IV*.

For a *case control study* it is a challenge to find adequate controls, as they should not have had the outbreak disease but a similar likelihood of having been exposed as the cases. So picking controls that live in the same neighborhood as the cases or are referred by them (friends and family) can solve this issue.

Generally, food-related infections are determined by high prevalence and low mortality rates. In the case of *Listeria monocytogenes* (invasive listeriosis) the situation is different [ZUNABOVIC et al., 2011] and the investigation of listeriosis outbreaks is quite

difficult. Most of the cases seem sporadic, the disease has a long incubation period, the severe infection of the central nervous system and involved hospitalizations and the high lethality rate hinder epidemiological outbreak investigation with traditional concepts. [PICHLER et al., 2010]

In 2009, we investigated an outbreak of *L. monocytogenes* sv 1/2a and the source was initially identified based solely on epidemiological findings. Since no reliable information was available on food consumed during the incubation period, all surviving Austrian outbreak cases were asked to collect grocery receipts of purchases after their discharge from hospital in order to collect information on their routine food consumption behavior, and compared them for matches [FRETZ et al., 2010a]. This generated a hypothesis to be tested by a case control study using case-case comparisons. For this study, a case was defined as a person in Austria from whom the *L. monocytogenes* outbreak clone 1 was isolated. Controls were patients from Austria with *L. monocytogenes* infections in 2009, whose isolates showed profiles other than that of the outbreak clone 1. Patients were asked about consumption of 12 cheese products in the six-month period prior to disease onset. Control persons were requested to provide information on consumption of the same cheese products in the year 2009 [PICHLER et al., 2010]. The epidemiological investigation revealed consumption of 'Quargel', a type of acid curd cheese available in different flavors, as a highly likely source of this outbreak. [FRETZ et al., 2010b]

To support the hypothesis additional laboratory testing of the suspected food is important. Finding bacteria with the same DNA fingerprint in a food sample and in the stool samples of people involved in the outbreak is a strong evidence of a source of infection. But often we do not get food samples for testing, as food items with a short shelf life are no longer available by the time the outbreak is known. If the suspected food is available, the pathogen may also be difficult to detect. It can have decreased in number since the outbreak or the bacteria started to change the DNA pattern as we experienced when the ripening environment was changed and a second clone emerg-

ed during the *L. monocytogenes* serovar 1/2a outbreak and caused further cases of listeriosis (see *review II*).

8. Controlling an outbreak

Once the source of a foodborne outbreak was found, new control measures have to be put in place immediately and it is important to ensure that the measures set are being carried out. Otherwise more people may get sick, if contaminated food stays in the shops, in restaurant kitchens, or in home pantries. Outbreak control measures might include specific cleaning and disinfecting procedures of food facilities, temporarily closing down a restaurant [PICHLER et al., 2009b] or processing plant [PICHLER et al., 2011], recalling food items from the market, informing the public how to make the food safe or to avoid it completely by throwing away the incriminated food (see *original article IV* and *review II*). After a certain time, these measures have to be evaluated and when indicated modified or expanded and disseminated to the public.

Public health officials can decide on control measures already due to strong epidemiological evidence on the disease's origin, spread, and development. Since the amendment of the Austrian Food Safety and Consumer Protection Law (LMSVG) [ANONYMOUS, 2006] in 2010 [ANONYMOUS, 2010], health authorities are allowed to order market withdrawals and to recall products even without microbiological proof [PICHLER et al., 2011]. This practice is seen as a prevention measure and can lead to earlier action in order to protect the public's health.

9. Communicate findings and prevention information

Good communication with the public and other stakeholders like state or local health officials, food inspectors, hospital staff, microbiologists, epidemiologists, managers of public institutions, and the media is of utmost importance. Updated information should be given to those who need to be informed depending on the specifics of the outbreak investigation. A clear and concise documentation of the progress of the outbreak investigation is essential for internal reports or scientific publication, as well as written communication can be released to attorneys if legal action should follow. Dis-

seminating the highlights of the outbreak investigation, the results and the prevention information is also relevant for training public health students or junior epidemiologists in the team.

10. Maintain surveillance

The very last step in outbreak investigation is to maintain monitoring surveillance data after the cases in the epidemic curve have declined and the outbreak has seemingly stopped. If cases start to increase again a new hypothesis and additional investigations are needed. Maybe a consecutive secondary outbreak came up as the reservoir and a (new) way of infection still exists and control measures to combat the pathogen have to be adapted appropriately.

Limitations of field epidemiology

It happens that outbreak investigation studies are critically named a ‘quick and dirty approach‘ as the number of cases and controls is not derived from any power calculations based on a sound hypothesis and assumptions. This seem to be true, but during an outbreak situation it is mostly important to fill up the data base with as many cases as may be needed to lead to statistically significant findings. Professionals who are doing outbreak investigation or are decision makers and working in risk management or public health services should not forget that for any results from these studies, there should be biologic plausibility. [DWORKIN, 2010]

4 PUBLICATIONS

Chapter 4 contains seven original publications dealing with the topic foodborne outbreak investigation and control. Different approaches and procedural methods to conduct an outbreak investigation are demonstrated by two different outbreaks due to the pathogen *Listeria monocytogenes* serovar 1/2a and 4b. Additionally, a survey evaluating the level of hygiene and food safety knowledge of food handlers working in restaurants and catering facilities is attached to enlighten a very important part of public health measures concerning staff training and thus preventing foodborne outbreaks.

Review I

*Much P, **Pichler J**, Kasper S, Allerberger F. 2009: Foodborne outbreaks, Austria 2007. Wien. Klin. Wochenschr. (2009)121: 77-85.* Impact Factor: 0.813

The first publication gives an overview of the foodborne outbreak situation in Austria in the year 2007. Information is given about the legal situation in Austria and the European Union and the numbers of cases, hospitalisations and deaths in 2007. Additionally, data concerning causing pathogens, involved food items, modes of transmission, and locations of exposure are documented.

Original article I

*Much P, **Pichler J**, Luckner-Hornischer A, Pusker P, Trampler R, Lassnig H, Kornschober C and Allerberger F. 2007: When Epidemiological Evidence Should Suffice. Food Protection Trends, Vol. 27, No. 12: 967-970.*

In the second paper we critically discussed outbreak investigation performance and the disregard of decision makers when only epidemiological evidence is given and microbiological proof of the incriminated food is missing.

Review II

Pichler J, *Appl G, Pietzka A, Allerberger F. 2011: Lessons to be Learned from an Outbreak of Foodborne Listeriosis, Austria 2009-2010. Food Protection Trends, Vol. 31, No. 5, May 2011, pp. 268-273.*

The third paper is a review article and deals with lessons learned from an outbreak of foodborne listeriosis happening in Austria from 2009 – 2010. The incriminated food was acid curd cheese containing *L. monocytogenes* serotype 1/2a and causing 8 deaths in three European countries.

Original article II

Fretz R, Sagel U, Ruppitsch W, Pietzka AT, Stöger A, Huhulescu S, Heuberger S, ***Pichler J****, Much P, Pfaff G, Stark K, Prager R, Flieger A, Feenstra O, Allerberger F. 2010: Listeriosis outbreak caused by acid curd cheese 'Quargel', Austria and Germany 2009. Euro Surveill. 2010;15(5):pii=19477.* Impact Factor: 5.491

Paper 4 and 5 are two online articles describing the before named outbreak and highlighting the importance of routine molecular typing of *Listeria* isolates as a tool to identify outbreaks. Also the considerable potential of cross-border cooperation for elucidating chains of infections in multinational outbreaks was demonstrated.

Original article III

Fretz R, ***Pichler J,*** *Sagel U, Much P, Ruppitsch W, Pietzka AT, Stöger A, Huhulescu S, Heuberger S, Appl G, Werber D, Stark K, Prager R, Flieger A, Karpíšková R, Pfaff G, Allerberger F. 2010: Update: Multinational listeriosis outbreak due to 'Quargel', a sour milk curd cheese, caused by two different L. monocytogenes serotype 1/2a strains, 2009-2010. Euro Surveill. 2010;15(16):pii=19543.* Impact Factor: 5.491

Original article IV

Pichler J*, Much P, Kasper S, Fretz R, Auer B, Kathan J, Mann M, Huhulescu S, Ruppitsch W, Pietzka A, Silberbauer K, Neumann C, Gschiel E, DeMartin A, Schuetz A, Gindl J, Al-*

lerberger F. 2009: An outbreak of febrile gastroenteritis associated with jellied pork contaminated with Listeria monocytogenes. Wien. Klin. Wochenschr. (2009)121: 149-156. Impact Factor: 0.813

The 6th original publication describes an outbreak of febrile gastroenteritis caused by jellied pork contaminated with *Listeria monocytogenes* serotype 4b in Lower Austria in the year 2008. The epidemiological investigation (cohort study) identified the consumption of mixed cold cuts (including jellied pork) at the wine tavern as the most likely vehicle of the foodborne outbreak (*P* value = 0.0015). This hypothesis was confirmed by microbiological investigation of jellied pork produced by the tavern owner. *L. monocytogenes* was isolated from leftover food in numbers of $3 \times 10^3 - 3 \times 10^4$ colony forming units/g and was indistinguishable from the clinical outbreak isolates. The outbreak was stopped effectively and all patients recovered.

Original article V

***Pichler J**, Ziegler J, Aldrian U, Allerberger F. 2014: Evaluating levels of knowledge on food safety among food handlers from restaurants and various catering businesses in Vienna, Austria 2011/2012. Food Control. 2014; 35(1), pp. 33–40.* Impact Factor: 2.738

The last publication is a survey about evaluating the knowledge on food safety and hygiene among food handlers from different restaurants and catering businesses in Vienna. The study identified substantial knowledge gaps concerning correct temperatures for cooking, holding and storing foods. The results indicate considerable potential for further improvement in the field of food safety and kitchen staff training in Austria in order to keep up with safe food handling practices.

4.1 Review I

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Foodborne outbreaks, Austria 2007

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Lebensmittelbedingte Krankheitsausbrüche, Österreich 2007

Zusammenfassung. Im Jahr 2007 wurden in Österreich 438 lebensmittelbedingte Krankheitsausbrüche, mit 1.715 Erkrankten (davon 286 Hospitalisierte und ein Todesfall) berichtet. Für 95% aller Ausbrüche waren *Salmonella* spp. und *Campylobacter* spp. verantwortlich. Von den 438 berichteten Ausbrüchen wurden 48 (11%) im Ausland erworben. Von 390 im Inland akquirierten lebensmittelbedingten Krankheitsausbrüchen wurden 376 durch Bakterien, 11 durch Viren (Noroviren und einmal Hepatitis-A-Virus), zwei durch Intoxikation (einmal durch Staphylococcus aureus Enterotoxin, einmal durch Alkaloide) verursacht. Bei einem Ausbruch wurde als Erreger unbekannt angegeben. Die bakteriell bedingten Ausbrüche teilen sich wie folgt auf: 264 (70%) durch *Salmonella* spp., 104 (28%) durch *Campylobacter* spp., sechs Ausbrüche durch enterohämorrhagische *Escherichia coli* (EHEC O145:H-, O157:H-, O157:H7, O182: H49, O91:H7, ONT:H4), jeweils ein Ausbruch durch *Shigella flexneri* und *Shigella sonnei*.

Hospitalisiert wurden 22% bzw. 14% der im Inland an einem Ausbruch durch *Salmonella* spp.- bzw. *Campylobacter* spp. erkrankten Personen. Das Lebensmittel Ei zeichnete alleine für 49% aller inländischen Ausbrüche mit berichteter Infektionsquelle verantwortlich. Fleisch (besonders Geflügelfleisch) war für 44% der inländischen Ausbrüche und Fisch für 2% verantwortlich. Das Verhältnis Haushaltsausbrüche zu allgemeinen Ausbrüchen beträgt 82,3% zu 17,7%. Bei 54 von 62 allgemeinen inländischen Ausbrüchen wurden folgende Orte der Exposition benannt: öffentliche Verköstigungen (z.B. Restaurant, Hotel) 24-mal, Familienfeier, Kindergarten, Take-away oder Grillfest 22-mal, Altersheime oder Krankenhäuser 8-mal. Der hohe Anteil an Haushaltsausbrüchen reflektiert unseres Erachtens nach vor allem die noch nicht vollständig ausgeschöpfte epidemiologische Qualität der Ausbruchsabklärungen, i.e. fehlende Zusammenführung einzelner kleiner lebensmittelbedingter Ausbrüche zu bezirks- und bundesländerübergreifenden Ausbrüchen.

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Summary. In 2007 Austria reported a total of 438 foodborne outbreaks affecting 1715 people, including 286 hospitalized patients and one death. *Salmonella* spp. and *Campylobacter* spp. accounted for 95% of all reported outbreaks. Forty-eight (11%) of the 438 Austrian outbreaks were acquired abroad. Of the 390 domestically acquired foodborne outbreaks, bacterial infection caused 376, viruses (norovirus and 1-time Hepatitis A Virus) caused 11, and intoxications (*Staphylococcus aureus* enterotoxins, alkaloid toxins) caused two. In one outbreak the causative agent was unknown. *Salmonella* spp. caused 264 (70%) of the bacterial outbreaks, *Campylobacter* spp caused 104 (28%), enterohemorrhagic *Escherichia coli* (EHEC O145:H-, O157:H-, O157:H7, O182: H49, O91:H7, ONT:H4) caused six, *Shigella flexneri* and *Shigella sonnei* each caused two.

The hospitalization rates were 22% for domestically acquired infections with *Salmonella* spp. and 14% for *Campylobacter* spp. Among outbreaks where the source was known, eggs were implicated in 49%, meat products (especially poultry) in 44% and fish in 2%. The ratio of household outbreaks to general outbreaks was 82.3% to 17.7%. In 54 of the 62 general domestic outbreaks the following locations of exposure were documented: commercial food suppliers (e.g. restaurants, cafeterias) 24 times, family celebrations, nursery schools, take-aways and barbecues 22 times, nursing homes and hospitals eight times. It is likely that the relatively high number of household outbreaks reflects an insufficient level of epidemiological investigation of outbreaks in Austria. More resources may be needed for identification of individual clusters that belong to larger foodborne outbreaks exceeding district or provincial borders.

Key words: Outbreak investigation, foodborne infection, zoonosis.

Introduction

Since June 12, 2004 Austria has implemented Directive 2003/99/EC of the European Parliament and the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents [1]. Protecting human health against disease and infection transmissible directly or indirectly between animals and humans is of paramount importance. Although there has been a steep increase in the number of reported foodborne bacterial

illnesses since the 1960s, health authorities did not immediately take steps to address the problem. In 1992 there were more than 13,000 microbiologically confirmed human salmonellosis infections reported in Austria, prompting the implementation of prevention measures. It is assumed that 95% of human salmonella infections are foodborne [2]. According to the 1950 National Epidemic Act, all cases of notifiable disease, including suspected and fatal cases, must be reported to health authorities [3]. The 2005 National Zoonoses Act requires that all foodborne outbreaks must be investigated epidemiologically and microbiologically, and that every investigation must provide a short report summarizing its findings and the measures implemented to correct the situation [4].

Microbiologically confirmed foodborne cases are considered to be the tip of the iceberg. However, noncompliance among physicians does not seem to be a major issue since there were 3587 reported cases of salmonellosis in 2007 and 4050 human primary isolates were sent to the National Reference Center for Salmonella at AGES [5, 6]. In estimating the burden of disease within a population it is important to recognize that many people do not visit a physician when they become ill with gastrointestinal disease and that not all physicians send in stool samples for microbiological verification. Microbiologically confirmed salmonella cases account for only 2.6–6.9% of the estimated total number of salmonella infections [2, 7] and thus it may be calculated that actual cases of salmonella infection in Austria in 2007 reached approximately 60,000–160,000. Although data may be missing from the true scale of Austrian foodborne infections and outbreaks, interpretation and evaluation of the available reported outbreaks for 2007 remains important.

Materials and methods

Definitions of foodborne outbreaks and cases

A "foodborne outbreak" refers to an incidence, observed under given circumstances, of two or more human cases of the same disease and/or infection, or a situation in which the observed number of cases exceeds the expected number and where the cases are linked, or are probably linked, to the same food source [1]. An outbreak includes all cases of the same disease that have an epidemiological relationship, such as the same exposure as a confirmed case (eating foods from the same source). All cases that cannot be counted as outbreak cases are classified as sporadic cases. The case definitions used in notification of communicable diseases to health authorities follow the guidelines of the decision of the European Commission 2002/253/EG [8].

Data collection

Every year, disease notification tables, which were developed by the European Food Safety Authority (EFSA), are sent to each of the nine Austrian provinces and the municipality for health in Vienna. Each foodborne outbreak for the previous year is recorded using the following criteria: identification code of the outbreak, causative agent, type of outbreak (general or household), country of infection (travel-associated or domestic origin), number of cases/hospitalizations/deaths, foodstuff implicated, type of evidence, setting/location of exposure, place of the origin of the problem, origin of the food stuff and other contributing factors [9]. In addition to the tables, each outbreak investigation should include a summary report.

Compilation of data from each province

The yearly tables submitted from each province are reviewed for credibility and completeness. Where there are missing data, provinces are requested to provide these for later addition to the tables. Outbreak cases that have an epidemiological link, i.e. those that carry the same outbreak identification code, are grouped together. Cases can be associated with an outbreak at various levels: within a district, within a province, or at the national or international level. An outbreak investigation may be able to combine multiple outbreaks into one larger epidemiologically linked outbreak; this can affect the case numbers associated with the outbreaks. In principle, outbreaks are counted and analyzed in the same year as their start date (i.e. the date on which the first case was reported).

Electronic data entry

The EFSA tables are in the form of a Microsoft® Office Excel 2003 document (Microsoft, USA). RegioGraph version 8 (Macon, Germany) is used for geographic evaluation of outbreaks.

Results

Case numbers

Austria reported 438 foodborne outbreaks in 2007; these involved 1715 cases and resulted in 286 hospitalizations and one death (Table 1). Although originally the nine

Table 1. Numbers of foodborne outbreaks and persons affected, Austria 2003–2007

Year	2003	2004	2005	2006	2007
Total outbreaks	7	539	606	609	438
Number of persons taken ill	232	1771	1910	2530	1715
Number of hospitalized persons	NA	224	368	493	286
Number of deaths	0	1	1	3	1
Household outbreaks	6	481	541	515	364
General outbreaks	1	58	65	94	74

NA not available.

Austrian provinces reported a total of 469 foodborne outbreaks (Table 2) involving 1810 people (346 hospitalizations and one death), some outbreaks were combined when epidemiological links were found. Examples include three outbreaks caused by *S.* Enteritidis phage type (PT) 8 acquired abroad and reported in more than one province, and five individual outbreaks of *S.* Enteritidis PT1 compiled into one outbreak involving five different provinces. In addition, four norovirus outbreaks were excluded since the transmission mode was from person to person.

Case numbers according to causative agents

Outbreaks due to salmonella (n = 305) and campylobacter (n = 108) caused 95% of all outbreaks but counted for only 70% of persons taken ill (n = 1203) in foodborne outbreaks. A further 26% of cases were due to norovirus (10 outbreaks, 281 cases) and *Staphylococcus aureus* enterotoxin (one outbreak, 166 cases) (Table 3). The remaining 4% of cases were due to enterohemorrhagic *Escherichia coli* (EHEC), shigella, alkaloid intoxication or were of unknown origin.

A total of 26.8% (962/3587) of reported salmonellosis cases, but only 4% of reported campylobacter cases (n = 6077), were involved in outbreaks. Among reported EHEC cases (n = 87) 20% were linked to an outbreak and among shigellosis cases (n = 141) 9% were linked to outbreaks (Table 4). No outbreaks due to yersiniosis or listeriosis were reported. Of 900 persons who became ill with norovirus, 31.2% (n = 281) were involved in an outbreak.

Travel-associated foodborne outbreaks

Of the 438 foodborne outbreaks, 48 (11%) were acquired abroad. The following countries were documented as locations of infection: Greece nine times, Turkey eight times, Croatia seven times, Bosnia, Serbia, Egypt and Slovenia each three times, Czech Republic, Italy, Hungary and Rumania each twice, and Tunisia, Spain, Morocco and India each once. A total of 44% of reported Austrian foodborne outbreaks acquired abroad could be traced to EU Member States (MS).

Two examples of successful outbreak investigations in 2007 are described:

- *International* Salmonella *Enteritidis PT8 outbreak associated with a Formula One race in Hungary 2007*: 31 visitors to this race were infected with *S.* Enteritidis PT8. Those taken ill came from Hungary, Sweden, Norway, Finland, the Netherlands and Austria [10]. The epidemiological and microbiological investigation in Hungary found that eight of the cases came from Austria, involving three different provinces. The source of infection was identified as a mixed buffet (suspect food item: chicken with rice and egg) for which food items were prepared and delivered from a restaurant in Budapest. The investigation found that the chicken had not been cooked to an adequate internal temperature and foods were insufficiently cooled during delivery and storage.
- *Hotel-associated outbreak of* Salmonella *Enteritidis PT6 in Turkey:* A minimum of 70 cases of *S.* Enteritidis PT6 were identified among 2879 Austrian high school students vacationing at the Turkish resort complex. The most likely source of infection was breakfast eggs that were prepared "sunny side up".

Domestic outbreaks

Of 390 reported domestic outbreaks, 328 (84%) were categorized as household outbreaks and 62 (16%) as general outbreaks. The number of new cases per 10,000 people (incidence) associated with domestic outbreaks and the number of domestic outbreaks per province are shown in Fig. 1. Where an outbreak has taken place in more than one province the outbreak is counted in each one, increasing the total number of outbreaks among all provinces in 2007 to 395.

Table 2. Numbers of reported foodborne outbreaks and persons affected per province in 2007 (domestically and internationally acquired infections)

	Reported outbreaks		General outbreaks		Household outbreaks		Numbers of persons affected		
	n	%	n	%	n	%	Illness	Deaths	Hospitalized
Burgenland	17	3.6	4	23.5	13	76.5	57	0	15
Carinthia	36	7.7	3	8.3	33	91.7	94	0	26
Lower Austria	64	13.6	8	12.5	56	87.5	417	0	36
Upper Austria	105	22.4	15	14.3	90	85.7	472	1	149
Salzburg	48	10.2	13	27.1	35	72.9	261	0	22
Styria	31	6.6	21	67.7	10	32.3	138	0	30
Tyrol	75	16.0	13	17.3	62	82.7	154	0	19
Vorarlberg	7	1.5	0	0	7	100.0	18	0	4
Vienna	86	18.3	6	7.0	80	93.0	199	0	45
Austria (total per province)	**469**	**100**	**83**	**17.7**	**386**	**82.3**	**1810**	**1**	**346**
Austria corrected	**438**		**74**	**16.9**	**364**	**83.1**	**1715**	**1**	**286**

Table 3. Domestically and internationally acquired infections according to pathogen, including numbers of persons affected or hospitalized

	Number of foodborne outbreaks	Number of persons taken ill	Average number of affected persons per outbreak	Number of hospitalized persons	Percentage of hospitalized persons among affected persons
Domestically acquired infections	**390**	**1514**	**3.9**	**253**	**16.7**
Salmonella spp.	264	777	2.9	172	22.1
S. Enteritidis	236	706	3.0	152	21.5
PT8	88	279	3.2	60	21.5
PT4	91	264	2.9	52	19.7
PT21	27	67	2.5	11	16.4
S. Typhimurium	16	42	2.6	10	23.8
Campylobacter spp.	104	233	2.2	33	14.2
Campylobacter jejuni	73	164	2.2	33	20.1
Campylobacter coli	2	6	3.0	0	0
EHEC	6	17	2.8	4	23.5
Shigella spp.	2	5	2.5	2	40.0
Staphylococcus aureus enterotoxin	1	166	166.0	2	1.2
Norovirus	10	281	28.1	6	2.1
Hepatitis A virus	1	22	22.0	13	59.1
Alkaloid intoxication	1	8	8.0	7	87.5
Unknown pathogen	1	5	5.0	0	0
Internationally acquired infections	**48**	**201**	**4.2**	**33**	**16.4**
Salmonella spp.	41	185	4.5	29	15.7
Campylobacter spp.	4	8	2.0	2	25.0
Shigella spp.	3	8	2.7	2	25.0
Total	**438**	**1715**	**3.9**	**286**	**16.7**

Table 4. Comparison of numbers of persons affected in outbreaks with numbers of reported cases and numbers of laboratory-confirmed cases, 2007

	Infected persons involved in an outbreak	Number of reported cases	Number of laboratory confirmed cases
Salmonella spp.	962	3587	4050
Campylobacter spp.	241	6077	NA*
EHEC	17	87	93
Yersinia spp.	0	150	146**
Shigella spp.	13	141	138
Listeria spp.	0	11	20
Norovirus	281	900	NA*

* *NA* Not available, not all isolates were analyzed by the National Reference Laboratories;** Of the 146 isolates, 117 were pathogenic.

Among the domestically acquired foodborne outbreaks, 376 (96%) were caused by bacteria, 11 (3%) by viruses (norovirus and hepatitis A) and two by toxins (*Staphylococcus aureus* enterotoxin and alkaloid intoxication). For one outbreak no causative agent was identified.

Salmonella spp. caused 264 (67%) of the outbreaks, *Campylobacter* spp caused 104 (28%), enterohemorrhagic *Escherichia coli* caused six (EHEC O145:H-, O157:H-, O157:H7, O182:H49, O91:H7, ONT:H4) and *Shigella flexneri* and *Shigella sonnei* each caused one. Among the 264 salmonella outbreaks, *S.* Enteritidis was identified as the causative agent in 89.4% (n = 236); the majority of the others were caused by *S.* Typhimurium (n = 16) or the serotype was unknown or not given. Among the 104 foodborne outbreaks caused by *Campylobacter* spp., *C. jejuni* was responsible for 73 outbreaks, *C. coli* was identified in two and on one occasion both species were isolated from patients stools in the same outbreak. The bacterial species was not identified in 28 of the out-

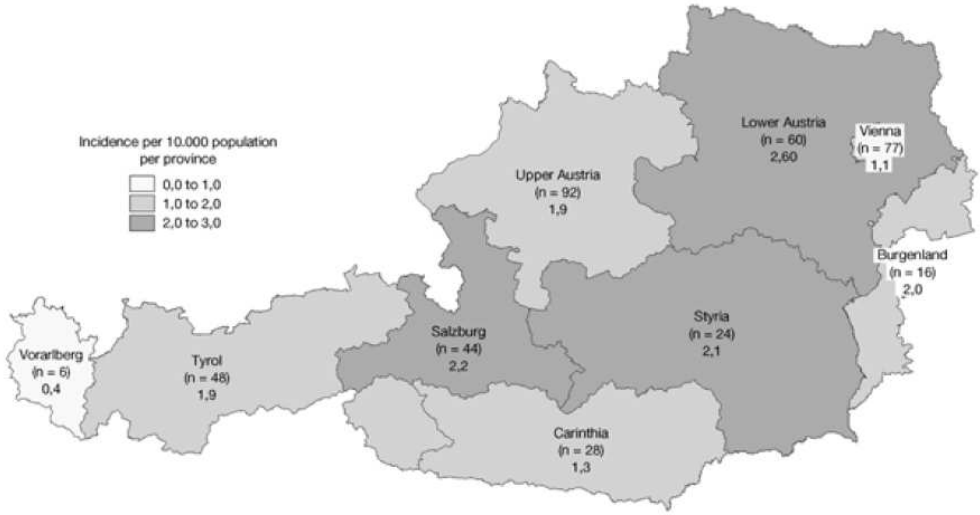

Fig. 1. Incidence (per 10,000 people) of all domestically acquired infections and outbreaks per province in 2007. National average of 1.8 outbreaks per 10,000. The number of outbreaks reported by each province differs from the total number of reported outbreaks in Austria to account for two outbreaks that involved more than one province

breaks. Hospitalizations in domestically acquired salmonella and campylobacter outbreaks were 22% and 14%, respectively.

Table 3 lists the internationally and domestically acquired foodborne outbreaks in order of pathogen importance and includes the numbers of persons taken ill and the numbers hospitalized. In 63% of domestically acquired outbreaks the source of infection was not identified. Eggs were the primary source of infection in 18%, meat, especially poultry, was responsible for 16% and fish/fishery products for 1%. A total of 114 (80%) outbreaks were caused by salmonella and 23 (16%) by campylobacter. More information can be found in Table 5.

The location of exposure was identified in 54 of 62 general domestically acquired outbreaks. Further categories can be seen in Fig. 2.

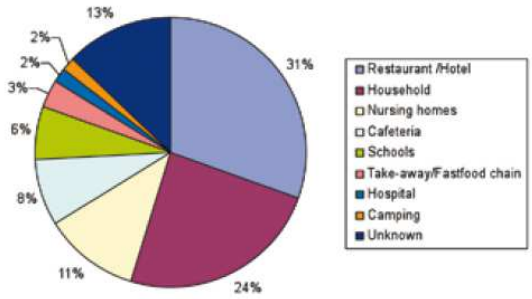

Fig. 2. Location of exposure – domestically reported outbreaks

Table 5. Food categories as source of domestically acquired infection

Food categories	N	Percentage	Food items
Unknown	246	63	
Eggs	71	18.2	36 × fried, scrambled or raw eggs, 14 × desserts (e.g. tiramisu, curd cheese), 5 × dumplings, 4 × mayonnaise, 3 × raw dough, 3 × ice cream, 2 × cake, 2 × pizza, 1 × egg dumpling, 1 × spread
Poultry meat	36	9.2	30 × chicken, 5 × turkey, 1 × duck
Meat	28	7.2	7 × pork, 1 × beef, 1 × lamb, 15 × unknown (e.g. 4 × sausage, 3 × minced meat, 2 × grilled sausage, 1 × kebab, 3 × barbecue, 1 × gravy meat, 1 × meat with rice)
Fish	3	0.8	1 × fish, 1 × fish soup, 1 × sushi
Milk	1	0.3	1 × milk
Other	5	1.3	water, infant formula, vegetables
Total	**390**	**100**	

Investigations of seven of the domestically acquired foodborne outbreaks in 2007 are described:

- *National outbreak of* Salmonella *Enteritidis PT1:* In June 2007 a descriptive epidemiological outbreak investigation identified 20 of 73 microbiologically confirmed *S.* Enteritidis PT1 cases as linked to eggs from a contaminated laying hen facility. The egg producer, who had 20,000 birds, provided eggs to a nationwide supermarket chain, which resulted in a nationwide spread of contaminated eggs. The vehicle of infection was identified in 10 persons who ate Schomlauer Nockerl at a local wine tavern. The dessert was prepared with raw eggs from the implicated egg farm. The farm was sanitized and no further PT 1 cases were reported in Austria in 2007.
- *A supermarket-associated outbreak of hepatitis A virus:* In one Austrian province, 22 people were infected with hepatitis A virus from one supermarket. The infection was caused by a butcher who tested positive for hepatitis A and who had contaminated food items through inadequate hygiene practices (hand washing) [11].
- *A* Staphylococcus aureus *enterotoxin outbreak at a school:* In Lower Austria, 166 students were intoxicated with *Staphylococcus aureus* enterotoxin present in their pasteurized lunch milk. The toxin was identified in the cows' milk and in milk equipment (tubing) at the dairy farm. Local investigators concluded that a breakdown in the application of HACCP guidelines caused the outbreak.
- *Alkaloid intoxication caused by the mistaken consumption of Laburnum blossoms:* In May 2007, Golden Chain blossoms (*Laburnum anagyroides)* were mistaken for Acacia blossoms and served as a dessert at a senior center [12]. Eight people suffered from vomiting and seven of these were hospitalized. The alkaloid toxins were found in leftover food and human isolates tested positive for the toxin.
- *Outbreak of* Salmonella *Enteritidis PT8 at a prison:* An *S.* Enteritidis PT8 outbreak affected at least seven people at a prison in Austria; however, the source of infection remained unknown and the outbreak was not reported to the local health authorities [12]. This outbreak was not included in the 390 domestically acquired foodborne outbreaks.
- *Case clusters of* Salmonella *Typhimurium definitive type (DT) 46:* In October 2007, 14 *S.* Typhimurium DT46 cases were recorded in two Austrian provinces. Five cases were identified as a household outbreak where the infection came from their own laying hens (14 birds). Although four more cases with a common workplace and cafeteria and two additional household outbreaks were identified within the same period, no common source of infection could be established and therefore these cases could not be classified as a national outbreak involving more than one province.
- *Case clusters of* Salmonella *Typhimurium DT104L:* At the beginning of 2007 there was an increase in the number of cases involving *S.* Typhimurium DT104L resistant to five antibiotics (ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline). Children 5–14 years old were the most affected. Molecular subtyping of human isolates using multiple locus variable number tandem repeat analysis (MLVA) revealed patterns indistinguishable from each other but different from that of an epidemiologically unrelated DT104L, therefore five seemingly unrelated household outbreaks and several sporadic single cases were classified as one Austrian-wide outbreak.

Discussion

Obtaining a true picture of the burden of gastrointestinal infections circulating in the community is difficult. Surveillance systems mostly focus on foodborne disease, the data coming mainly from large outbreaks in restaurants, hospitals etc, whereas sporadic cases, particularly milder infections in the home, go largely unreported [13]. Community-based studies in the UK [14], the Netherlands [15] and Austria [16] suggest that foodborne infections represent only a fraction of the total burden of gastrointestinal infections. The 2003 WHO report stated that, among all outbreaks reported in Europe, 60% in 1999 and 69% in 2000 were due to person-to-person rather than foodborne transmission [17]. The UK community-based study, 1993–1996, estimated that only one in 136 cases of gastrointestinal illness is detected by surveillance and that for every one reported case of campylobacter, salmonella, rotavirus and norovirus another 7.6, 3.2, 35 and 1562 cases, respectively, occur in the community. The incidence of non-foodborne infections in the UK is estimated at around 4.5 million cases per year, the largest proportion of which are norovirus infections transmitted easily from person to person within community groups [18].

The Zoonoses Report published by EFSA and the European Centre for Disease Prevention and Control (ECDC) in 2007 estimated that one third of the populations in developed countries are affected by foodborne diseases every year [19, 20]. In a 1999 review Sattar et al stated that as many as 99% of these infections go unreported because of the relatively mild symptoms; the true impact of foodborne illness is thus difficult to determine [21].

Evidence-based measures for the prevention of foodborne diseases require information on the chain of infection [22]. "If foodborne zoonotic outbreaks are investigated, then the causative pathogens can be identified within food products and preventative measures can be implemented during food production or food processing" [4]. Through the 2005 Zoonoses Act, the heads of every Austrian province are mandated to conduct complete epidemiological investigations of every reported foodborne outbreak [4].

The increase in the number of foodborne outbreaks reported in Austria from seven in 2003 to 609 in 2006 reflects the dramatic improvements made by the surveillance system in the past few years. The reduction of reported outbreaks in 2007 (n = 438) already shows the

improved quality of epidemiological outbreak investigations. Since an outbreak investigation may be able to combine independently reported cases or individual outbreaks into one epidemiologically linked outbreak, this reduces the number of individual outbreaks and sporadic cases respectively. In addition, implementation of control measures has led to reduction of salmonella contamination in eggs and has decreased the number of reported salmonellosis cases from 7582 in 2003 to 3587 in 2007. The proportion between household outbreaks and general outbreaks in 2007 remains unchanged from that of 2006: 86% to 14% [23]. This high proportion of household outbreaks is indicative of the need to further improve the quality of epidemiological outbreak investigations; for example, where there is failure to identify epidemiologically linked regional outbreaks as belonging within a larger outbreak. According to the national bulletin on notifiable diseases from the Ministry of Health, Family and Youth, 11,348 people became ill with bacterial or viral foodborne infections in 2007 [6]. Of these, only 1715 people (15%) could be assigned to the 438 foodborne outbreaks reported in that year. The remaining 9633 were categorized as sporadic single cases; this number may be reduced through improved epidemiological outbreak investigation. In 2007, the incidence of all domestically acquired foodborne infections for all provinces was 0.4–2.6 cases per 10,000. It is interesting to note that the range of incidences from all provinces in Austria in 2007 (Fig. 1) is lower than that reported for 2006 (0.5–7.5) [24]; the reason for this decrease may also be due to improved efforts to conduct outbreak investigations among all provinces.

The 2003 WHO report concluded that about 40% of reported foodborne outbreaks in the WHO European Region occur in private homes [17]. The potential for food poisoning at home is indicated by the prevalence of food-related pathogens in products purchased from retail premises. The ECDC review estimated that campylobacter were most commonly detected in fresh poultry meat, with an average of 35% positive samples. Salmonella was most commonly found in fresh poultry and pork meat, with 5.6% and 1.0% positive samples. Chapman et al showed that 0.4–0.8% of meat products purchased from butchers in the UK were positive for *Escherichia coli* O157 [25]. Household outbreaks have the advantage that members within a single household generally bear the same family name and are therefore more easily linked. Nevertheless, outbreaks caused by contaminated foodstuffs that are distributed throughout Austria often result in a number of seemingly sporadic single cases where no epidemiologic link has been established. As a result of an outbreak investigation in 2004, 36 microbiologically confirmed *S.* Enteritidis PT36 cases which involved four provinces could be linked to one contaminated laying hen facility [26]. The flock was culled and the facility was sanitized. Following this outbreak investigation, there were no further cases of *S.* Enteritidis PT36.

Worldwide, bacterial foodborne zoonotic infections are the most common cause of reported cases and outbreaks of human infectious intestinal disease, with salmonella and campylobacter accounting for over 90% of all reported cases of bacteria-related food poisonings [27]. Similarly to 2006, salmonella and campylobacter were responsible for 95% of all Austrian outbreaks in 2007 [24], although only 305 outbreaks associated with salmonella were reported, compared with 452 outbreaks in 2006; in addition, the number of persons taken ill in salmonella outbreaks decreased by half from 1822 to 962 over the same period.

As in previous years, the number of reported campylobacter cases increased in 2007, to a total of 6077; in contrast, fewer campylobacter outbreaks were reported and the number of campylobacter outbreak-related cases decreased by 20% from 302 in 2006 to 241 in 2007. In general in 2007, household outbreaks caused by campylobacter affected an average of only 2.2 people, whereas salmonella outbreaks affected 3.2 people.

A 2007 report on infectious disease outbreaks in Germany evaluated data from 30,578 German outbreak reports for 2001–2005 [28]. The most common settings among the 10,008 entries for 9946 outbreaks in 2004 and 2005 were households (53%). Among these outbreaks, 90% were caused by pathogens of the intestinal tract (e.g. salmonella, norovirus, rotavirus, hepatitis A virus, enteropathogenic *E. coli*, campylobacter). Whereas German households were reported as the most frequent settings for outbreaks associated with salmonella, rotavirus and campylobacter (accounting for 38%, 25% and 14% of total outbreaks, respectively), this was not the case for norovirus, where hospitals and nursing homes were cited as the setting for 66% of reported outbreaks and the household for only 13%. Indications are that norovirus is now the most significant cause of infectious gastrointestinal illness in the developed world, both outbreak-related and endemic [27, 29, 39].

The most important pathogens associated with foodborne outbreaks and the resulting hospitalizations are shown in Table 3. In 2007, 236 foodborne outbreak cases (19.6%) were hospitalized with salmonella or campylobacter infection. Similarly to the outbreak cases, 16.5% of all individual reported cases of salmonella and campylobacter were hospitalized (cases are classified according to the International Statistical Classification of Diseases and Related Health Problems, ICD-10). In Austria in 2006, 493 hospitalized persons were involved in foodborne outbreaks [24]; in 2007 this number decreased to 286.

The three most common sources of infection in Austria were eggs, meat (especially poultry) and fish; these data correspond with those of the Community Summary Report of Zoonoses in 2006 (eggs: 17.8%, meat: 14.5%, fish: 4.6%). In Austria, the source of infection was known in only 37% of the outbreaks; no source of infection was given by reporting MS in 42.6% of outbreaks [31].

The three most common locations of exposure are similar in Austria and the EU as a whole: in 2007 restaurants/hotels in Austria were implicated in 31% locations of exposure, households in 24% and the location of exposure was unknown in 13%; the average EU location of

exposure included restaurants/hotels in 19.8%, households in 46.4% and unknown locations in 12% [31].

Globalization has expanded food trade, migration and tourist travel, which further increases the need for international monitoring and outbreak investigation [22]. The level of detail in data reported by EU MS varies greatly in relation to disease monitoring. The EU has recognized the need for a centralized and coordinated system for outbreak data collection within MS. Foodborne disease outbreaks were summarized at the Community level for the first time beginning in 2004 [32] and in 2005 reporting became mandatory. Year to year, as this centralized reporting system improves, an increase in the number of reported outbreaks may be expected [32]. At European level, a total of 5710 outbreaks were reported for 2006, which was 6.6% more than in 2005 [33], the increase possibly attributable to implementation of improved reporting structures within MS. However, the degree of detail in reported data received from MS remains varied. The completeness of outbreak reporting systems depends on two components: the ability to detect outbreaks at the local level and accuracy of reporting to the national authority. Most MS indicated that outbreaks are under-reported in relation to these two factors [32]. The thorough disease notification structure in Austria may also explain why single outbreak numbers are higher for Austria than for other countries included in the EFSA Community Report.

In the study by de Jong and Ekdahl, high correlation was found between the numbers of reported salmonellosis cases among returning travelers and the prevalence of salmonella in laying hen flocks in the destination countries. Although the highest prevalences of salmonella were found in laying hen flocks in Portugal, Poland and Spain, the annual incidence of salmonellosis cases reported in those countries is much lower [34]. An EU average of 1.2 outbreaks per 100,000 people was reported among the 22 EU MS in 2006. The highest number of outbreaks per 100,000 was reported in Latvia, with 13.5 outbreaks per 100,000; Austria reported 7.4 outbreaks per 100,000 and Germany 1.7 outbreaks per 100,000 [32]. However, based on de Jong and Ekdahl's findings in laying hen flocks, countries such as Portugal, Spain and Greece should have also reported high numbers of outbreaks per 100,000. According to EFSA, Portugal reported 0.1 outbreaks per 100,000 in 2006, Spain reported 0.8 outbreaks per 100,000 and Poland reported 1.5 outbreaks per 100,000. This indicates that the quality of reported data may be under representative. Furthermore, de Jong and Ekdahl, using the Swedish Tourist Database, have calculated the estimated burden of salmonella disease in EU MS. Numbers of returning travelers who had fallen ill with salmonella were used to estimate an annual incidence of the disease within each country. Based on these calculations, the risk of contracting salmonella in Portugal was 79.9 per 100,000 travelers, the risk in Poland was 76.5 per 100,000 travelers and in Spain 72 per 100,000 [35]. Although in 2006 Portugal, Poland and Spain reported a lower number of outbreaks per 100,000 people in comparison with Austria, there was a higher risk of infection with salmonella among the travelers visiting these countries. This may indicate that the level of reporting in these countries is not as detailed as the level of reporting in Austria, and not that there is a higher rate of outbreaks in Austria.

The 2003 WHO report cites factors such as the use of contaminated raw food ingredients, contact between raw and cooked foods, and poor personal hygiene in food handlers as "critical for a large portion of foodborne diseases" [17]. A key aspect stated in the WHO report is that "foodborne illness is almost 100% preventable". In order to achieve that position an overview of the sources of infection and transmission routes is essential. Thorough investigation of foodborne outbreaks is a critical part of this process. The readiness of the treating physician to submit patient samples for microbiological testing is also important; without regular laboratory confirmation of infectious diseases, the implementation of adequate public health measures to prevent additional disease outbreaks cannot be achieved.

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4.2 Original article I

Food Protection Trends, Vol. 27, No. 12, Pages 967–970

When Epidemiological Evidence Should Suffice

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SUMMARY

Each year *Salmonella* transmitted via eggs cause many foodborne outbreaks. Because of European legislation (Zoonoses Directive 2003/99/EC) and national requirements (e.g., the Zoonoses Act 128/2005 in Austria), more and more of these outbreaks are investigated. Frequently the infectious vehicle can be found by epidemiological studies and the source of infection confirmed by microbiological testing of fecal and environmental samples from incriminated flocks of laying hens, but proof of the infectious vehicle cannot be obtained because the outbreak strain cannot be detected in the incriminated food. In Austria, two outbreaks have been traced back to one egg production plant. We report on three further outbreaks linked to this egg production plant that again did not lead to proper actions by health authorities because of missing microbiological proof in the eggs originating from the incriminated flocks.

INTRODUCTION

Because of European legislation (Zoonoses Directive 2003/99/EC) and national requirements (e.g., the Zoonoses Act 128/2005 in Austria) more and more foodborne outbreaks are investigated *(2, 3)*. Recently Schmid et al. reported on outbreaks of *Salmonella* Enteritidis phage type (PT) 4 and PT6 in Austria in November 2005 and June 2006 *(7)*. Although epidemiological findings clearly implicated egg producing plant A as the source of these 58 cases, no restrictions were placed on the incriminated flocks. Regardless of sampling of fecal droppings and dust between January and July 2006 revealed 11 different *Salmonella enterica* subspecies *enterica* strains, laying hens were kept in production until they stopped laying because of age in September 2006. Microbiological investigation of 1,200 eggs (examined after shell disinfection) collected in May 2006 had yielded no *Salmonella*. In June 2006, a cluster of 23 cases of *S.* Enteritidis PT6 infection was again associated with this egg production plant A *(7)*. Schmid et al. complained that scientific evidence provided by analytical epidemiological studies — in her case, a cohort study — is often disregarded by decision makers *(7)*. We now report on three further

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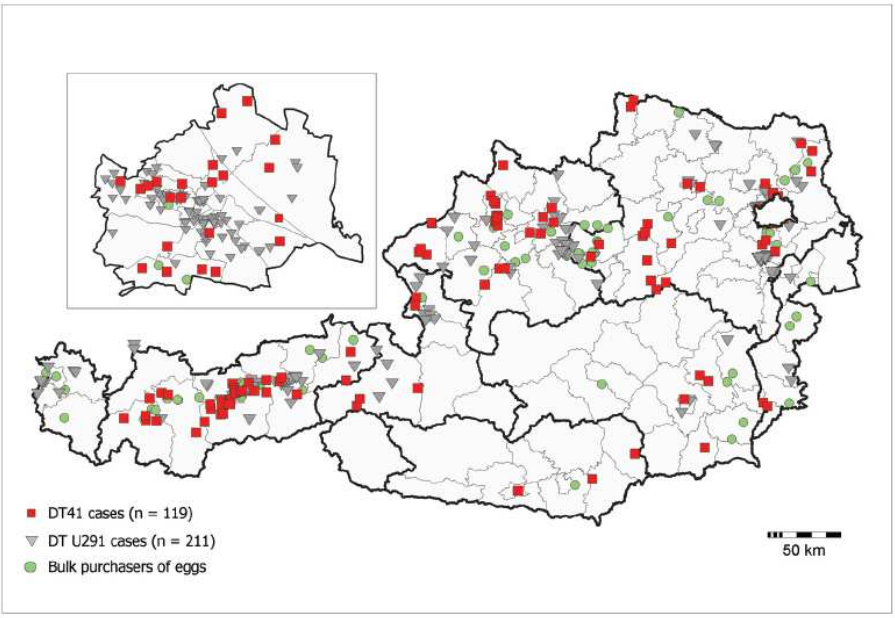
FIGURE 1. Geographical distribution of human outbreaks – and sporadically cases caused by *S.* Typhimurium DT41 and *S.* Typhimurium DT U291, and the location of bulk purchasers of the eggs produced at plant A in Austria, January 2003 to February 2007



outbreaks linked to this egg production plant A, outbreaks that again did not lead to proper actions by health authorities because of absence of microbiological proof in the incriminated food.

OUTBREAK INVESTIGATIONS

Outbreak I

In May 2006, ten persons were affected by a *S.* Typhimurium definitive type 41 (DT41) outbreak in the Austrian province of Tyrol. The epidemiologically most likely source for this outbreak was a cream cake produced with "pasteurized" eggs and sold at a local coffeehouse/bakery; all ten laboratory-confirmed cases had consumed this food item. The pasteurized eggs could be traced to a producer who purchased from the previously mentioned egg production plant A, a laying hen farm that also supplies eggs to egg packing station B. Raw eggs used at the coffeehouse were traced to this packing station B. A sample of pasteurized egg from a container in use at the bakery yielded *S.* Enteritidis PT4, the same phage type causing the outbreak reported by Schmid et al. *(7)*. Again, microbiological investigation of 12 table eggs collected in May 2006 at the coffeehouse yielded no *Salmonella*. *S.* Typhimurium DT41 was one of the 11 different serotypes discovered by sampling of fecal droppings and dust at the farm in February 2006. We hypothesize that the pasteurized egg was possibly cross contaminated with *S.* Typhimurium DT41 (originating from contaminated table eggs) in the bakery or that the bakery did not use pasteurized egg as claimed. *S.* Typhimurium DT41 was repeatedly discovered in dust samples of egg producing plant A.

Outbreaks II and III

In October 2003, two outbreaks with *S.* Typhimurium DT U291, affecting a total of 37 cases, occurred in Tyrol. A hotel-associated outbreak comprised 24 laboratory-confirmed cases and six epidemiologically related clinical cases (without laboratory confirmation). The patients were Austrian and German tourists and employees. Case series data indicated that illness was associated with consumption of a house-made tiramisu, which was served in the hotel on the evening of October 4 and the following morning and which was finished off by employees during the next two days. All cases had consumed this house-made tiramisu.

On October 21/22 a family outbreak affected seven persons. These seven laboratory-confirmed cases caused by *S.* Typhimurium DT U291 were linked by common time and place of exposure to a home made tiramisu consumed on October 20. Epidemiological investigations revealed that the eggs used for preparing the tiramisus in both outbreaks were bought from egg handlers who purchased their eggs from the same egg wholesaler. A total of 68 egg producing farms who supplied egg packing station B, which served this

wholesaler (information provided by the packing station B), were screened in June 2004 for *Salmonella* (testing pooled fecal samples). While 12 of 68 (16%) farms tested positive for *Salmonella*, none yielded *S.* Typhimurium DT U291. In February (as in July) 2006, when *S.* Enteritidis PT4 was being sought as described by Schmid et al. *(7)*, dust samples of one flock of laying hens of the egg producing plant A yielded *S.* Typhimurium DT U291. Eggs of plant A had been sold to egg packing station B.

DISCUSSION

Foodborne outbreaks often wax and wane, thereby lulling decision makers into the misconception that the problem will disappear by itself. In all the above mentioned outbreaks, decision makers misinterpreted negative results of microbiologically tested food samples as evidence against a causal association between egg producing plant A and the outbreaks. Our report of three further *Salmonella* outbreaks connected to egg production plant A underlines the importance of epidemiological evidence for a targeted approach against foodborne diseases.

For none of these three outbreaks were analytical epidemiological studies performed by the respective local health authorities. Nevertheless, the epidemiological findings for outbreak I (all ten laboratory confirmed cases had consumed cream cake produced with eggs from egg producing plant A, known to harbor the causative microorganism) and for outbreaks II and III (a total of 31 laboratory confirmed cases and six cases without laboratory confirmation had consumed one of two tiramisus prepared with eggs from packing station B served by plant A) underline the causative role of egg producing plant A, as previously shown in a cohort study by Schmid et al. *(7)*. Case control studies or cohort studies are resource consuming endeavours, and local health authorities in Austria are often reluctant to perform such proper analytical epidemiological investigations. We consider the results of the descriptive epidemiological investigations of outbreaks I, II, and III sufficient to warrant proper health action, especially in connection with the microbiological demonstration of the causative agents in the epidemiologically related egg producing plant A.

While field investigations of outbreaks frequently lead to discovery of the source of infection, the sources of infection of the large number of sporadic cases of foodborne illness often remain cryptic. Epidemiological outbreak investigation may also yield valuable hints to elucidate the source of seemingly unrelated sporadic cases. Fig. 1 presents all culture-confirmed human cases of salmonellosis due to *S.* Typhimurium DT41 (n=119) and *S.* Typhimurium DT U291 (n=211) documented in Austria from January 2003 to February 2007 and all bulk purchasers of egg producing plant A according to location. The finding that the areas of illness and egg distribution are closely related leads us to hypothesize that these seemingly sporadic cases may also be causally connected to egg producing plant A. As none of the listed strains have been detected in eggs in Austria between 2003 and 2007, proper public health action has not been taken so far. According to EU Regulation (EC) No 178/2002, the food business operators have primary legal responsibility for ensuring food safety for their products *(1)*. Lack of adequate prophylactic action therefore could lead to liability of the producer should "conclusive evidence" develop later.

Our findings further support the conclusion of Schmid et al. *(7)* that regulatory response should follow a strong epidemiological association from a well-conducted investigation even in the absence of confirmatory microbiological testing of food items. In the United States such procedure is widely accepted, as was seen in a recent *Salmonella* outbreak epidemiologically associated with Peter Pan peanut butter, where the epidemiological evidence sufficed for the FDA to recall the product and in which the causative microorganism was through subsequently found product and environmental sampling *(6)*. In Europe, as November 1 of 2007, European legislation will mandate that when, as a result of epidemiological investigation of foodborne outbreaks, *Salmonella* ssp. are identified in a flock of laying hens as the source of infection for humans, the food business operator is no longer allowed to market his products as table eggs *(5)*: "… these eggs originating from flocks which were identified as the source of infection in a specific human foodborne outbreak, may be used for human consumption only if treated in a manner that guarantees the destruction of all *Salmonella* serotypes with public health significance in accordance with Community legislation on food hygiene" *(5)*. Such eggs have to be considered as Class B eggs *(5)*, which are eggs of second quality that are allowed to be delivered only to the food industry and non-food industry *(4)*.

In our opinion, the finding that plant A was the source of not only 58 cases as shown by the cohort studies of Schmid et al., but also of 47 further cases as shown by our descriptive epidemiological studies and microbiological testing of fecal and environmental samples and possibly of 330 sporadic culture-confirmed salmonellosis cases, underlines the appropriateness of the decision of EC authorities to respond to epidemiological evidence alone in outbreaks associated with eggs *(7)*. Absence of microbiological proof in epidemiologically incriminated food must not preclude proper action by health authorities.

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4.3 Review II

ARTICLES

Food Protection Trends, Vol. 31, No. 5, Pages 268–273

Lessons to be Learned from an Outbreak of Foodborne Listeriosis, Austria 2009–2010

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ABSTRACT

From June 2009 until February 2010, an outbreak of invasive listeriosis affected 25 persons in Austria, 8 in Germany and 1 in the Czech Republic. The source of this outbreak with 8 fatalities was initially identified based solely on epidemiological findings. Grocery receipts of purchases from 7 patients were collected prospectively during 3 weeks after discharge from hospital and compared for matches. This generated a hypothesis tested by a case control study, which revealed Austrian 'Quargel' cheese as the source of infection. At least two patients had consumed the contaminated product after it was withdrawn from the market. Risk communication with the public is of utmost importance, especially if hard-to-reach elderly are involved. Of 63 food samples of the product analyzed officially, 20 were positive for *L. monocytogenes* and 10 samples harbored ≥100 CFU/g (maximum 92,000 CFU/g). A leftover specimen, stored in a patient's refrigerator, yielded 2,100,000 CFU/g of *L. monocytogenes* indistinguishable from the patient's isolate. *L. monocytogenes* was first documented in the plant in a sample of smear fluid in May 2009, which temporarily coincided with major construction work. European legislation requires 'Quargel' not to exceed 100 CFU/g when products are placed on the market during their shelf life. A routine sample from a market had already shown 400 CFU/g in July 2009. We feel that a general zero tolerance policy would not have provided additional safety in this outbreak situation.

INTRODUCTION

Listeriosis is a rare but serious infection caused by *Listeria monocytogenes*. This organism can be found throughout the environment, in soil, vegetation, and animals. The main route of transmission is believed to be through consumption of contaminated food *(1, 22, 29)*. However, infection can also, albeit very rarely, be transmitted directly from infected animals to humans *(1)*. In neonatal infections, *L. monocytogenes* can be transmitted from mother to child in utero or during passage through the infected birth canal. There are also rare reports of nosocomial transmission attributed to contaminated material or patient-to-patient transmission via healthcare workers *(1)*. The bacterium is particularly successful in causing foodborne disease, because it survives food processing technologies that rely on acidic conditions or high salt concentrations and, unlike many pathogens, can continue to multiply slowly at low temperatures, allowing growth to occur even in properly refrigerated foods *(1)*.

In recent years, an increasing rate of listeriosis has been reported in several

A peer-reviewed article

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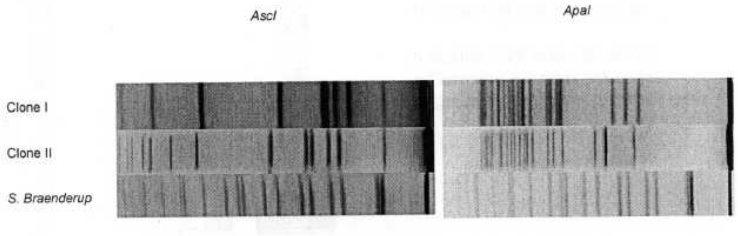
FIGURE 1. Pulsed-field gel electrophoresis (PFGE) patterns of *L. monocytogenes* serotype 1/2a clone 1 and clone 2 using AscI and ApaI as restriction enzymes (*Salmonella* Braenderup included for comparison)



European countries *(6, 9, 13, 14, 17, 19, 20, 28)*. These increases primarily reflect a higher rate of bacteremic listeriosis in those > 65 years of age and are not otherwise correlated with geography, gender, ethnicity, socioeconomic factors or infectious serotypes *(9, 17)*. At present, the available pathogenesis and subtyping data generally fail to provide adequate insight about the virulence of field isolates and the likelihood that a given strain will cause illness *(18)*. In Austria, the introduction of routine subtyping of human *L. monocytogenes* isolates in 2008 has shown that up to 25% of clinical isolates belong to clusters, indicating the possibility of foodborne outbreaks. The source of infection of outbreaks of listeriosis often remains undetected *(10, 30)*.

On 14 August 2009, the Austrian Reference Centre for Listeria in Vienna noticed the occurrence of a new pulsed-field gel electrophoresis (PFGE) pattern (clone 1) in human isolates of *L. monocytogenes* serotype 1/2a. An initial report on this cluster and an update on this foodborne outbreak have previously been published as rapid communications in Eurosurveillance *(11, 12)*. The investigation of foodborne outbreaks caused by zoonotic agents provides unique opportunities to improve the prevention and control of communicable diseases. The European Zoonoses Directive 2003/99/EC and the Austrian Zoonoses Act BGBl I 2005/128 even specify that health authorities must investigate foodborne outbreaks and that the investigation should provide data on the epidemiological profile, the foodstuffs potentially implicated and the feasible causes of the outbreak *(2, 3)*. We report on the epidemiological and microbiological investigations that finally elucidated an Austrian cheese product as the source of this incident affecting Austria, Germany and the Czech Republic and discuss the lessons to be learned from this outbreak of foodborne listeriosis.

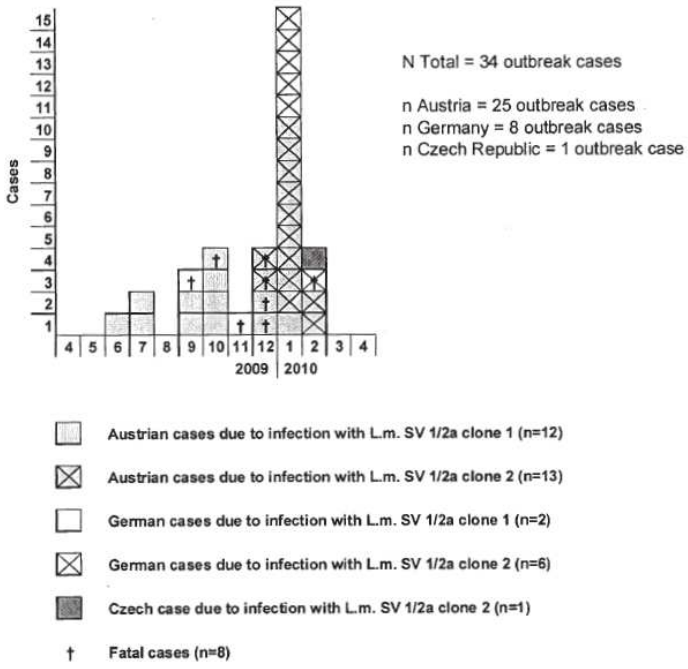
OUTBREAK

In August 2009, the binational Austrian-German Consiliar Laboratory for *Listeria* in Vienna (Austria) noticed a cluster of human isolates of *L. monocytogenes* serotype 1/2a with a new pulsed-field gel electrophoresis (PFGE) pattern (clone 1) (Fig. 1). Subtyping was performed according to the "Standardized PulseNet Protocol for Molecular Subtyping of *L. monocytogenes* by pulsed-field gel electrophoresis (PFGE)" *(15)*. Finally, fourteen cases (12 Austrian and two German) with this new clone 1, including five with fatal outcome (two of them German), were identified, with onset of disease ranging from June 2009 to January 2010. An epidemiological investigation revealed 'Quargel' cheese produced by an Austrian manufacturer as the source of infection. Approximately 16 tons of 'Quargel' were produced per week by the manufacturer. Fifty-three percent of the product was exported to the German market and small amounts to the Czech Republic and Slovakia. This cheese is made of curdled milk, which ripens for one day at 28°C after addition of starter cultures and for another two days at 14°C after being sprayed with *Brevibacterium linens*. The shelf life after packing and marketing is two months. The product was voluntarily withdrawn from the Austrian, German, Slovakian and Czech markets on 23 January 2010. An environmental *L. monocytogenes* 1/2a isolate from the production plant, from a gully (drainage pit) sample collected in December 2009, became available on 19 January 2010 and proved indistinguishable from the outbreak strain by genotyping. Microbiological investigations also confirmed the presence of this new strain (clone 1) in 'Quargel' samples taken at the factory in 2010: Two of 64 isolates available for testing (44 isolates cultured from cheese produced in 2010 and provided by the manufacturer, 20 isolates cultured from samples officially gained during outbreak investigation) showed the new PFGE pattern associated with the outbreak.

The 62 remaining food isolates showed a different PFGE pattern that had not been seen previously in Austrian isolates either (clone 2) (Fig. 1). It was indistinguishable from the pattern of a human isolate from a listeriosis patient who was hospitalized at the time and who claimed to have eaten 'Quargel' cheese. Only two of 46 human *L. monocytogenes* isolates documented at the Austrian Reference Centre in 2009 yielded this PFGE-pattern, both coming from patients with a food history positive for 'Quargel'. Ultimately, this second outbreak clone of *L. monocytogenes* serotype 1/2a accounted for 13 Austrian cases (two with fatal outcome), six German cases (one death), and one Czech case; onset of disease ranged from December 2009 until the end of February 2010. The epidemic curve shows all cases associated with the two different outbreak clones by onset of illness (Fig. 2).

In total, the outbreak involved 34 cases of invasive listeriosis, 25 of which originated from seven of nine Austrian provinces. Four of these patients presented with meningitis, two with clone 1 and two with clone 2. A further eight patients were from four of 16 German federal states, and one patient was from the Czech Republic. Eight of the 34 cases in this outbreak had a fatal outcome. The median age of the

FIGURE 2. Outbreak cases of listeriosis by onset of illness and final outcome, Austria, Germany and the Czech Republic, 2009–2010 (n = 34)



cases was 72 years (range: 57–89 years), and 26 patients were male. There were no materno-neonatal cases. Underlying diseases were not different from those generally described for patients with listeriosis *(1)*. In Austria, all but one case can be explained by consumption of the contaminated product before it was withdrawn from the market on 23 January; one patient who was hospitalized for meningitis on 26 February 2010 had eaten the cheese (purchased before withdrawal from the market) on February 13. A leftover specimen, stored in the patient's refrigerator and sampled on 3 March, yielded 2,100,000 CFU/g of *L. monocytogenes*. Despite high media coverage, the patient and his wife were not aware of the product recall. In Germany, at least one person with fatal outcome ate the incriminated cheese after the product was withdrawn from the market *(5)*.

A total of 63 food samples of the 'Quargel' cheese products were microbiologically analyzed. Twenty samples were found positive for *L. monocytogenes*. Ten of the 20 samples yielded less than 100 CFU/g, and ten samples harbored more than 100 CFU/g: 160 CFU/g, 170 CFU/g, 240 CFU/g, 1,500 CFU/g, 4,500 CFU/g, 6,050 CFU/g, 18,000 CFU/g, 18,000 CFU/g, 30,000 CFU/g, and 92,000 CFU/g, respectively.

The source of this outbreak was initially identified based solely on epidemiological findings. We collected grocery receipts of purchases made by seven patients in December 2009, after their discharge from hospital, and compared them for matches. This generated a hypothesis to be tested by a case control study using case-case comparisons. For this study, a case was defined as a person in Austria from whom the *L. monocytogenes* outbreak clone 1 was isolated. Controls were patients from Austria with *L. monocytogenes* infections in 2009, whose isolates showed profiles other than that of the outbreak clone 1. Patients were asked about consumption of 12 cheese products in the six-month period prior to disease onset. Control persons were requested to provide information on consumption of the same cheese products in the year 2009. The overall response rate was 83.3% in the case group (ten of 12 possible cases at the time) and 72.2% in the control group (24 of 33 possible controls at the time, i.e., listeriosis patients with isolates that showed profiles other than that of the outbreak clone 1). Consumption of the 'Quargel' cheese was identified as the only significant risk factor highly associated with the illness in question. Nine of the ten patients with clone 1 had consumed the product; the tenth provided no answer concerning this food item. Of 22 controls (none with clone 2), all but two denied having eaten this specific cheese; the remaining two provided no answer concerning this food item. The computed odds ratio was 76.6 (95% confidence interval (CI): 9.3-infinity; P value < 0.001).

DISCUSSION

Listeriosis is a foodborne illness of major public health concern because of the severity of its consequences (infections of the central nervous system, septicaemia, and abortion), the high case-fatality ratio (20–23% of cases), and the long incubation time *(1)*. Although exposure to *L. monocytogenes* is common, listeriosis is rare, and any outbreak should therefore be used to gain new knowledge on prevention of this disease. Despite the existence of technical literature on methods for outbreak investigations, the situation–specific nature of outbreaks makes it impossible for a gold standard to exist for investigations of clusters of foodborne illness *(16)*. There are no prespecified formulae to dictate the path that an outbreak investigation is supposed to take *(16)*. Nevertheless, in the absence of controlled human experiments, these outbreaks provide a unique opportunity to gain new scientific knowledge on foodborne illness.

The outbreak described shows impressively that the waning of an outbreak (i.e., disappearance of an outbreak clone) does not necessarily imply that the underlying problem has disappeared. The shift to a different outbreak clone in December 2009/January 2010 was probably caused by the change to a new commercial yeast-ripening culture (ACL2; Cargill, Minneapolis, MN) used in the cheese factory in late November 2009

because of a shortage of the original culture FAA1 (Cargill).

In this outbreak, the producer and the local food inspection authorities were not aware of the legal European requirement for zero tolerance in 'Quargel' when the plant facility was inspected. Therefore, failure of the producer's HACCP system and possibly inadequate training of local food inspectors have to be blamed for this foodborne outbreak that finally cost eight lives. Laboratory documents provided to the justice department by an employee of the cheese production plant (who reported himself in February 2010) revealed that microbiological tests on 'Quargel' delivered to Germany had already shown presence of 400 CFU/g in July 2009.

In Austria, the role of environmental testing in a possible HACCP regulatory framework and its potential to lower public health risk is still a matter of debate. Several sources in the literature question the role of testing as an HACCP verification tool, comparing it to "looking for the proverbial needle in the haystack" *(8)*. Others contend that environmental testing is essential to ensure that the processing facility is not causing incidental contamination that could easily go undetected in finished product tests *(26)*. In the described outbreak, an *L. monocytogenes* isolate from an environmental sample was the first available strain confirming the epidemiological finding of the outbreak source. This could be seen as an argument in favor of mandatory environmental *Listeria* spp. testing in production of soft cheeses like 'Quargel'.

The investigation was not able to elucidate decisively when and how *L. monocytogenes* entered the plant. Soil on workers' shoes and clothing, contaminated raw material and human carriers are among a multitude of possibilities *(27)*. Schoder et al. *(23)* hypothesize that *L. monocytogenes* was introduced into the plant during major construction work. From 23 February 2009 until 27 May 2009, a ripening room in direct proximity to the central production facility was remodeled, which temporarily coincides with the first documented detection of *L. monocytogenes* in a sample of smear fluid on 12 May 2009, allegedly after years of no microbiological proof of *L. monocytogenes*. Sample testing from the recall campaign (performed on behalf of the incriminated company after January 27, 2010) revealed that all 16 batches of the smear-ripened cheese tested were positive for *L. monocytogenes*, a finding that is compatible with ripening smear as the causative vehicle of spread inside the facility *(23)*. "*The lots were tested after delivery* [to the laboratory], *at the end of shelf life (that is, up to 50 days post production), and to mimic a worst case scenario, at time points exceeding the shelf life unless the sensorial properties remain unchanged. Each lot was positive qualitatively at each time point and, with exceeding times of storage, L. monocytogenes multiplied dramatically. The highest value found was* 2.8×10^8 *colony forming units (CFU) per gram of cheese*" *(23)*. Investigations after closure of production revealed the outbreak clone 2 even in ground beetles (Family Carabidae) collected from insect traps in the production facility *(24)*. Carry over of the bacteria via beetles is just one of many possible ways the *Listeria* could have been introduced during major construction work. In May 2010, the producer decided to close down the incriminated cheese production facility permanently.

This outbreak underlines the considerable potential of molecular subtyping of human *L. monocytogenes* isolates as a tool to recognize clusters and also emphasizes the immense importance of cross-border cooperation for elucidating chains of infections in multinational outbreaks. Further improvements in pathogen identification techniques, especially in inter-laboratory exchange of typing data, may increase the chances that foodborne illnesses will be linked to specific food products and firms in the near future *(29)*.

Industrial food production combined with international marketing of food, the low proportion of a population affected after consuming food contaminated by *L. monocytogenes*, and the fact that the food evidence has usually already been eaten generally hinder epidemiological outbreak investigation with traditional concepts *(7)*. Furthermore, the long incubation period of up to 70 days makes it difficult to generate a hypothesis on the food source of a listeriosis outbreak. To our knowledge, this was the first outbreak in which grocery bills were collected prospectively — after patients' discharge from hospital — and successfully used to reveal the food consumption patterns of listeriosis patients, indicating sour milk curd cheese 'Quargel' as a possible source for this outbreak, to be tested by analytical epidemiology.

Spriggs and Isaac *(25)* identified outbreaks of foodborne illness as the major force for change in regulations and markets. In this outbreak situation, the time span between epidemiological elucidation of the outbreak source (January 15, 2010) and the "voluntary" product recall (January 23, 2010) was criticized as inappropriately long. At this time, Austrian law required microbiological proof before any public health action could be taken on an unsafe food product. As a direct consequence of this outbreak, on 21 April 2010, Austria amended its Food Safety and Consumer Protection Act, allowing health authorities in future to order market withdrawals and to recall products even without microbiological proof of their being unsafe.

Recalling the food and advising people not to eat the contaminated product controlled this outbreak. At least two cases were due to consumption of the contaminated product after it was withdrawn from the market. Risk communication with the public is of utmost importance, especially if the outbreak involves hard-to-reach elderly, a risk group that is known to be reluctant to dispose of food even after it has exceeded the "best before" date. The case of the Austrian patient with meningitis who still had a leftover specimen of the causative food in his refrigerator also underlines the importance of visiting households of listeriosis patients in order to obtain food samples and to advise other household members on precautionary measures. Some sporadic cases may be unrecognized common-source outbreaks *(21)*. A single leftover food sample could prove an invaluable clue for elucidating the source of infection and thereby preventing further illness.

The United States has a zero tolerance policy for *L. monocytogenes* in ready-to-eat foods, but in Europe, there is some tolerance for it in certain foods *(21)*. COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005

on microbiological criteria for foodstuffs requires a limitation of < 100 CFU/g in ready-to-eat foods able to support the growth of *L. monocytogenes* (other than those intended for infants and for special medical purposes), during the shelf life when products are placed on the market; the same products have to prove absence of *L. monocytogenes* in 25 g before the food has left the immediate control of the producing food business operator *(4)*. Although the infective dose of *L. monocytogenes* is unknown and different population groups may require different degrees of protection, we feel that the present European legislation is sufficient, and a general zero tolerance policy would not have provided additional safety in this outbreak situation.

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4.4 Original article II

Listeriosis outbreak caused by acid curd cheese 'Quargel', Austria and Germany 2009

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We report an outbreak of listeriosis in Austria and Germany due to the consumption of 'Quargel' cheese produced by an Austrian manufacturer. At the time of writing this report, the outbreak was known to account for 14 outbreak cases in 2009, including four cases with lethal outcome. On 23 January 2010, the cheese product was voluntarily withdrawn from the market.

On 14 August 2009, the binational Austrian-German Consiliar Laboratory for Listeria in Vienna noticed the occurrence of a new pulsed-field gel electrophoresis (PFGE) pattern in human isolates of *Listeria monocytogenes* serotype 1/2a. This consiliar laboratory receives all human isolates from Austria as required by law. In Germany, submission of isolates is voluntary. According to the available information at the time of writing this report, the outbreak clone accounted for 12 of the 46 Austrian cases in 2009 (serotype 1/2a (n=29), 4b (n=9), 1/2b (n=8)). Onset of illness is shown in the Figure. The 12 Austrian outbreak cases (two of them fatal) affected six of nine Austrian provinces. The mean age was 74.5 years (range: 58-88 years), eleven patients were male. In addition, two of 92 available human isolates from Germany in 2009 (total number of cases 389) showed this new PFGE-pattern. The German outbreak cases were two women in their 70s who died in November and December 2009 respectively. They had not visited Austria during the likely period of incubation (up to 70 days).

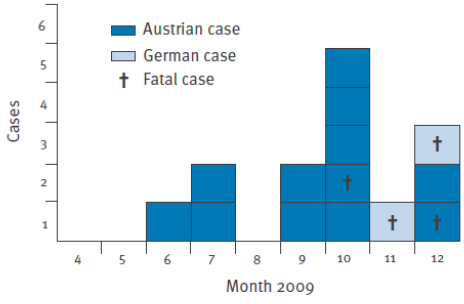
Since no reliable information was available on food consumed during the incubation period, all surviving Austrian outbreak cases were asked to collect grocery receipts for the three weeks after 3 December, i.e. after they were discharged from hospital, in order to collect information on routine food consumption behaviour. This epidemiological investigation revealed consumption of 'Quargel', a type of acid curd cheese available in different flavours, as a highly likely source of this outbreak. Three of seven outbreak cases providing receipts had bought product X produced by the Austrian manufacturer. Regular consumption of Quargel product X was confirmed by eight of nine participating outbreak cases, and consumption of Quargel cheese products was reported by heteroanamnesis for one German outbreak case (data on the second case remain unavailable).

Approximately 16 tons of Quargel per week are produced by the Austrian manufacturer. Fifty-three per cent of the product is exported to the German market and small amounts to the Czech Republic, Poland and Slovakia. This cheese is made of curdled milk, which ripens after addition of starter cultures for one day at 28°C, and after being sprayed with *Brevibacterium linens* for another two days at 14°C. The shelf life after packing and marketing is two months.

An environmental *L. monocytogenes* 1/2a isolate from the production plant, collected in December 2009, became available in January 2010 and proved indistinguishable from the outbreak strain by genotyping. Quargel cheese products sampled at the plant on January 13 yielded three different strains of

FIGURE

Outbreak cases of listeriosis by onset of illness, Austria and Germany, 2009 (n=14)



L. monocytogenes 1/2a, including the outbreak clone, in numbers of less than 100 colony-forming units (cfu) per gram. Food products collected on 18 January 2010 yielded greater than 100 cfu/g *L. monocytogenes*. The product was voluntarily withdrawn from the market on 23 January. On the same day, the public was informed about the incident and warned about cheese already bought. The plant stopped production. Investigation of the source of contamination is ongoing.

Conclusion

Industrial food production combined with international marketing of food and the low attack rate of *L. monocytogenes* hinder epidemiological outbreak investigation with traditional concepts [1]. Genotyping of *L. monocytogenes* isolates from clinical specimens can discriminate single-source clusters of food-borne infection and contribute to the identification and investigation of outbreaks. The outbreak described in this report probably would not have been identified without molecular typing [2]. The effectiveness of microbiological surveillance is entirely dependent upon the consistent and timely submission of all Listeria isolates from clinical laboratories to public health laboratories. In Austria, clinical laboratories are required by law to submit all clinical isolates of *L. monocytogenes* to AGES for PFGE analysis. In Germany, submission of *L. monocytogenes* isolates from clinical specimens by clinical laboratories is not required. The high case fatality ratio of listeriosis makes a strong case for the importance and priority of improved surveillance in Europe [3]. Our outbreak report underlines the value of routine molecular typing of Listeria isolates and also points out the considerable potential of cross-border cooperation for elucidating chains of infections.

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4.5 Original article III

Update: Multinational listeriosis outbreak due to 'Quargel', a sour milk curd cheese, caused by two different L. monocytogenes serotype 1/2a strains, 2009-2010

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This article has been published on 22 April 2010

We previously reported an outbreak of listeriosis in Austria and Germany due to consumption of 'Quargel' cheese. It comprised 14 cases (including five fatalities) infected by a serotype 1/2a *Listeria monocytogenes* (clone 1), with onset of illness from June 2009 to January 2010. A second strain of *L. monocytogenes* serotype 1/2a (clone 2) spread by this product could be linked to further 13 cases in Austria (two fatal), six in Germany (one fatal) and one case in the Czech Republic, with onset of disease from December 2009 to end of February 2010.

Clone 1

As reported earlier, the binational Austrian-German Consiliar Laboratory for Listeria in Vienna noticed a cluster of human isolates of *Listeria monocytogenes* serotype 1/2a in August 2009 with a new pulsed-field gel electrophoresis (PFGE) pattern [1]. Fourteen cases (12 Austrian and two German), including five with fatal outcome (two of them German), were identified. Onset of disease ranged from June 2009 to January 2010. An epidemiological investigation revealed 'Quargel' cheese produced by an Austrian manufacturer as the source of infection. The product was withdrawn from the Austrian, German, Slovakian and Czech markets on 23 January 2010 [1].

Microbiological investigations confirmed the presence of this new strain (clone 1) in 'Quargel' samples taken at the factory in 2010: Two of 64 isolates available for testing (44 isolates cultured from cheese produced in 2010 and provided by the manufacturer, 20 isolates cultured from samples officially gained during outbreak investigation) showed the new PFGE pattern associated with the outbreak.

Clone 2

The 62 remaining food isolates showed a different PFGE pattern, that had not previously been seen in Austrian isolates either (clone 2). It was indistinguishable from the pattern of a human isolate from a listeriosis patient hospitalised at the time, who claimed to have eaten 'Quargel' cheese. Only two of 46 human *L. monocytogenes* isolates documented at the Austrian Reference Centre in 2009 yielded this PFGE-pattern, both coming from patients with a food-history positive for 'Quargel'. Ultimately, this second outbreak clone of *L. monocytogenes* serotype 1/2a accounted for 13 Austrian cases (two with fatal outcome), six German cases (one death), and one Czech case; onset of disease ranged from December 2009 until end of February 2010. The epidemic curve shows all cases associated with the two different outbreak clones by onset of illness (Figure).

Outbreak analysis

In total, the outbreak involved 34 cases of invasive listeriosis: 25 outbreak cases originated from seven of nine Austrian provinces. Four of these patients presented with meningitis: two with clone 1 and two with clone 2. A further eight patients were from four of 16 German federal states, and one patient was from the Czech Republic. Eight of the 34 cases in this outbreak had a fatal outcome. The median age of the cases was 72 years (range: 57-89 years), and 26 patients were male. There were no materno-neonatal cases. Underlying diseases were not different from those generally described for patients with listeriosis [2].

A total of 63 food samples of the 'Quargel' cheese products were microbiologically analysed. 20 samples were found positive for *L. monocytogenes*. 11 of the 20

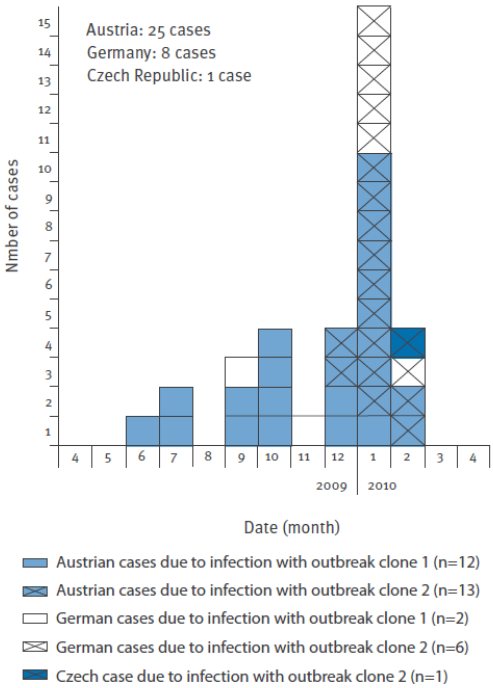
samples yielded less than 100 colony-forming units per gram (CFU/g), and nine samples harboured more than 100 CFU/g. All but one case can be explained by consumption of the contaminated product before it was withdrawn from the market on 23 January: one patient who was hospitalised for meningitis on 26 February 2010 had eaten the cheese (purchased before withdrawal from the market) on February 13. A leftover specimen, stored in the patient's refrigerator and sampled on 3 March, yielded 2,100,000 CFU/g of *L. monocytogenes*.

Case control study

The source of this outbreak was initially identified based solely on epidemiological findings [1]. We collected the cases' grocery receipts of purchases they made in December 2009, after their discharge from hospital, and compared them for matches. This generated a hypothesis to be tested by a case control study using case-case comparisons. For this study, a case was defined as a person in Austria from whom the *L. monocytogenes* outbreak clone 1 was isolated. Controls were patients from Austria with *L. monocytogenes* infections in 2009, whose isolates showed other profiles than the outbreak clone 1. Cases were asked about consumption of 12 cheese products in the six-month period prior to disease onset. Control persons were requested to provide information on consumption of the same cheese products in the year 2009. The overall response rate was 83.3% in the case group (ten of at the time 12 possible cases) and 72.2% in the control group (24 of at the time 33 possible controls, i.e. listeriosis-patients with isolates that showed other profiles than the outbreak clone 1). Consumption of the 'Quargel' cheese was identified as the only significant risk factor, highly associated with the illness in question. Nine of the ten cases with clone 1 had consumed the product; the tenth case provided no answer concerning this food item. Of 22 control cases (none with clone 2) all but two denied having eaten this specific cheese; the remaining two provided no answer concerning this food item. The computed odds ratio was 76.6 (95% confidence interval (CI): 9.3-infinity; P value <0.001).

FIGURE

Outbreak cases of listeriosis by onset of illness, Austria, Germany and Czech Republic, 2009-2010 (n=34)



Conclusions

The described outbreak provides some valuable lessons: Firstly, it underlines the considerable potential of molecular subtyping as a tool to identify outbreaks. Without routine PFGE typing of human isolates, this outbreak would have been missed. Secondly, it shows impressively that the waning of an outbreak (i.e. disappearance of an outbreak clone) does not necessarily imply that the underlying problem has disappeared. The shift to a different outbreak clone in December 2009/ January 2010 was probably caused by a change (in late November 2009) of the commercial ripening culture used in the cheese factory due to short supply of the original culture. Thirdly, our outbreak also emphasises the considerable potential of cross-border cooperation for elucidating chains of infections in multinational outbreaks. Industrial food production combined with international marketing of food and the low attack rate of *L. monocytogenes* hinder epidemiological outbreak investigations with traditional concepts [2]. Finally, the case of our patient with meningitis who had a leftover specimen of the causative food still in his refrigerator, underlines the importance of visiting households of listeriosis patients in order to obtain food samples and to advise other household members on precautionary measures. A single leftover food sample could prove an invaluable clue for elucidating the source of infection and thereby preventing further illness.

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4.6 Original article IV

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An outbreak of febrile gastroenteritis associated with jellied pork contaminated with *Listeria monocytogenes*

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Ein Ausbruch von fieberhafter Gastroenteritis durch *Listeria monocytogenes*-kontaminierte Presswurst

Zusammenfassung. Im September 2008 erfuhr die Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES) von einem Ausbruch infektiöser Gastroenteritis; die Blutkultur eines 71-jährigen Hospitalisierten hatte *Listeria monocytogenes* erbracht. Sieben von 19 Teilnehmern eines Tagesausfluges nach Bratislava stellten Stuhlproben zur Verfügung; aus drei Proben konnte *L. monocytogenes* angezüchtet werden. Alle Isolate waren vom Serovar 4b und zeigten idente DNA-Fingerabdrücke. Eine Kohortenstudie zeigte, dass der Erkrankungsausbruch auf jene 16 Personen der Reisegruppe beschränkt war, die am 6. September im Rahmen eines abschließenden Heurigenbesuches zu Abend aßen. Von 15 Personen, die von einer kalten Platte aßen, erkrankten 12 (80%) binnen 24–48 h. Im Median waren die Erkrankten 62 Jahre alt. Ein 72 Jähriger erholte sich von der Gastroenteritis, wurde jedoch am Tag 19 nach dem Abendessen mit bakterieller Meningitis hospitalisiert. Die epidemiologische Abklärung belegte den Verzehr der gemischten Platte (inklusive Presswurst) als wahrscheinlichste Ursache des Krankheitsausbruchs (p = 0,015). Diese Hypothese wurde durch den mikrobiologischen Nachweis des Erregers in der am 3. September im Heurigenbetrieb hergestellten Presswurst untermauert: *L. monocytogenes* fand sich in Keimzahlen von 3×10^3 bis 3×10^4 koloniebildenden Einheiten /g und war von den Humanisolaten nicht unterscheidbar. Die Symptome der 12 Patienten umfassten Fieber (12x), Durchfall (9x), Kopfweh (5x), Erbrechen (4x), Körperschmerzen (2x), und Halsweh (1x). Die aktive Fallsuche erbrachte zudem einen Fall von Rhombencephalitis (weiblich, 48 Jahre alt); von einer 4-Personengruppe hatten am 6. September nur die Patientin und ihr asymptomatischer Gatte saure Presswurst mit Zwiebel konsumiert. Bislang war in Österreich ein Ausbruch von *L. monocytogenes*-assoziierter Gastroenteritis noch nicht beschrieben worden. Das Auftreten von eitriger Meningitis (diagnostiziert am Tag 19 nach Verzehr von kontaminierter Presswurst) bei einem Durchfallpatienten belegt ein signifikantes Risiko für systemische Listeriose bei älteren Patienten mit febriler Gastroenteritis durch *L. monocytogenes*; eine antibiotische Therapie sollte in derartigen Fällen gesicherter Listerien-Gastroenteritis in Betracht gezogen werden.

Summary. In September 2008, the Austrian Agency for Health and Food Safety (AGES) learned of an outbreak of diarrheal illness that included a 71-year-old patient hospitalized for gastroenteritis with a blood culture positive for *Listeria monocytogenes*. Three stool specimens provided by seven of 19 persons attending a day trip to a foreign city, including a final break at an Austrian tavern, yielded *L. monocytogenes*. All isolates were of serovar 4b and had fingerprints indistinguishable from each other. A cohort study revealed that the outbreak of gastroenteritis occurred among 16 persons who had eaten dinner at the wine tavern on September 6. Of the 15 persons who ate from platters of mixed cold-cuts, 12 (80%) developed symptoms of febrile gastroenteritis within 24–48 h. The median age of those who became ill was 62 years. A 72-year-old patient recovered from gastroenteritis but was hospitalized with bacterial meningitis on day 19 after the dinner. The epidemiological investigation identified the consumption of mixed cold-cuts (including jellied pork) at the wine tavern as the most likely vehicle of the foodborne outbreak ($P = 0.015$). This hypothesis was confirmed by microbiological investigation of jellied pork produced by the tavern owner on September 3. *L. monocytogenes* was isolated from leftover food in numbers of 3×10^3–3×10^4 colony forming units/g and was indistinguishable from the clinical outbreak isolates. Symptoms reported by the 12 patients

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included unspecified fever (12x), diarrhea (9x), headache (5x), vomiting (4x), body aches (2x) and sore throat (1x). Active case finding identified one case of rhombencephalitis (female, age 48) among another group of four guests, among whom only the patient and her asymptomatic husband had eaten jellied pork on September 6. This is the first outbreak of *L. monocytogenes*-associated gastroenteritis reported in Austria. The occurrence of a secondary case of meningitis (diagnosed on day 19 after consumption of jellied pork) indicates a significant risk of systemic listeriosis among elderly patients with febrile gastroenteritis caused by *L. monocytogenes*; antibiotic therapy should therefore be considered in such cases of documented listerial gastroenteritis.

Key words: *Listeria monocytogenes*, gastroenteritis, meningitis, rhombencephalitis, food-borne, outbreak.

Introduction

The clinical manifestation of infection with *Listeria (L.) monocytogenes* is usually described as an illness of the central nervous system, as sepsis, or – in pregnant women – as a flu-like illness; it has limited effect on the general population. *L. monocytogenes* causes invasive illness mainly in certain well defined high-risk groups, including immunocompromised persons, pregnant women, neonates and the elderly [1]. Nevertheless, listeriosis can occur in otherwise healthy individuals, particularly during an outbreak. Because of its severity, the high case-fatality ratio (20–23% of cases) and the long incubation time, listeriosis is a foodborne illness of major pubic health concern [1]. Several large outbreaks of invasive listeriosis associated with contaminated foods such as coleslaw, meat products and soft cheese have established the importance of foodborne transmission in invasive listeriosis [2]. Although listeriosis is known to be transmitted through food, it has only recently begun to be considered as a gastrointestinal disease [3]. For most foodborne pathogens, such as salmonella and campylobacter, gastrointestinal illness is the presenting and only symptom in the majority of cases. Approximately 10 years ago it was demonstrated that foodborne listeriosis can also present as a gastrointestinal illness with fever [4–6]. This article reports on an Austrian outbreak of febrile gastroenteritis associated with homemade jellied pork contaminated with *L. monocytogenes*.

Background

On September 6, 2008, a group of 19 persons (members of a music band and their partners) made a one-day excursion by coach. The day included lunch and a boat trip to a foreign city outside Austria. Three persons returned by boat early, the remaining 16 persons were picked up at the city by coach and stopped for dinner at a wine tavern in rural Austria. Two of the group left before dinner; the bus driver and his accompanying spouse were invited to join the meal. The restaurant was a traditional Austrian wine tavern, open for periods of 14 days approximately six times a year and offering 70 seats to up to 300 guests per day. The tavern opened for a two-week period on September 4. In addition to providing home-grown wine, the tavern offered homemade salads and meat products (jellied pork, blood sausage and hog roast were produced in-house on September 3) and commercially purchased bread and cheeses.

On September 6, the group ordered platters of mixed cold-cuts (meat products, cheeses and spreads). On the afternoon of the following day, a 71-year-old fell ill with body aches, diarrhea, shivering and vomiting. On September 8, the patient was hospitalized and a blood culture from the febrile (38.2°C) patient yielded *L. monocytogenes*. According to the patient, up to 13 members of the excursion group suffered from diarrheal illness. On September 16, the Austrian Agency for Health and Food Safety (AGES) was granted permission to investigate the outbreak. To identify the source and cause of the illness, we conducted epidemiologic and microbiologic investigations.

Patients, materials and methods

Outbreak confirmation

On September 16, AGES requested collection of stool samples from all persons attending the day trip. Seven persons provided samples: specimens from three persons (male aged 50, female aged 72, male aged 73) yielded *L. monocytogenes*; four were negative for listeria (female aged 41, female aged 41, female aged 68, male aged 72). All isolates were *L. monocytogenes* serovar (sv) 4b and indistinguishable from each other by repetitive element sequence-based PCR (rep-PCR), pulsed-field gel electrophoresis (PFGE) using *Asc*I and *Apa*I as restriction enzymes, and amplified fragment length polymorphism (AFLP), but clearly different from the patterns of the *L. monocytogenes* sv 4b strains from human cases in Austria isolated since 1999.

Epidemiological investigation

In this cohort study, the cohort consisted of 19 persons travelling to the foreign city and two persons joining them for the meal at the wine tavern. Calculations were made for two different factors of exposure. Persons were interviewed by phone; the owners of the wine tavern were interviewed face to face. Data were analyzed using EpiInfo Version 6, Statcalc (November 1993).

Cases were defined as follows: Confirmed case: a person who had dinner at the wine tavern on September 6, 2008 and developed diarrhea or fever within the following seven days, with an isolate of *L. monocytogenes* from stool, blood or cerebrospinal fluid (CSF). Possible case: a person who had dinner at the wine tavern on September 6, 2008 and developed diarrhea or fever within the following seven days, but who did not have an isolate of *L. monocytogenes* from stool, blood or CSF although epidemiologically linked to the outbreak.

Active case finding

Efforts were made to contact other guests who had visited the wine tavern on September 6. Obeying an obligation to keep the tavern's identity confidential, we were able to identify only one other group of guests (n = 4) visiting the tavern on that day.

On September 6, a total of eight persons were working in the tavern, all of them claiming "regular" consumption of the food served to guests. None of the staff reported clinical symptoms.

Microbiological investigation

Human samples: Stool samples were examined bacteriologically after cold enrichment in Fraser enrichment broth (Heipha-Biotest, Eppelheim, Germany) at 4–8 °C over 14 days in ambient atmosphere. Every three days and at day 14 an aliquot of broth was streaked onto selective chromogenic agar plates (ALOA listeria agar according to Ottaviani–Agosti, Heipha-Biotest, Germany) and incubated at 35 °C in ambient atmosphere for 48 h. Suspected colonies (blue) were subcultured on Columbia blood-agar (bioMerieux, Marcy l'Etoile, France) and identified phenotypically using Api-Listeria (bioMerieux). Blood and CSF were processed as described elsewhere [7].

Food: Samples of suspect food items served on September 6 (jellied pork, blood sausage, hog roast) plus cheeses and spreads had been kept refrigerated and were available for laboratory testing. Conventional methods as described in the Austrian standard ÖNORM EN ISO 11290-1/2 (2005) were used [8]. In brief, a two-step process was used for qualitative detection: 25 g of specimen was enriched in half Fraser broth at 30 °C for 24 h before 0.1 ml of this enrichment was transferred to Fraser broth (37 °C, 48 h) and finally subcultured on Palcam agar plates and ALOA listeria agar plates (AES Chemunex, Bruz, France). Suspected colonies were processed as described above. For quantification, aliquots of 1 ml of 1:10 dilutions (in buffered peptone water) were spread on Palcam agar plates (140 mm diameter) and ALOA plates (390 mm diameter).

Pulsed-field gel electrophoresis

PFGE was performed according to the "Standardized PulseNet Protocol for Molecular Subtyping of *Listeria monocytogenes* by Pulsed-Field Gel Electrophoresis (PFGE)" [9].

In brief, cultures for DNA isolation were grown overnight on brain-heart infusion agar (Oxoid, Basingstoke, UK) at 37 °C. Cells were transferred from plates to tubes containing Tris-EDTA buffer and the bacterial density adjusted to OD_{610} = 1.35. The bacterial suspension was embedded into plugs of 1.2% SeaKem Gold agarose (Cembrex, Ponte Tresa, Switzerland), lysed overnight in a 54 °C water bath with constant agitation and washed with preheated deionized water and Tris-EDTA buffer. Plugs were sliced (2–2.5 mm slices) and the DNA digested with 25 units of *Asc*I (New England BioLabs, Ipswich, MA) at 37 °C for at least 4 h or with 200 units of *Apa*I (Roche, Basel, Switzerland) at 30°C for at least 5 h. *Salmonella* Braenderup strain H9812 was used as a reference standard. Plug preparation and restriction digestion of DNA with *Xba*I (Fermentas Life Sciences, Ontario, Canada) was performed according to the PulseNet protocol "One-Day (24–28 hours) Standardized Laboratory Protocol for Molecular Subtyping of *Escherichia coli* O157:H7, non-typhoidal *Salmonella* serotypes and *Shigella sonnei* by PFGE" [10]. The DNA restriction fragments in plugs were separated by electrophoresis on a 1% SeaKem Gold agarose gel in 0.5 x solution of Tris-borate-EDTA buffer (Bio-Rad, Hercules, CA) in a CHEF-DR III PFGE system (Bio-Rad). The electrophoretic parameters were as follows: initial switch time 4.0 s, final switch time 40.0 s, run time 21 h (gel size 21 cm x 14 cm), angle 120°, gradient 6.0 V/cm, temperature 13°C. After electrophoresis the gels were stained for 30 min in 500 ml deionized water containing 50 µl ethidium bromide (10 mg/ml) and destained for at least 2 h in deionized water. Gels were photographed under UV transillumination using the Gel Doc 2000 documentation system (Bio-Rad) and patterns analyzed using Bionumerics software (Applied Maths, Sint-Martems-Latem, Belgium).

Amplified fragment length polymorphism

Genomic bacterial DNA (gDNA) for subsequent typing analysis was extracted from bacterial cells grown overnight at 37 °C on RAPID'L.Mono (Bio-Rad) using the GenEluteTM Bacterial Genomic DNA kit (Sigma, St. Louis, MO) according to the manufacturer's instructions. The concentration of purified gDNA was determined spectrophotometrically at 260 nm and the quality by determination of the 260/280 ratio. AFLP analysis was as described by Vos et al. [11]. Briefly, 200 ng gDNA were digested with the restriction enzymes *EcoR*I (Invitrogen, Carlsbad, CA) and *Mse*I (Invitrogen) for 4 h. The reaction was heated to 70°C for 15 min. *EcoR*I and *Mse*I adaptors (Invitrogen) were ligated to the DNA fragments overnight at 20 °C. The ligation solution was diluted 1:10 and a prePCR was performed with *EcoR*I-0 and *Mse*I-0 primers (Invitrogen). The prePCR was diluted 1:10 and 1 µl was added to the subsequent specific PCR containing 5 pmol *EcoR*I primer and 50 pmol *Mse*I primer, each labeled with IRD-800 (MWG-Biotech, Ebersberg, Germany). Among several AFLP primer combinations tested, the set E-0/M-15 and E-02/M-03 were finally chosen for comparing the bacterial strains. SAGA-MX (LI-COR Biosciences, Lincoln, NE) AFLP analysis software was used to analyze the AFLP gels and transform the AFLP band pattern into a binary code. TreeCon, a free software package made available by the University of Ghent, Belgium, was used for binary matrix analysis with distance estimation and construction of the dendrogram [12].

Repetitive element sequence-based PCR

DNA was prepared as described above for AFLP. Primers REP 1R-I (5'-IIIICGICGICATCIGGC) and REP 2-I (5'-ICGICTTATCIGGCCTAC) were used for rep-PCR as described by Jersek et al. [13]. A total reaction volume of 20 µl containing 50 pmol of each primer (Invitrogen), 100 ng gDNA and 10 µl RedTaqReady Mix (Sigma) was used for amplification in a DNA thermocycler (Mastercycler gradient eps, Eppendorf, Germany) under the following conditions: an initial denaturizing step at 95°C for 7 min; 30 cycles at 90°C for 30 s, at 40°C for 1 min, at 65°C for 8 min; 1 cycle at 65° for 16 min. Samples (15 µl) of PCR products were separated on 1.5% agarose gels in 1 x Tris-borate-EDTA buffer at 100V for 3 h. After ethidium-bromide staining, gels were photographed in a GelDoc2000 Gel documentation system (Bio-Rad). Resulting images were analyzed using Bionumerics software package version 5.1 (Applied Maths).

Serologic investigation

For the patient with rhombencephalitis, antibodies were detected in the Gruber–Widal reaction using listeria suspensions O and –H (Dade Behring Marburg GmbH, Marburg, Germany). Positive serological findings must be treated with caution and, in cases other than rhombencephalitis, exact diagnosis requires detection of the pathogen.

Results

Music band: None of the three persons (female aged 45, male aged 52, male aged 64) finishing the one-day trip early, and leaving the visited city by boat instead of using the prebooked coach, fell ill during the following month. A total of 16 persons, comprising 14 members of the music band and the bus driver and his wife (mean age 59.8, median age 62, age range 41–74), had dinner at the wine tavern between 6 pm and 9 pm on September 6. According to recall by the 16 people, all except one person ate mixed cold-cuts; the person who did not eat at all did not become ill. Twelve of the 15 persons consuming mixed cold-cuts, including the driver and his spouse, fell ill with febrile gastroenteritis: nine on September 7 and three on September 8. Three patients with diarrhea were

hospitalized for 4, 6 and 9 days, respectively. Symptoms included unspecified fever (12/12 patients), diarrhea (9/12), headache (5/12), vomiting (4/12), body aches (2/12) and sore throat (1/12). The 71-year-old index case, who fell ill with fever (38.2 °C), diarrhea and shivering, was hospitalized on September 8 (for 4 days) and had a blood culture positive for *L. monocytogenes* sv 4. A 72-year-old patient, who fell ill on September 8 with fever, diarrhea, nausea and malaise, appeared to be recovering but developed severe headache on day 14 after the tavern visit. A stool specimen on September 16 was negative for listeria. This case was hospitalized on September 25 and diagnosed with bacterial meningitis; a CSF sample yielded *L. monocytogenes* sv 4. All patients recovered.

Seven stool samples on September 18 from five patients with diarrhea (male aged 50, male aged 72, male aged 73, female aged 68, female aged 41) and from two patients with fever but no reported symptoms of diarrhea (female aged 41, female aged 72) were tested bacteriologically for *L. monocytogenes*. Three samples were positive for sv 4b: from two patients with diarrhea [male aged 50, male aged 73] after three days of cold enrichment; from one patient with fever, headache and shivering but no reported diarrhea [female aged 72], after 14 days of cold enrichment.

Staff: On September 6, the staff of the wine tavern comprised eight persons: five family members, a family member's girl friend and two employees (mean age 38.25, median age 34.5, age range 19–56). None of these persons reported symptoms and all allegedly consumed the homemade products "regularly". Eight stool samples from the staff on October 6 were tested: two (male aged 56, female aged 54) were positive for *L. monocytogenes* sv 4b after cold enrichment for 3 and 14 days, respectively; six specimens were negative for listeria (female aged 19, female aged 27, female aged 49, male aged 32, male aged 33, male aged 36).

Other guests: We were able to trace one other group of four guests attending the tavern on September 6, two (female aged 48, male aged 52) of whom ate jellied pork (dressed with raw onions and vinegar). Approximately 30 h after consumption of the jellied pork, the 48-year-old woman noticed muscle pain, arthralgia and shivering but no signs of diarrhea; symptoms lasted for approximately 48 h. On October 4, 28 days after the incriminated dinner, she developed peripheral facial paralysis and on day 33 fever (unspecified) and severe headache. Lumbar puncture on October 9 showed 501 cells/µl CSF (89% segmented neutrophils), glucose 47 mg% (versus 93 mg% in serum) and elevated CSF protein (59 mg/dl); samples of CSF remained sterile by bacteriological culture. The peripheral leucocyte count was 11.27 x 10^9 cells/l. Magnetic resonance tomography imaging (MRI) revealed rhombencephalitis. The initial MRI (Fig. 1) showed a marked hyperintensity in the right pons in T2-weighted images, this region was also hyperintense in dark-fluid sequences (not shown) corresponding well with a right-sided rhombencephalitis. After seven days of treatment the extent of the inflammation was significantly smaller (Fig. 1). A serum specimen on November 14 contained antibodies against O4b antigen at a titer of 1:80. The patient recovered.

Food: Food was sampled by local health authorities on September 17. None of the commercially purchased food (various cheeses including unpasteurized sheep cheese) or homemade spreads yielded listeria. None of 30 raw-milk samples from the sheep of the producer of the unpasteurized cheese yielded *L. monocytogenes*. No salad samples were available for testing. Homemade jellied pork was found positive for *L. monocytogenes*: two of six meat loaves (each 3 kg) produced on September 3 were available for testing: one loaf was taken from the daily kitchen and yielded 3200 colony-forming units per gram (CFU/g) and 30,000 CFU/g when tested in two sep-

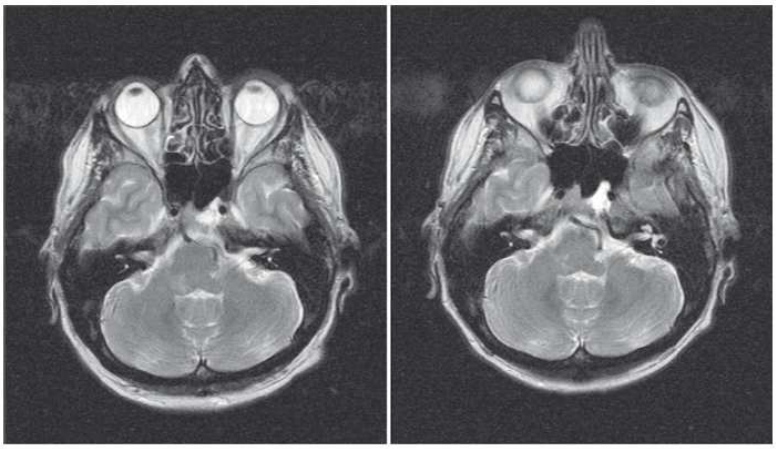

Fig. 1. Magnetic resonance tomography imaging of a 48-year-old patient showing a marked hyperintensity in the right pons in T2-weighted images, indicating a right-sided rhombencephalitis (left picture). After 7 days of treatment, the extent of the inflammation was significantly smaller (right picture)

arate accredited laboratories. The second loaf, stored at –20°C since September 17 yielded 4700 CFU/g. Hog roast yielded 230 CFU/g, blood sausage 10 CFU/g. Isolates from jellied pork and hog roast were indistinguishable from the human isolates; blood sausage yielded the *L. monocytogenes* sv 4b outbreak strain plus a *L. monocytogenes* sv 1/2b strain not found in any of the patients. According to the tavern owner's testimony, the samples of blood sausage and hog roast had been taken using the same kitchen knife as used for cutting samples of the jellied pork (without any intermediate cleaning steps).

Fingerprinting: Table 1 summarizes the results of molecular subtyping of human isolates from stool, CSF and blood and those of food samples. Epidemiologically unrelated human *L. monocytogenes* sv 4b isolates collected in Austria in 2008 are included for comparison. Fig. 2 and Fig. 3 show the DNA macrorestriction patterns of the isolates, determined by *Asc*I restriction of genomic DNA and separation of the restriction fragments by PFGE.

Analytical epidemiological investigation

The defined cohort comprised 19 persons travelling to the foreign city and two further persons joining them for dinner at the wine tavern (n = 21). Using our case

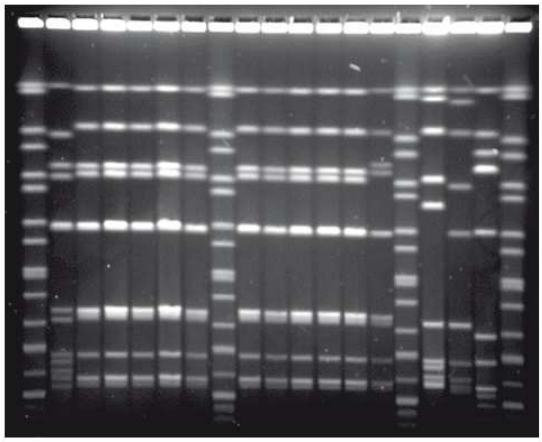

Fig. 2. DNA macrorestriction patterns of the isolates, determined by *Asc*I restriction of genomic DNA and separation of the restriction fragments by pulsed-field gel electrophoresis. Lanes 1, 8, 15, and 19 show the molecular-size standards (*Salmonella* Branderup), lane 2 shows the internal reference strain DSM 19094 (sv 1/2b); lanes 3 to 7 and 9 to 10 show isolates from stool specimens of persons with non-invasive listeriosis; lanes 11 to 13 isolates from the implicated jellied pork; and lanes 14 ,16, 17 and 18 isolates of serotype 4b unrelated to this outbreak

Table 1. Summarized data on molecular subtyping of human isolates from stool, cerebrospinal fluid and blood and those of food samples. Epidemiologically unrelated *L. monocytogenes* sv 4b isolates collected in Austria in 2008 are included for comparison

Strain no.	Date of sample	Source of *Listeria monocytogenes* sv 4b	Strain ID as used at the national listeria reference center	PFGE type using *Asc*I	PFGE type using *Apa*I	Rep-PCR type	AFLP
1	18.09.2008	Human (patient stool)	L33 807915/08	A	A	A	A
2	18.09.2008	Human (patient stool)	L38 807920/08	A	A	A	A
3	18.09.2008	Human (patient stool)	L39 807921/08	A	A	A	A
4	09.10.2008	Human (staff stool)	L67 808472/08	A	A	A	A
5	09.10.2008	Human (staff stool)	L68 808473/08	A	A	A	A
6	08.09.2008	Human (patient blood)	L40 807931/08	A	A	A	A
7	25.09.2008	Human (patient cerebrospinal fluid)	L45 806269/08	A	A	A	A
8	17.09.2008	Food isolate I (jellied pork)	W95 8080752	A	A	A	A
9	17.09.2008	Food isolate II (jellied pork)	W96 8080753	A	A	A	A
10	17.09.2008	Food isolate III (jellied pork)	W97 8080751	A	A	A	A
11	–	reference strain	DSM 15675	B	B	B	B
12	18.07.2008	epidemiologically unrelated (patient stool)	L27 805995/08	C	C	C	C
13	22.10.2008	epidemiologically unrelated (patient blood)	L75 809365/08	D	D	D	D

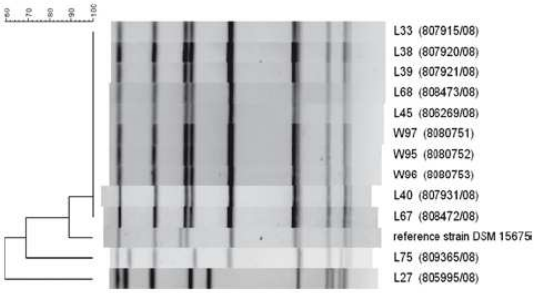

Fig. 3. Comparison of the *Asc*I pulsed-field gel electrophoresis patterns for the *Listeria monocytogenes* strains listed in Tabel 1. Cluster analysis was performed with the Bionumerics software (Applied Maths, Sint-Martems-Latem, Belgium) using the following settings: 0.5% optimization, 0.8% position tolerance, Dice similarity coefficient. For dendrogram construction the UPGMA algorithm was used

definitions, five confirmed cases and seven possible ones were identified. Calculations were performed on all cases for two different factors of exposure (lunch at foreign city; dinner at wine tavern). Ten of the 19 persons who had lunch in the foreign city fell ill (52.6%) and the two who did not eat lunch also fell ill (100%). This accounts for a relative risk of 0.53 (CI = 0.34–0.81) and a *P* value of 0.49. Twelve of the 15 persons who had dinner at the wine tavern fell ill (80%) and among the six persons who did not eat the dinner no-one fell ill (0%). The resulting *P* value of 0.0015 is highly significant. The relative risk was not calculable without "fudging". Using 11 instead of 12 for the diseased/exposed and 1 instead of 0 for the diseased/non-exposed gave a relative risk of 5.5 (CI = 0.88–34.46) and a *P* value of 0.016. Table 2 summarizes the epidemiological findings of this cohort study.

Discussion

L. monocytogenes is often isolated from food and can be isolated from the stools of healthy persons. Grif et al. studied the incidence of fecal carriage of *L. monocytogenes* in healthy volunteers and found that 0.2% were shedding *L. monocytogenes* by conventional culture and up to 3.6% were positive by PCR techniques [14]. In a related study, PCR results indicated an incidence of 5–9 exposures to *L. monocytogenes* per person/year [15]. On average, the incidence of culture-confirmed fecal carriage in healthy adults was two episodes of *L. monocytogenes* carriage per person/year; fecal shedding was of short duration (maximum 4 days).

In 1997, Dalton et al. provided clear proof of a listeria gastroenteritis syndrome [4]. In the preceding years, small outbreaks of primary gastrointestinal disease already had suggested that a typical gastroenteric illness could result from ingestion of *L. monocytogenes* [16, 17]. Including the Austrian outbreak described here, nine outbreaks of foodborne gastroenteritis for which *L. monocytogenes* was the most likely etiology have been published [3, 4, 6, 16, 17, 19–21]. How commonly listeria gastroenteritis occurs remains unclear, since *L. monocytogenes* is not detected in routine stool cultures. In Canada, Schlech et al. studied the incidence of sporadic, febrile gastroenteritis caused by *L. monocytogenes* over a two-year period [18]. They found 17 of 7775 stool specimens to yield *L. monocytogenes* and concluded that routine screening of stool samples for listeria remains unwarranted [18].

Outbreaks provide a unique opportunity to gain information on the number of organisms necessary to cause human illness, although leftover food from meals causing outbreaks is often no longer available at the time of the investigation [3]. However, chocolate milk causing an outbreak of gastroenteritis in Illinois in 1994 contained *L. monocytogenes* 1.2–8.8 x 10^8 CFU/ml [4], and leftover precooked, sliced turkey causing an outbreak of acute febrile gastroenteritis in 16 of 44 healthy persons at a catered party in the USA in 2001 yielded *L. monocytogenes* 1.6 x 10^9 CFU/g [19]. In the Austrian outbreak described here, we found 3200–30,000 CFU/g in the epidemiologically incriminated jellied pork. To our knowledge, this is only the third reported outbreak of *L. monocytogenes*-associated gastroenteritis where leftover food was available for estimation of the number of organisms present in the incriminated food. From the data available on outbreaks in Italy and in Illinois, it appears that febrile gastroenteritis in normal hosts requires the ingestion of a high dose of several million bacteria [4, 19]; the relatively low number found in our outbreak may explain why none of eight members of staff who had eaten the products fell ill.

In 1992 consumption of pork tongue in aspic (jelly) was associated with one of the largest documented out-

Table 2. Attack rates (AR) and relative risks (RR) of two different exposure factors

Exposure factors		Diseased	Non-diseased	Sum	AR %	RR (95% CI)	*P*-value
Lunch in foreign city	Exposed	10	9	19	52.6	0.53 (0.34-0.81)	0.49
	Non-exposed	2	0	2	100		
	Sum	*12*	*9*	*21*			
Dinner at wine tavern	Exposed	12	3	15	80	Not calculable	0.0015
	Non-exposed	0	6	6	0		
	Sum	*12*	*9*	*21*			

breaks of systemic listeriosis in France [22]. This outbreak was also due to sv4b and resulted in 63 deaths in nonpregnancy-asociated cases, 22 fetal deaths and seven neonate deaths, giving an overall case fatality ratio of 33%.

Khan et al. examined the behavior of *L. monocytogenes* in a preparation of pork muscle sarcoplasmic proteins inoculated to contain approximately 1 x 10^4 CFU/ml of *L. monocytogenes* and held at 4°C [23]. According to these investigators, the pathogen grew readily in preparations of pork protein, reaching levels of approximately 1 x 10^5 and 1 x 10^7 CFU/ml, respectively, after 12 days of refrigerated storage. Hence, it appears that raw pork can support rapid growth of listeria, particularly when the product has undergone temperature abuse, as frequently occurs at the retail level. Standard thermal treatment is more than sufficient for producing listeria-free meat products; however, all cooked meats can become contaminated with listeria organisms during slicing and further handling.

Most foodborne outbreaks of listeriosis have been caused by sv 4b, although there does not appear to be a direct correlation between this serotype and greater virulence. The fact that our gastroenteritis outbreak was also caused by sv 4b, as was the outbreak described by Aureli et al. [3], contradicts the hypothesis that "serotype 1/2 b is more likely to cause the milder form of gastroenteritis with fever" [24]. A foodborne outbreak of gastroenteritis reported by Salamina et al. was caused by *L. monocytogenes* sv 1/2b [16]; an outbreak reported by Frye et al. was due to *L. monocytogenes* sv 1/2a [19].

Generally, the median incubation period of systemic listeriosis is estimated to be three weeks, although outbreak cases of systemic listeriosis have occurred 3–70 days following a single exposure to an implicated product [25]. Our meningitis patient with culture negative gastroenteritis developed severe headache approximately 14 days after consumption of the jellied pork.

For the gastroenteritis patients described by Aureli et al., the median time elapsing between consumption of the meal and onset of symptoms was 24 h [3]. Concerning gastroenteritis, Salamina et al. [16] and Dalton et al. [4] reported shorter incubation periods (18 h and 20 h [range 9–32], respectively), whereas Miettinen et al. [9] reported a longer period (median incubation 28 h). Our finding of an incubation period of 1–2 days in our gastroenteritis patients correlates well with these findings.

Aureli et al. had observed an attack rate of 72% [3], Dalton et al. of 75% [4]. In the Austrian outbreak described here, 12 of 15 exposed persons developed febrile gastroenteritis, giving an attack rate of 80%.

In the outbreak of febrile gastroenteritis associated with corn contaminated by *L. monocytogenes* sv 4b described by Aureli et al., 19% (292 persons) of those reporting symptoms were admitted to hospital (median duration of hospital stay, 3 days); none had a diagnosis of sepsis, all were children [3]. In the outbreak associated with milk contaminated by *L. monocytogenes* sv 1/2a described by Dalton et al., four persons (aged 1, 7, 49 and 77) were hospitalized for a total of eight hospital days, none of them died [4]. In the Austrian outbreak, the three patients hospitalized with diarrhea stayed in the clinics for 4, 6 and 9 days, respectively. In Austria in 2007, the average hospital stay of patients with salmonellosis was 7.9 days, that of patients with campylobacteriosis 4.6 days (unpublished data). It is clear that gastroenteritis caused by *L. monocytogenes* can require hospital stays of similar length to those caused by classic gastroenteric pathogens.

In the Austrian outbreak described, five (33%) of 15 stool samples (7 from symptomatic and 8 from asymptomatic patients) were positive for *L. monocytogenes* within 3–14 days of cold enrichment. The five positive samples were from three symptomatic patients and two who were asymptomatic. Aureli et al. reported that of 141 stool samples tested, 123 (87%) were positive for *L. monocytogenes* [3]. According to Dalton et al., *L. monocytogenes* was isolated from 11 (27%) of 41 stool samples collected 16–19 days after consumption of the milk [4]. Aureli et al. already emphasized the importance of cold enrichment (a technique to selectively enhance the growth of psychrophilic bacteria) during the processing of stool samples [3].

In our Austrian outbreak, a single positive blood culture triggered awareness of the possibility of listeriosis as the cause of this cluster of febrile gastroenteritis. Aureli et al. reported that one of 40 blood cultures from patients with gastroenteric listeriosis was positive for *L. monocytogenes* [3]. During another outbreak of gastroenteritis that occurred in Italy in 1996 among 39 persons (previously healthy, young, non-pregnant adults) who had attended a private supper, four were hospitalized with acute febrile gastroenteritis, two of whom had blood cultures positive for *L. monocytogenes* [16]. According to Dalton et al., blood cultures from a person hospitalized with febrile gastroenteritis caused by *L. monocytogenes* remained sterile [4].

Current regulations in Europe allow low-level contamination (<1 x 10^2 CFU/g) of raw or processed foods if there has been no documented association with human disease. Our findings of higher levels of listeria (3 x 10^3–3 x 10^4 CFU/g) in the incriminated jellied pork support such a dose-based regulatory approach rather than an absolute proscription of this pathogen in food.

Outbreaks of *L. monocytogenes*-associated gastroenteritis are probably under-recognized because of a lack of awareness of this syndrome and because stool samples are infrequently screened for *L. monocytogenes*. Healthcare workers and public health officials should consider *L. monocytogenes* as a possible cause of febrile gastroenteritis, particularly in cases where routine screening fails to identify an enteric pathogen. The factors leading to febrile gastroenteritis as opposed to invasive disease are currently poorly understood [26]. Here we have described the first outbreak of *L. monocytogenes*-associated gastroenteritis documented in Austria. The occurrence of a secondary case of meningitis (diagnosed on day 19 after consumption of jellied pork) indicates a significant risk for systemic listeriosis among elderly patients with febrile gastroenteritis caused by *L. monocytogenes*; antibiotic therapy should therefore

be considered in such cases of documented listerial gastroenteritis. Although there are no data on the efficacy of treatment with antimicrobials in this illness [27], from our findings it could be argued that in both symptomatic and asymptomatic persons known to have ingested a food implicated in an outbreak and who have a high risk of invasive disease because of underlying illness, pregnancy, or age (elderly) it might be prudent to administer several days' treatment with oral ampicillin or trimethoprim-sulfamethoxazole.

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4.7 Original article V

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Evaluating levels of knowledge on food safety among food handlers from restaurants and various catering businesses in Vienna, Austria 2011/2012

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ABSTRACT

The aim of this study was to detect the most important gaps in knowledge on food safety among food handlers in Vienna, Austria and to identify possible differences in levels of knowledge between food handlers from restaurants and catering companies. The survey was conducted from May 2011 to January 2012 in Vienna and 234 food handlers participated. The average knowledge score for all food handlers was 76%. We revealed no significant difference between the two sample groups; food handlers from catering businesses scored similar (75%) to those from restaurants (76%). Persons with a training at their current workplace (internal and external) scored significantly higher (82%) then persons without an on the job training (71%). Food handlers passing the mandatorily required yearly food safety training had a higher knowledge than persons without this on-the-job training ($p \leq 0.001$); 23% of the food handlers didn't participate in any training during the last year. The study identified substantial knowledge gaps concerning correct temperatures for cooking, holding and storing foods. Data from this project underline the value of harmonized action in the field of food safety, but also indicate considerable potential for further improvement in Austria.

1. Introduction

Foodborne diseases and outbreaks are crucial contributors to morbidity and mortality worldwide (Flint et al., 2005). The total health-related cost of foodborne illness in the United States is $ 51.0 billion in the basic cost-of-illness model (including values for medical care, productivity losses and mortality) and $ 77.7 billion in the enhanced model, including added pain and suffering (Scharff, 2012). No published data for Austria and the European Union are available concerning socio-economic costs of foodborne infections. Globally, the incurred costs and dimensions of foodborne diseases are still unknown but they seem to be substantial (Kuchenmüller et al., 2009).

The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Foodborne Outbreaks – published by the European Food Safety Authority and the European Centre for Disease Prevention and Control – reported 5262 foodborne outbreaks with 43,473 cases, 4695 hospitalizations, and 25 deaths in the European Union for the year 2010 (European Food Safety Authority & European Centre for Disease Prevention and Control, 2012). Thirty-one percent of the outbreaks with strong evidence were associated with restaurants, cafes, pubs, bars and hotels and 17% were linked to catering for schools, kindergartens, residential institutions, temporary mass events and workplace canteens (European Food Safety Authority & European Centre for Disease Prevention and Control, 2012). In Austria, 193 outbreaks involving 838 people, 155 hospitalizations and two fatal cases were reported for 2010. 12 out of 161 domestically acquired outbreaks were associated with eating establishments and 198 persons were involved; 38% of the outbreaks had an unknown setting (Much, Voss, Pichler, & Allerberger, 2011).

In Austria, a large proportion of people is 'eating out' and the number of meals consumed outside the home at lunchtime is likely to increase: 24% of the Austrian population eat regularly in workplace canteens, 21% in restaurants and 16% at fast food premises (Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, 2010).

Many different factors may lead to foodborne infections and outbreaks; high risk factors for foodborne disease are food from unsafe sources, poor personal hygiene, inadequate cooking,

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improper holding times and temperatures, and cross contamination (U. S. Food and Drug Administration, 2009). Lack of knowledge concerning food safety among food handlers may result in the transmission of foodborne pathogens to the public during food preparation (DeBess, Pippert, Angulo, & Cieslak, 2009). A recent meta-analysis has shown that food safety training increases knowledge and improves attitudes about hand hygiene practices and that refresher training and recurrent emphasis on good food handling behavior may have ongoing positive effects on hand washing practices among food handlers (Soon, Baines, & Seaman, 2012). However other studies found that although special training may improve knowledge of food safety, this does not always result in better and safer food handling behavior (Baş, Şafak Ersun, & Kıvanç, 2006; Park, Kwak, & Chang, 2010). Clayton et al. and Green et al. pointed out that behavioral change and new food safety practices will only be implemented if adequate resources (e.g. structural environment, sufficient staff and time) and a supportive management culture exist (Clayton, Griffith, Price, & Peters, 2002; Green & Selman, 2005). Apart from food safety training, several other factors and food handler characteristics like age (Martins, Hogg, & Otero, 2012), level of education and work experience may affect the knowledge scores (Angelillo, Viggiani, Rizzo, & Bianco, 2000; Martins et al., 2012).

In Austria, little is known about level of knowledge of food safety and practices of food handlers in restaurants and catering companies. The aim of this study was to detect the most important gaps in food safety knowledge among food handlers in Vienna (capital and largest city of Austria, total population: 1.73 million) and to identify possible knowledge differences between food handlers from restaurants and catering companies concerning food safety. Furthermore the study should elucidate any relationships among different food handler characteristics like education, work experience, previous food safety training and food safety knowledge. The results of this survey can improve the efficacy of educational brochures and training materials for different target groups.

2. Materials and methods

2.1. Questionnaire development and data collection

The aim of the survey was to obtain baseline information on knowledge, behavior, and personal hygiene practices of food handlers, as well as data on food establishment characteristics. The basis of the questionnaire was developed within an international hygiene study on food handling knowledge from the University of Illinois at Chicago School of Public Health, Division of Epidemiology and Biostatistics (Dworkin, Udompat, Panchal, & Liu, 2011; Panchal, Liu, & Dworkin, 2012). Besides Chicago and Vienna, the questionnaire was applied in two other studies, conducted in Neuchâtel, Switzerland (Panchal, Bonhôte, & Dworkin, 2013) and Bolzano, Italy. The English questionnaire was translated into German and adapted to the Austrian food law and food handling requirements. Meetings with experts from the Vienna Chamber of Commerce and the University of Vienna, Department of Nutritional Sciences led to questionnaire adjustments. After pilot testing was completed, the final survey instrument was launched.

The knowledge assessment part of the questionnaire consisted of 42 knowledge questions addressing relevant topics like appropriate handling of high risk food groups, correct storage, processing and cooking of food, proper temperatures for heating and cooling food, correct hand washing and whether to work while ill. Additionally, participants were asked about their personal hygiene, socio-economic data, work experience, work tasks and their history of food safety training. Data on food service establishment characteristics like restaurant size, food service style, cuisine-type and average entrée price were also collected (data not shown). The questionnaire included true–false, yes–no, multiple-choice and fill-in-the-blank questions and the possibility to answer 'do not know'. The interviewers insisted that the questionnaires were completed during their visit to preclude the participants from looking up information and being supported by workmates. The food handlers were able to ask the interviewer in case of any language difficulties or ambiguity concerning the questions. No compensation was offered to the food handlers for participating. The German questionnaire was translated back into English for this publication.

2.2. Study samples

The survey was conducted from May 2011 to January 2012 in Vienna and involved 234 food handlers. The study population consisted of 157 restaurant food handlers and 77 food handlers from catering businesses. Of the 77 catering food handlers, 40 worked for the Austrian Armed Forces and 37 for private catering companies. For the restaurant study sample a member list of 2037 restaurants in Vienna was used, provided by the Vienna Chamber of Commerce. Out of this list, 279 restaurants were selected as a random sample to be approached and 235 agreed to take part in the survey in the first approach. Each of the 235 restaurants (food service styles fast food, informal and formal) was contacted by phone to arrange a date for an on-site interview; 64% of the restaurant managers agreed to participate in the survey with at least one food worker. Finally, 157 food handlers (managers and ordinary workers) from 150 different restaurants in Vienna were interviewed by a self-administered questionnaire. The 40 food handlers from the Austrian Armed Forces, working with a cook & chill technique, were interviewed in four different facilities in Vienna, including one central kitchen and three finalizing kitchens. In three different private catering businesses 37 persons out of a total staff of 89 persons agreed to be interviewed.

For statistical analysis participants were condensed to two sample groups: restaurant food handlers and food handlers from various catering businesses (private caterings and participants from the Austrian military services). All 234 participants of the survey were assured of confidentiality.

2.3. Statistical methods

Statistical analysis was performed using SPSS 20.0 for Windows (SPSS Inc, Chicago, IL). The relative knowledge score (the percentage of the correct answers) was calculated by dividing the sum of correct answers by the total number of valid responses. "Relative" refers to the relation of correct answers in terms of total (valid) responses. In the following discussion of the knowledge score, the relative reference is implied. *T*-tests were performed to compare the knowledge scores between two groups-categorical variables, such as gender. Analysis of Variance (ANOVA) models were carried out to compare knowledge scores across levels of categorical variables between more than two groups, such as age or level of education. To identify knowledge gaps among food handlers, chi square tests were used to compare the percentage of correct responses to each of the 42 questions across the two sample groups (food handlers from restaurants, food handlers from catering businesses). To identify effects to the food handler knowledge score, multivariate analysis was carried out using a general linear model. The backward selection method with a level of significance <0.05 was used to determine food handler characteristics and food establishment variables that remained in the final multivariate model.

3. Results

3.1. Descriptive analysis (Table 1)

3.1.1. Sample group: restaurant food handlers

The mean age of the 157 participating food handlers from restaurants was 42 years (age range 18–73 years), 75% ($n = 117$) were male and 62% ($n = 98$) were born in Austria. Fifty-eight percent ($n = 91$) of the food handlers had had formal culinary training as cook or waiter. Twenty-nine percent ($n = 45$) had a work experience of 8–16 years, 16–25 years and more than 25 years had each 43 participants (28%). Seventy-two percent ($n = 111$) had participated in food safety training at their current workplace and 55% ($n = 84$) were non-certified hygiene managers.

One hundred fifty-seven food handlers from 150 restaurants participated (response rate = 64%). Reasons stated by restaurants for not participating in our study included refusals without comments ($n = 48$; 20%), closures ($n = 4$; 2%), and other reasons like changed management or language barriers ($n = 33$; 14%).

3.1.2. Sample group: catering businesses

The mean age of the 77 cooperative food handlers from catering businesses was 30 years with a range of 16–58 years, 87% ($n = 66$) were male and 79% ($n = 61$) were born in Austria. Fifty-one percent ($n = 37$) had had a formal culinary training as cook or waiter and 36% ($n = 21$) of the participants had worked for 2–8 years as food handlers. Eighty-three percent ($n = 63$) of the food handlers had participated in a food safety training at their current workplace and 70% ($n = 48$) were non-certified hygiene managers.

3.2. Knowledge scores

Overall, the knowledge score was 76%. Food handlers from restaurants had a knowledge score of 76%, those from catering businesses 75%. Within the study sample catering businesses food handlers from private catering businesses scored significantly better (79%) then the food handlers from the Austrian military services (72%, $p = 0.005$).

3.3. Bivariate analysis of the food handlers' characteristics (Table 1)

Bivariate analysis of **all food handlers** showed that their scores were significantly associated with age ($p = 0.040$), education ($p < 0.001$), country of birth ($p = 0.033$), mother tongue ($p < 0.001$), previous participation in food safety training courses ($p = 0.003$) and training at current workplace ($p = 0.001$). Younger food handlers (15–29 years) scored 73% and participants aged 30 years and older (three age categories) scored 77%, respectively. Kitchen staff with an apprenticeship (cook or waiter) scored 79% and those with the certificate "Meisterprüfung", the highest Austrian degree of an apprenticeship, scored 78%. A low level of food safety knowledge had participants with other apprenticeships (66%). Previous participation in food safety training courses showed significant effects on the knowledge scores; participants who had undergone food safety training courses achieved higher knowledge scores (78%) than those who attended no training courses (74%). Persons with a training at their current workplace (internal and external) scored significantly higher (82%) than persons without an on the job training (71%).

Bivariate analysis of the **restaurant food handler** characteristics showed education ($p = 0.004$), country of birth ($p = 0.013$) and mother tongue ($p = 0.027$) as significantly associated with the knowledge scores. Food handlers with compulsory schooling only scored lower (70%) than participants with formal culinary training (cook or waiter) or a university degree (78%, respectively).

Bivariate analysis of **catering business food handler** characteristics demonstrated that their knowledge scores were significantly associated with age ($p = 0.002$), education ($p < 0.001$), mother tongue ($p = 0.003$), work experience ($p = 0.044$), previous participation in food safety training courses ($p = 0.005$) and a training at their current workplace ($p < 0.001$). Catering business food handlers aged 15–29 years had lower scores (71%) than those aged between 30 and 39 years (84%). Kitchen staff with an apprenticeship (cook or waiter) scored 81% and those with the certificate "Meisterprüfung", the highest Austrian degree of an apprenticeship, scored 82%. Both scored significantly higher than participants with other apprenticeships (60%). Native German speakers achieved a significantly higher score (78%) than those with another mother tongue (69%). Work experience showed a significant influence on the knowledge scores: participants with a work experience of up to 2 years scored 71%, while food handlers with a work experience of 16–25 years scored 83%. The scores of participants without previous participation in a food safety training course (71%) was lower than the scores of certified hygiene managers (83%) and of food handlers who participated in other food safety training courses (81%). Training at current workplace showed significant effects on the scores; participants with internal training achieved 77%, those who participated in external training 82%, and those with both (internal and external training) 86%. Food handlers without any training at the actual workplace differed significantly with scores of 60%.

3.4. Knowledge gaps – a comparison between the sample groups

Significant differences of food handlers' individual knowledge levels from restaurants and catering businesses were revealed by questions concerning optimal temperatures for cooking, holding and cooling foods, and of hygiene practices (Table 2).

Time and temperature: More catering business food handlers responded correctly to questions about the minimum internal temperatures for cooking hamburger and ground beef mixtures (46%), and minimum temperature for cooking chicken (52%) than did the restaurant food handlers (15%, 32%, respectively; $p \leq 0.004$). Significantly more food handlers from restaurants than from catering businesses answered correctly about the maximum temperature for storing cold foods like cold cuts or cooked rice (83% versus 63%, $p = 0.001$). A similar picture shows the statement 'If fish has been stored at a temperature that is to warm, but then is properly cooked to the correct temperature, it becomes safe to eat'; 82% of the restaurant food handlers and 64% of those from catering businesses knew the correct answer ($p = 0.003$).

Cross contamination: Ninety-three percent of the restaurant food handlers and 75% of those from catering businesses answered correctly to the statement 'It is safe to put frozen chicken breast on the counter to thaw' ($p < 0.001$). Eighty percent of restaurant food handlers and 66% of food handlers from catering businesses knew that if vegetables for a salad were splashed with raw chicken juice, they should not be rinsed, but instead must be thrown away ($p = 0.019$). Ninety-four percent (respectively) of restaurant food handlers and 86% and 82% of food handlers from catering businesses recognized that the statements 'Raw eggs may be stored above a prepared but uncovered salad in the refrigerator' and 'Raw meat can be stored above ready-to-eat-food' are false ($p \leq 0.003$). All of the catering business food handlers answered the following question correctly with "no": 'Is it okay to pick up ice cubes with bare hands?' Also the restaurant food handlers largely answered correctly (99%). Similar scores achieved the study participants from catering businesses (99%) regarding the question "Is it okay to pick up ice cubes with an ice scoop?" Those from restaurants knew less often the correct answer (89%, $p = 0.010$).

Table 1
Food handler characteristics (restaurant, catering) and differences in the knowledge scores (KS).

		Overall ($n = 234$)		KS[a]	Within[b]	Restaurant ($n = 157$)		KS[a]	Within[b]	Catering ($n = 77$)		KS[a]	Within[b]	Between[c]
						N	Valid *N* %	Rel. score %	*p*-value*	*N*	Valid N %	Rel. score %	*p*-value*	*p*-value*
Total		234	100%	76		157	100%	76		77	100%	75		
Gender	Male	183	78.5%	76	0.103	117	75%	77	0.057	66	87%	75	0.864	0.307
	Female	50	21.5%	74		40	25%	74		10	13%	74		0.895
Age	15–29 years	73	31.2%	73	0.040*	26	17%	77	0.773	47	61%	71	0.002*	0.043*
	30–39 years	46	19.7%	77		34	22%	75		12	16%	84		0.002*
	40–49 years	65	27.8%	77		57	36%	76		8	10%	80		0.238
	≧50 years	50	21.4%	77		40	25%	76		10	13%	80		0.305
	Mean (years)	38				42				30				
	Min–max (years)	16–73				18–73				16–58				
Education	Compulsory schooling only	29	12.6%	71	0.000*	13	8%	70	0.004*	16	22%	71	0.000*	0.643
	Apprenticeship in culinary training	105	45.7%	79		71	45%	78		34	47%	81		0.044*
	Other apprenticeship	21	9.1%	66		9	6%	74		12	16%	60		0.009*
	Meisterprüfung[d]	23	10.0%	78		20	13%	77		3	4%	82		0.277
	College degree	26	11.3%	72		24	15%	73		2	3%	69		0.600
	University degree	26	11.3%	77		20	13%	78		6	8%	74		0.467
Country of birth	Austria	159	67.9%	77	0.033*	98	62%	77	0.013*	61	79%	76	0.451	0.320
	Foreign country	75	32.1%	74		59	38%	74		16	21%	73		0.769
First language	German	157	67.4%	77	0.000*	104	66%	77	0.027*	53	70%	78	0.003*	0.741
	Other language	76	32.6%	72		53	34%	74		23	30%	69		0.031*
Work experience	Up to 2 years	18	8.5%	72	0.123	5	3%	74	0.344	13	22%	71	0.044*	0.625
	2–8 years	40	18.8%	75		19	12%	73		21	36%	78		0.158
	8–16 years	53	24.9%	77		45	29%	77		8	14%	79		0.420
	16–25 years	51	23.9%	77		43	28%	76		8	14%	83		0.010*
	25 years and more	51	23.9%	78		43	28%	77		8	14%	81		0.224
Food safety training course	'Yes, certified hygiene manager'	25	11.2%	78	0.003*	19	12%	76	0.256	6	9%	83	0.005*	0.156
	'Yes, other training'	66	29.6%	78		51	33%	78		15	22%	81		0.192
	'No'	132	59.2%	74		84	55%	75		48	70%	71		0.021*
Training at current workplace	'Yes, internal'	132	57.1%	77	0.001*	75	48%	77	0.374	57	75%	77	0.000*	0.782
	'Yes, external'	34	14.7%	77		32	21%	76		2	3%	82		0.374
	'Yes, both'	8	3.5%	82		4	3%	79		4	5%	86		0.164
	'No'	54	23.4%	71		42	27%	74		12	16%	60		0.000*
	'I don't know'	3	1.3%	75		2	1%	81		1	1%	64		0.290

*Statistical significance $p < 0.05$.
[a] Knowledge score, relative percentages (based on valid values only).
[b] Differences within the characteristics.
[c] Differences between food handlers from restaurants and catering businesses.
[d] Highest Austrian degree of an apprenticeship.

Table 2
Frequencies of correct responses to knowledge questions asked of food handlers from restaurants and catering businesses from Vienna, 2011. Questions marked by an asterisk indicate statistical significance at $p < 0.05$.

Questions (*Answers*)	Correct responses (valid *N* %)			
	Question types	Overall ($n = 234$)	Restaurants ($n = 157$)	Catering businesses ($n = 77$)
Time and temperature (10)				
If fish (such as raw tuna) has been stored at a temperature that is too warm, but then is properly cooked to the correct internal temperature, it becomes safe to eat.* (*false*)	True/False	77%	82%	64%
Cold food should be stored chilled (at 13 °C or lower). (*false*)	True/False	67%	69%	63%
Chilling of hot food has to happen fast. To pass the critical temperature range faster, it is recommended to put the food into smaller containers for storing in the refrigerator. (*true*)	True/False	71%	68%	76%
How should foods be stored in the refrigerator regarding to their expiration dates? (*first in–first out*)	Fill-in-the-blank	96%	97%	93%
Germs that make people sick grow well between which temperatures? Minimum (*4–6 °C*)	Fill-in-the-blank	7%	9%	4%
Germs that make people sick grow well between which temperatures? Maximum (*65 °C*)	Fill-in-the-blank	12%	11%	14%
Hamburger and other ground beef mixtures such as meatloaf should be cooked to at least what temperature on a meat thermometer?* (*75 °C*)	Fill-in-the-blank	25%	15%	46%
What is the proper minimum internal temperature to cook chicken?* (*75 °C*)	Fill-in-the-blank	39%	32%	52%
If hot roast beef has been held in a steam table below 57 °C for over 4 h, it should be (*thrown away*).	Multiple-choice	55%	55%	55%
Cold food (cold cuts, cooked rice) must be kept at a maximum temperature of* _4_°C (*tolerable 6 °C*).	Fill-in-the-blank	77%	83%	63%
Cross contamination (14)				
Cooked rice, which was stored incorrectly, may contain germs that can make people sick. (*true*)	True/False	83%	85%	77%
It is safe to put frozen chicken breast on the counter to thaw.* (*false*)	True/False	87%	93%	75%
Raw eggs may be stored above a prepared but uncovered salad in the refrigerator.* (*false*)	True/False	91%	94%	86%
A food handler who has a small infected cut on his or her finger prepares a sandwich without using single-use gloves. The sandwich is not stored chilled. The person who eats that sandwich could become ill with vomiting and diarrhea. (*true*)	True/False	86%	87%	86%
Gloves used to handle ready-to-eat food should be thrown in the trash when interruptions occur in operations. (*true*)	True/False	95%	96%	95%
If you are ill with diarrhea, it is okay to handle raw food as long as that food will be cooked. (*false*)	True/False	85%	84%	87%
When you suffered from vomiting or diarrhea but don't feel really ill, you are allowed to handle ready to serve food like sandwiches or salad on that day. (*false*)	True/False	89%	89%	88%
Vegetables for a salad splashed with a few drops of raw chicken juice should not be rinsed, but must be thrown away.* (*true*)	True/False	76%	80%	66%
Raw meat can be stored above ready to serve food.* (*false*)	True/False	90%	94%	82%
Raw meat can be stored anywhere in a refrigerator as long as it is tightly sealed in plastic film. (*true*)	True/False	57%	53%	64%
Is it okay to put ice cubes in a glass by…				
Using tongs? (*yes*)	Yes/No	92%	93%	90%
Using an ice scoop?* (*yes*)	Yes/No	92%	89%	99%
Scooping the glass into the ice cubes? (*no*)	Yes/No	86%	87%	82%
Picking up ice cubes with your bare hands? (*no*)	Yes/No	99%	99%	100%
Hand hygiene (7)				
At work if you only urinated, and did not have a bowel movement, you do not need to wash your hands. (*false*)	True/False	90%	90%	88%
Do you need to have thoroughly washed hands if you use				
Wax paper to handle food? (*yes*)	Yes/No	94%	94%	96%
A spatula or tongs to handle food? (*yes*)	Yes/No	80%	78%	86%
Single-use gloves to handle food? (*yes*)	Yes/No	78%	76%	84%
To wash your hands had you better use warm or cold water? (*warm*)	Multiple-choice	97%	97%	97%
About how many seconds should you lather your hands with soap? At least__* (*10 s*).	Fill-in-the-blank	77%	72%	89%
On what should you dry your hands? (*paper towel or air dryer*)	Multiple-choice	87%	89%	83%

(*continued on next page*)

Table 2 (*continued*)

Questions (*Answers*)	Correct responses (valid *N* %)			
	Question types	Overall ($n = 234$)	Restaurants ($n = 157$)	Catering businesses ($n = 77$)
Cleaning and sanitizing (2)				
Properly labeled detergents may be kept in the same areas where food is prepared, if they have an own storage area and are only used for intermediate cleaning. (*true*)	True/False	31%	29%	35%
The difference between cleaning and sanitizing is: (*Cleaning is to remove food or other types of soil from a surface but sanitizing is to reduce the number of germs on a clean surface to safe levels*)	Multiple-choice	94%	94%	94%
Other (9)				
Is it true that if not completely cooked, these foods could cause serious illnesses?				
Raw beef (*true*)	True/False	53%	51%	57%
Raw poultry (*true*)	True/False	96%	97%	95%
Raw eggs (*true*)	True/False	87%	88%	84%
You can be sure food is safe to eat when it smells and tastes normal. (*false*)	True/False	43%	41%	47%
Eating ground meat that is not completely cooked can cause diarrhea.* (*true*)	True/False	83%	90%	70%
Beef may be placed directly on the counter to defrost. (*false*)	True/False	91%	93%	86%
Beef may be placed in hot water to defrost.* (*false*)	True/False	85%	90%	74%
Where should meat thermometers be inserted to accurately check the meat's temperature? (*the thickest part of the meat*)	Multiple-choice	96%	97%	94%
Which type of thermometer is best to check the temperature of a chicken breast? (*a metal stem thermometer*)	Multiple-choice	79%	80%	77%

Hand hygiene: A high percentage of all the food handlers were able to correctly identify aspects of good hand hygiene practice such as necessity to wash hands after going to the toilet (90%), to wash hands before using wax paper to handle foods (94%) or to use warm water for washing hands (97%). Only 72% of the restaurant food handlers and 89% of the catering business food handlers knew, that the minimum duration of lathering the hands with soap is 10 s ($p = 0.005$).

Cleaning and sanitizing: Ninety-four percent of all food handlers were aware of the difference between cleaning and sanitizing; 31% of them falsely thought that it is not allowed to keep properly labeled detergents in the same area as where food is prepared. For the two questions in this category no significant differences existed in the scores of the two sample groups.

Other questions: Most food handlers (96%) knew the correct part of the meat to check the temperature and the fact that raw poultry can cause serious illness. Of all food handlers 43% falsely thought that food is safe when it smells and tastes normal. Fifty-three percent falsely claimed that raw beef could not cause serious illness. Restaurant food handlers had significantly better scores (90%, respectively, $p \leq 0.001$) concerning 'Eating ground meat that is not completely cooked can cause diarrhea' and 'Is it wrong to place beef in hot water to defrost?' than food handlers from catering businesses (70% and 74%).

Table 3
Influence of food handler characteristics on the knowledge score.

Multivariate analysis	Estimate	*p*-value
Intercept	72.08	< 0.0001
Education		
University degree	*Ref.*	
Compulsory schooling only	−6.22	0.009*
Apprenticeship in culinary training	1.03	0.596
Other apprenticeship	−10.69	0.000*
Meisterprüfung[a]	0.37	0.883
College degree	−4.91	0.042*
First language		
Other language	*Ref.*	
German	7.39	0.001*
Main effect; interactions		
First language*operating mode		
German*catering businesses	*Ref.*	
German*restaurants	−1.71	0.273
*Other*catering businesses*	*Ref.*	
Other*restaurants	4.53	0.042*

*Statistical significance $p < 0.05$.
Ref. = Reference value.
[a] Highest Austrian degree of an apprenticeship.

3.5. Factors associated with the knowledge scores

In the final multivariate model three variables (education, mother tongue and mother tongue in interaction with the operating mode) were found to be significantly associated with the knowledge scores (Table 3). The main effect of 'education' had a highly significant influence on the scores ($p < 0.001$), food handlers with a non-gastronomy specific apprenticeship, less educated food handlers (compulsory schooling only) and food handlers with a college degree had significantly lower knowledge scores than food handlers with a university degree (score differences: 10.7, 6.2 and 4.9 percentage points; $p < 0.05$). The main effect of 'mother tongue' showed a very significant influence ($p = 0.002$); German speaking food handlers scored significantly higher than food handlers speaking other first languages (score difference: 7.4 percentage points). The interaction between mother tongue and operating mode was tendentially significant ($p = 0.065$). German speaking food handlers had throughout a better knowledge score, regardless whether they work in restaurants or in catering businesses. But restaurant food handlers speaking other first languages scored higher than those food

handlers from catering businesses (score difference: 4.5 percentage points, $p = 0.042$).

4. Discussion

The main objective of our study was to survey the level of food safety knowledge of food handlers in general and to identify possible differences between food handlers from restaurants and catering businesses. The average knowledge score of all food handlers was 76%. They were slightly superior to the kitchen staff from Chicago (71%) (Panchal et al., 2012) and Neuchâtel, Switzerland (71%) (Panchal et al., 2013).

We identified no significant differences in knowledge between the two study samples; food handlers from restaurants scored similarly (76%) to those from catering businesses (75%). However, there are differences in food safety knowledge within the study sample catering businesses; food handlers from private catering businesses scored significantly better (79%) then the food handlers from the Austrian military services (72%, $p = 0.005$).

The second aim of our study was to identify the differences between food handlers with a certificate in food safety and food handlers without any food safety training courses. Certified hygiene managers had average scores of 78% compared to 74% for non-certified food handlers ($p < 0.01$). In Austria the certificate 'hygiene manager' can be obtained in multiple institutions after completion of several training modules concerning microbiological basics, cleaning and disinfection, good hygiene practice and Hazard analysis and critical control points (HACCP). There are also other certificates to be gained in food safety training courses but they are not mandatory to work in a kitchen. Promoting participation in such training courses seems to be an efficient tool to improve the food safety knowledge in Austria. Our findings are similar to the results of the hygiene study in Chicago, where the knowledge score of certified food handlers was 78% and that of non-certified food handlers only 66% (Panchal et al., 2012). However, training for food handlers and also the definition of 'certified food handler' have different meanings in the US and in Austria. A comparison of the mean knowledge scores between the two countries has to be done with caution.

In Austria, in order to work in a restaurant as cook or waiter, one has to do culinary training for over 3 years, either in a professional school in combination with in-service training in a kitchen or as a student in a gastronomy or vocational school with school leaving examinations focusing on gastronomy and additionally do several traineeships in different restaurants. Kitchen auxiliaries (semi-skilled workers) with on-the-job training assist professional food handlers. In all European Union member states, food handlers have to do refresher training courses once a year (Regulation EC, 2004). The owner of the restaurant and not the kitchen manager is responsible for the execution and the extent of the training; it is up to him whether the training is internal or external just like the duration. Persons do not have to pass an exam; they only need to attend to get credit. Jevšnik et al. reported for Slovenia that due to lack of time or poor knowledge such training is not carried out in small and medium-sized enterprises as intended by the law (Jevšnik, Hlebec, & Raspor, 2008). Also our results show that despite of mandatory training at the current workplace 23% of the food handlers had not participated in any training; persons with training at current workplace scored 82% knowledge level versus 71% in persons without on-the-job training. This underlines the entitlement of this legal requirement.

To prevent restaurant-associated foodborne disease, it is important to determine gaps in food safety knowledge of food handlers in order to guide effective educational and behavioral interventions. Shapiro et al. identified that a lack of food handlers' knowledge of proper cooking and storage temperatures could lead to multiplication of bacterial pathogens in food, with resultant outbreaks of illness (Shapiro et al., 1999). The aim of our study was to identify these knowledge gaps and indeed poor knowledge was found concerning the ideal temperatures for cooking, holding and storing foods. In almost all questions concerning time and temperature, the knowledge was not sufficient and many food handlers were not aware of basic temperature control requirements. Only 7% knew the minimum temperature and 12% knew the maximum temperature at which pathogenic microorganisms can multiply. These results are better than the results of the hygiene study in Neuchâtel (Panchal et al., 2013) and worse than the food handlers from Chicago scored (Panchal et al., 2012), where respective 2% or 17% of the food handlers knew the minimum and 11% or 23% knew the maximum temperature for good bacterial growth. When asking food handlers about the minimum internal temperature for cooked hamburger and ground beef mixtures our study showed similar knowledge scores (25%) to the study from Chicago (17%) and considerably better ones to those from Neuchâtel (2%) (Panchal et al., 2013, 2012). Among the fill-in-the-blank questions that specifically addressed cooking temperatures many food handlers demonstrated remarkably low knowledge and an important risk factor for foodborne infections was highlighted. We recommend the creation of targeted educational material with special focus on time and temperature in order to improve the knowledge of the food handling staff and therefore decrease the number of foodborne illnesses.

Our survey has some limitations. It was limited to food handlers in the city of Vienna and must not be representative for food handlers throughout Austria. The catering businesses were chosen in a non-random manner by a convenience approach dependent on volunteering and willingness of participants. This may have introduced selection bias. This survey is also limited by the fact that these data are self-reported; we only observed the process of answering the self-administered questionnaire. So the overall response rate was rather low and we had to deal with many missing values. Another limitation is a consequence of the heterogeneity of the "category size" of the different variables (age, education, work experience, etc.) which, however, reflects the reality of the different settings. So some categories have a limited number of participants, which can weaken the results of the statistical tests used. Participation bias could even lead to a possible overestimation of knowledge, as the better-informed food handlers might have been more likely to participate. The census of the data is a snap shot of the situation, i.e. people working in catering businesses can have obtained their knowledge in this kind of business but also in restaurants and vice versa.

5. Conclusion

Our survey demonstrates a limited level of knowledge among food handlers concerning the optimal temperatures for cooking, holding and storing foods and we recommend targeted educational material to create to keep up with safe food handling practices and new food technologies. Data from this project underline the potential of mandatory on-the-job training of food handlers as required by EU regulation, to significantly improve knowledge scores.

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5 DISCUSSION

The investigation of foodborne outbreaks caused by zoonotic agents provides unique opportunities to improve the prevention and control of communicable diseases [PICHLER et al., 2011]. The aims of an outbreak investigation are to stop the outbreak, to understand what have happened and why and to prevent future outbreaks.

Long-term prevention of foodborne outbreaks requires the cooperation of many partners in the food production chain – the so-called 'stable to table' approach. Some prevention measures include quality assurance programs at egg producing poultry farms, inspection systems at meat processing plants and diaries, use of pasteurization, cooking, and other steps to kill pathogens in food processing plants, food safety and hygiene training for food handlers, proper hand-washing procedures, and the importance of food safety education for consumers. [CENTRES FOR DISEASE CONTROL AND PREVENTION, 2013]

In the past, intensified sampling of foodstuffs alone during an outbreak turned out not to be constructive. In many cases, when an outbreak investigation starts, the food vehicle or the contaminated batch of food was no longer available for microbiological analysis. Therefore an epidemiological study can gain insight information in order to set preventive measures to avoid such situations in the future. The gained information from successfully investigated national and international outbreaks in the last years underlines the need and the benefit of epidemiological investigations. [MUCH et al., 2011b]

Listeriosis is a foodborne illness of major public concern [WARRINER and NAMVAR, 2009] due to the severity of its consequences, the high case-fatality-ratio, and the long incubation period. Although exposure to *L. monocytogenes* is common, listeriosis is rare, and in the absence of controlled human experiments any outbreak provides a unique opportunity to gain information on the number of organisms necessary to cause human illness. [PICHLER et al., 2011]

Changes in food consumption behaviour have led to new technologies and the production of food with long shelf life which are typical '*Listeria* risk foods', as the bacteria

have time to multiply, and the food does not undergo a listericidal process such as cooking before consumption. Exposure to *L. monocytogenes* cannot be avoided completely, but proper food preparation and correct storing can decrease the risk of infection. [ALLERBERGER, 2007; ALLERBERGER and WAGNER, 2010]

The described investigation of the *L. monocytogenes* sv 1/2a outbreak, caused by 'Quargel', an acid curd cheese, provided some valuable lessons: Firstly, it underlines the considerable potential of molecular subtyping as a tool to identify outbreaks. Without routine PFGE typing of human isolates, this outbreak would have been missed. Secondly, it shows impressively that the waning of an outbreak (i.e. disappearance of an outbreak clone) does not necessarily imply that the underlying problem has disappeared. Thirdly, outbreaks provide a unique opportunity to gain information on the number of organisms necessary to cause human illness. The patient who still had a leftover specimen of the causative food in his refrigerator underlines the importance of visiting households of listeriosis patients in order to obtain food samples and to advise other household members on precautionary measures. A single leftover food sample could prove an invaluable clue for elucidating the source of infection and thereby preventing further illness. Fourthly, risk communication with the public is of utmost importance, especially if the outbreak involves hard-to-reach elderly, a risk group that is known to be reluctant to dispose of food even after is has exceeded the 'best before' date. Fifthly, with the idea of collecting and analyzing patients' grocery bills we used a new epidemiological tool to successfully find the source of infection. Sixthly, this led to an important result in the analytical epidemiological study and therefore to an amendment in the Austrian Food Safety and Consumer Protection Law. Since 21 April 2010, health authorities can take measures (e.g. withdrawal from the market, recall products) even in absence of microbiological proof, i.e. with epidemiological proof only. Finally, our outbreak also emphasizes the considerable potential of cross-border cooperation for elucidating chains of infections in multinational outbreaks. Industrial food production combined with international marketing of food and the low attack rate of *L. monocytogenes* hinder epidemiological outbreak investigations with traditional concepts. [PICHLER et al., 2011]

Outlook - on a European level: start of first joint study on listeriosis

In 2010, EFSA initiated an European-wide food survey and ECDC invited the public health reference laboratories from its food and waterborne disease surveillance network to start storing isolates from human *Listeria* strains. A working group is responsible for a joint molecular typing study on food and human isolates. This study will highlight the epidemiology of listeriosis and will provide data concerning the sources of infections, and therefore serve as an invaluable repository of information for the Member States to better target preventive measures in the food safety area. [EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL, 2011]

The routine characterization of human, food and environmental isolates and the use of these centralised databases for subtyping results will support national and European-wide detection and control of foodborne outbreaks in the future.

6 SUMMARY

Foodborne diseases and outbreaks are crucial contributors to morbidity and mortality worldwide. For the prevention of foodborne infections it is of utmost importance to know the pathogens, their reservoirs and possible infection ways. Such knowledge can prompt reasonable and purposive interventions with the aim of eliminating the pathogen, to disrupt the chain of infection in order to stop the outbreak and to prevent this kind of infections in the future. The aim of the present thesis was to demonstrate the public health relevance of foodborne outbreak investigation in Austria and to find answers for the important question: "Why are we investigating foodborne outbreaks?"

In the introduction the current legal basis for outbreak investigation in Austria and the European Union is described and a historical overview of foodborne outbreaks from 2006 on is illustrated. The second chapter places emphasis on the bacteria *Listeria monocytogenes*, which can cause a foodborne outbreak, but outbreaks are very rarely documented. Information concerning occurrence, reservoir, mode of transmission, symptoms, therapy, and prevention is given. Additionally, Austrian data from humans, animals, and food is presented from the year 2012. Chapter 3 deals with the topic epidemiology of communicable diseases and the different steps of foodborne outbreak investigations are illustrated. In addition, unusual ways of outbreak investigations for rare pathogens are shown by two different outbreaks caused by *Listeria monocytogenes*.

By the means of seven original publications dealing with the topic foodborne outbreak investigation and control, different approaches and procedural methods to conduct an outbreak investigation are demonstrated. Furthermore, a survey evaluating the level of hygiene and food safety knowledge of food handlers working in restaurants and catering facilities is attached to enlighten a very important part of public health measures concerning staff training and thus preventing foodborne outbreaks.

The first publication provides an overview of the foodborne outbreak situation in Austria in the year 2007. Information is given about the legal situation in Austria and

the European Union as well as the numbers of cases, hospitalisations and deaths in 2007. Additionally, data concerning causing pathogens, involved food items, modes of transmission, and locations of exposure are presented.

In the second paper we critically discussed outbreak investigation performance and the disregard of decision makers when only epidemiological evidence is given and microbiological proof of the incriminated food is missing.

The third paper is a review article and deals with lessons learned from an outbreak of foodborne listeriosis happening in Austria from 2009 – 2010. The incriminated food was acid curd cheese containing *L. monocytogenes* serotype 1/2a and causing 8 deaths in three European countries.

Paper 4 and 5 are two online articles describing the before named outbreak and highlighting the importance of routine molecular typing of *Listeria* isolates as a tool to identify outbreaks. Also the considerable potential of cross-border cooperation for elucidating chains of infections in multinational outbreaks was demonstrated.

The 6th original publication describes an outbreak of febrile gastroenteritis caused by jellied pork contaminated with *Listeria monocytogenes* serotype 4b in Lower Austria in the year 2008. The epidemiological investigation with a cohort study identified the consumption of mixed cold cuts (including jellied pork) at the wine tavern as the most likely vehicle of the foodborne outbreak (*P* value = 0.0015). This hypothesis was confirmed by microbiological investigation of jellied pork produced by the tavern owner. *L. monocytogenes* was isolated from leftover food in numbers of $3 \times 10^3 – 3 \times 10^4$ colony forming units/g and was indistinguishable from the clinical outbreak isolates. The outbreak was stopped effectively and all patients recovered.

The last publication is a survey about evaluating the knowledge on food safety and hygiene among food handlers from different restaurants and catering businesses in Vienna. The study identified substantial knowledge gaps concerning correct temperatures for cooking, holding and storing foods. These results indicate considerable potential for further improvement in the field of food safety and kitchen staff training in Austria in order to keep up with safe food handling practices.

As shown by our publications included in this thesis, a well conducted outbreak investigation may improve the knowledge of how the contamination occurred at certain steps in the food supply chain, if it will occur again, and how it can be reduced or prevented. Outbreak investigations give the involved authorities and experts the chance to work together and often points for improvement in the public health system are revealed.

The result of thorough outbreak investigations should be better practices in food industry, regulations and enforcement by the regulatory agencies, and consumer understanding, in order to reduce the number of foodborne illnesses in the future.

7 ZUSAMMENFASSUNG

Lebensmittelbedingte Krankheiten und Ausbrüche sind bedeutende Faktoren für die Berechnung der Morbidität und Mortalität weltweit. Um lebensmittelbedingte Infektionen zu vermeiden ist es wichtig die krankmachenden Keime, ihr natürliches Habitat und mögliche Infektionswege zu kennen. Dieses Wissen ist wichtig für vernünftige und zweckmäßige Maßnahmen mit dem Ziel die Pathogene abzutöten, die Infektionskette zu unterbrechen und somit den Ausbruch zu stoppen und weitere Infektionen dieser Art in Zukunft zu vermeiden. Das Ziel dieser Dissertation war die Bedeutung der Abklärung von lebensmittelbedingten Krankheitsausbrüchen in Österreich für die öffentliche Gesundheit aufzuzeigen und Antworten auf die bedeutende Frage zu finden: „Wozu untersuchen wir lebensmittelbedingte Krankheitsausbrüche?“

Der erste Teil dieser Dissertation behandelt die gesetzlichen Grundlagen für die Ausbruchsuntersuchung in Österreich sowie der Europäischen Union und ein historischer Überblick für lebensmittelbedingte Krankheitsausbrüche von 2006 an wird aufgezeigt. Das zweite Kapitel legt den Fokus auf das Bakterium *Listeria monocytogenes,* das lebensmittelbedingte Ausbrüche verursachen kann, allerdings sind diese nur selten dokumentiert. Informationen über Herkunft, Reservoir, Übertragung, Symptome, Therapie und Prävention werden dargestellt. Zusätzlich werden österreichische Daten von Untersuchungen von Menschen, Tieren und Lebensmitteln im Jahr 2012 präsentiert. Das dritte Kapitel behandelt die Epidemiologie von übertragbaren Krankheiten und die verschiedenen Schritte in der Untersuchung von lebensmittelbedingten Krankheitsausbrüchen. Darüber hinaus werden ungewöhnliche Wege für die Ausbruchsabklärung von seltenen Krankheitserregern mit Hilfe zwei verschiedener Listerienausbrüche veranschaulicht.

Anhand der sieben beigefügten Arbeiten, die sich mit den Themen Abklärung und Kontrolle von lebensmittelbedingten Krankheitsausbrüchen befassen, werden verschiedene Ansätze und Methoden zur Ausbruchsabklärung präsentiert. Zusätzlich zeigt eine Studie über das Hygienewissen von Küchenmitarbeitern in Wiener Restaurants

und Gemeinschaftsverpflegungsbetrieben die Wichtigkeit von Mitarbeitertrainings und Schulungen im Bereich der Lebensmittelhygiene, um Krankheitsausbrüche zu vermeiden.

Die erste Publikation gibt einen Überblick über die Situation der lebensmittelbedingten Krankheitsausbrüche in Österreich für das Jahr 2007. Informationen der rechtlichen Grundlage in Österreich und der Europäischen Union und die Anzahl der Erkrankten und Hospitalisierten sowie der Todesfälle in diesem Jahr sind angeführt. Zusätzlich werden Daten über verursachende Erreger, involvierte Lebensmittel, Infektionswege und –orte präsentiert.

In der zweiten Arbeit wurde die Durchführung der Ausbruchsabklärung in Österreich kritisch diskutiert und die geringe Wertschätzung der Entscheidungsträger gegenüber ausschließlich epidemiologischer Beweisführung angesprochen, wenn mikrobiologische Testergebnisse fehlen.

Die dritte Publikation ist eine Übersichtsarbeit und zeigt die gemachten Erfahrungen bei der Abklärung eines lebensmittelbedingten Krankheitsausbruchs verursacht durch *Listeria monocytogenes* Serotyp 1/2a in Österreich in den Jahren 2009 – 2010. Das Infektionsvehikel war kontaminierter Quargel, ein Sauermilchkäse mit Rotschmiere, der acht Todesfälle in drei europäischen Ländern verursachte.

Die Artikel 4 und 5 sind Online-Publikationen und beschreiben den oben genannten Ausbruch und die Wichtigkeit von molekularen Typisierungen von Listerienisolaten in der Routineanwendung um Ausbrüche rechtzeitig zu identifizieren. Zudem wird das große Potential von grenzüberschreitender Zusammenarbeit bei der Abklärung von internationalen Ausbrüchen dargelegt.

Die sechste Arbeit zeigt einen Ausbruch von fieberhafter Gastroenteritis verursacht durch Listerien vom Serotyp 4b im Jahr 2008. Die epidemiologische Abklärung mittels Kohortenstudie hatte die Konsumation von gemischten Platten (inkl. Presswurst) beim niederösterreichischen Heurigen als Ursache der Infektion identifiziert (*P*-Wert = 0,0015). Diese Hypothese wurde durch die mikrobiologische Untersuchung der selbstgemachten Presswurst bestätigt. *L. monocytogenes* wurde mit Werten von 3 x 10^3 – 3 x 10^4 koloniebildenden Einheiten/g in Lebensmittelrestbeständen gefunden und

war ident mit den klinischen Ausbruchsisolaten. Der Ausbruch wurde effizient gestoppt und alle Patienten erholten sich vollständig.

Die letzte Publikation ist eine Studie zur Erhebung des Wissens über Lebensmittelsicherheit von Küchenpersonal in Wiener Restaurants und Betrieben der Gemeinschaftsverpflegung. Die Untersuchung zeigte erhebliche Wissenslücken bei den korrekten Temperaturen zum Kochen, Warmhalten und Lagern von Speisen auf. Die Ergebnisse verweisen auf ein beträchtliches Potential an Verbesserungsmaßnahmen beim Wissen über Lebensmittelhygiene und bei der Schulung von Küchenmitarbeitern in Österreich, um fortwährend eine hohe Lebensmittelsicherheit zu garantieren.

Wie die Publikationen in dieser Dissertation zeigen, können gewissenhafte Untersuchungen auf die Ursachen der Kontamination an bestimmten Stufen der Lebensmittelkette hinweisen und wie diese Verunreinigungen zukünftig reduziert bzw. vermieden werden könnten. Ausbruchsuntersuchungen geben den involvierten Behörden und Experten die Möglichkeit zur Zusammenarbeit und deuten auch auf Bereiche hin, wo das öffentliche Gesundheitssystem noch adaptiert werden könnte.

Das Ergebnis von sorgfältig durchgeführten Ausbruchsuntersuchungen ist eine verbesserte Handlungsweise in der Lebensmittelindustrie, bei der Vollziehung der Vorschriften durch die Behörden und für den Konsumenten – all das soll zur Reduzierung von lebensmittelbedingten Krankheiten beitragen.

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ZUNABOVIC M, DOMIG KJ, KNEIFEL W. 2011. Practical relevance of methodologies for detecting and tracing of *Listeria monocytogenes* in ready-to-eat foods and manufacture environments - A review. LWT-Food Sci Technol. 2011; 44(2): 351-362.

CURRICULUM VITAE

Name	Juliane Pichler
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Employer	Austrian Agency for Health and Food Safety (AGES) Spargelfeldstraße 191, 1220 Vienna email: juliane.pichler@ages.at, homepage: http://www.ages.at/ phone: +43-50555-25722

EDUCATION

since March 2009

Doctorate in Nutritional Sciences. Doctoral thesis: "Public health relevance of foodborne outbreak investigation in Austria with special emphasis on *Listeria monocytogenes*" (Supervisor Univ.-Prof. Mag. Dr. Karl-Heinz Wagner)
Department of Nutritional Sciences, University of Vienna, Austria

October 1999 – March 2006

Study of Nutritional Sciences (emphasis on marketing and psychology of nutrition). Diploma thesis: "Consumer Protection in Austria and the European Union with emphasis on food safety" (Supervisor Univ.-Prof. Mag. Dr. Ibrahim Elmadfa). Graduated as Magistra rerum naturalium (Mag. rer. nat.)
Department of Nutritional Sciences, University of Vienna, Austria

September 1989 – June 1994

Management Assistant for Travel & Tourism
School for Tourism and Hotel Management, Kleßheim, Austria

TRAINING

23 September – 10 October 2007

EPIET - European Programme for Intervention Epidemiology Training - Introductory Course at Lazareto, Menorca/Spain.
Three weeks training course in outbreak investigation methods, epidemiological surveillance planning, establishment and evaluation of surveillance systems, descriptive and analytical epidemiology.

18 – 20 October 2007

ESCAIDE - European Scientific Conference on Applied Infectious Disease Epidemiology - in Stockholm/Sweden

TEACHING

since 2010: teaching during the introductory course for the epidemiological investigation of foodborne outbreaks and the application of the software *Epi Info*™ for infectious disease outbreak investigation.

TUTORING

Tutor for the master thesis „Erhebung des Wissens über Lebensmittelsicherheit von Küchenpersonal in Wiener Restaurants und Betrieben der Gemeinschaftsverpflegung – Teilnahme an einer internationalen Hygienestudie“ (2012/2013).

WORK EXPERIENCE

July 2011 ongoing

Position: **Scientific expert**

Main activities:

- National Reporter for Zoonoses in Austria for the EFSA-reports ‘Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union’ since 2005 – ongoing
- Compilation of the ‘Austrian Zoonoses Report’ (2005 – ongoing)
- Project leader of the EFSA Project ‘Implementation and testing of electronic submission in XML, Excel and CSV formats of zoonoses, antimicrobial resistance and food-borne outbreak data and updating the historical datasets’
- Scientific expert for the EFSA Project ‘Implementation and testing of electronic submission in XML format of zoonoses, zoonotic agents, animal population, antimicrobial resistance and food-borne outbreaks data in the European Union’
- Scientific expert for the 3-years-project ‘Future Food for Men and Women’ funded by the Austrian Research Promotion Agency (FFG)
- Project leader of ‘Hygiene knowledge in food handlers in the City of Vienna’ (international hygiene study carried out in different restaurants and catering businesses in Vienna)
- Data Harmonization in the laboratory information system (LISA) within AGES

AGES – Austrian Agency for Health and Food Safety

Division for Data, Statistics and Risk Assessment – Department for Data Management, Vienna, Austria

April 2006 – June 2011

Position: **Graduate nutritionist**

Main activities:

- Risk assessment regarding food safety along the food chain (from the stable to the table) primarily concerning foodborne zoonoses
- National Reporter for Zoonoses in Austria for the EFSA-reports ‘Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union’ since 2005 – ongoing
- Compilation of the ‘Austrian Zoonoses Report’ (2005 – ongoing)

- National contact point for communicable diseases in Austria for the Country Information Project (CIP) of the European Centre for Disease Prevention and Control (ECDC) since 2008. Project leader for CIP 1, 2, 3. Responsible for updated information about the country involvement and activities in the field of communicable diseases, quality control of translations of documents to be published and support for the dissemination of ECDC outputs and information
- Cooperation in investigation of several foodborne outbreaks in Austria and conduct descriptive and analytical epidemiological studies
- Scientific publications of investigated foodborne outbreaks (e.g. *Salmonella*, *Listeria*)
- Production of consumer-friendly infectious diseases and nutrition information pamphlets
- Scientific management and supervision of EU-wide baseline surveillance surveys for Austria concerning the prevalence of Salmonella, MRSA and Campylobacter in different animals (laying hens, broilers, turkeys, and fattening pigs)
- Scientific management and supervision of annual national monitoring programs on selected zoonoses, zoonotic agents and antimicrobial resistance in cattle, small ruminants, and poultry

AGES – Austrian Agency for Health and Food Safety
Competence Center for Infectious Disease Epidemiology (CC-INFE), Vienna, Austria

June 1999 – August 1999
Hotel bar owner - responsible for purchases, personnel scheduling and accounting
Posthotel St. Moritz, St. Moritz, Switzerland

December 1998 – April 1999
Senior bartender - responsible for the cocktail bar and accounting
Club Sarazena, Pontresina, Switzerland

September 1997 – September 1998
Bartender - Working in the cocktail bar and restaurant, event planner
Restaurant/Bar Palavrion, Zürich, Switzerland

November 1994 – June 1997
Flight attendant – responsible for service and safety on board
Tyrolean Airways, Innsbruck, Austria

LIST OF PUBLICATIONS

Scientific papers/book chapter:

Pichler J, Ziegler J, Aldrian U, Allerberger F. 2014: Evaluating levels of knowledge on food safety among food handlers from restaurants and various catering businesses in Vienna, Austria 2011/2012. Food Control. 2014; 35(1), pp. 33–40.

Much P, Voss S. A, **Pichler J**, Allerberger F. 2011: Lebensmittelbedingte Krankheitsausbrüche, Österreich 2010. Public Health Newsletter of the Ministry of Health http://www.bmg.gv.at/cms/home/attachments/4/7/1/CH1305/CMS1315918293057/lmbka_2010_endgueltig_korr.bmg_korr.much20110912.pdf

Pichler J, Appl G, Pietzka A, Allerberger F. 2011: Lessons to be Learned from an Outbreak of Foodborne Listeriosis, Austria 2009-2010. Food Protect Trends, Vol. 31, No. 5, May 2011, pp. 268-273.

Much P, **Pichler J**, Allerberger F. 2010: Lebensmittelbedingte Krankheitsausbrüche, Österreich 2009. Public Health Newsletter of the Ministry of Health http://www.bmg.gv.at/cms/home/attachments/0/7/3/CH1187/CMS1294145806307/1111lmbka_2_112010.pdf

Fretz R, **Pichler J,** Sagel U et al. 2010: Update: Multinational listeriosis outbreak due to 'Quargel', a sour milk curd cheese, caused by two different L. monocytogenes serotype 1/2a strains, 2009-2010. Euro Surveill. 2010;15(16):pii=19543

Fretz R, Sagel U, Ruppitsch W, Pietzka AT, Stöger A, Huhulescu S, Heuberger S, **Pichler J**, Much P, Pfaff G, Stark K, Prager R, Flieger A, Feenstra O, Allerberger F. 2010: Listeriosis outbreak caused by acid curd cheese 'Quargel', Austria and Germany 2009. Euro Surveill. 2010;15(5):pii=19477

Allerberger F, **Pichler J**, Öhlinger R, Gelpi E, Budka H.: Nahrungsmittelbedingte Infektionskrankheiten und Intoxikationen. In: Klinische Ernährungsmedizin (Ledochowski M., Ed.) Springer Verlag, Wien, 2010, pp. 347-408.

Much P, **Pichler J**, Fretz R, Allerberger F. 2009: Lebensmittelbedingte Krankheitsausbrüche, Österreich 2008. Public Health Newsletter of the Ministry of Health http://www.bmg.gv.at/cms/home/attachments/0/3/3/CH1186/CMS1253518446773/lmbedingte_ausbrueche_2008.pdf

Pichler J, Much P, Kasper S, Fretz R, Auer B et al. 2009: An outbreak of febrile gastroenteritis associated with jellied pork contaminated with Listeria monocytogenes. Wien. Klin. Wochenschr. (2009)121: 149-156.

Much P, **Pichler J**, Kasper S, Allerberger F. 2009: Foodborne outbreaks, Austria 2007. Wien. Klin. Wochenschr. (2009)121: 77-85.

Much P, **Pichler J**, Kasper S, Lassnig H, Kornschober C, Buchner A, König C, Allerberger F. 2009: A foodborne outbreak of Salmonella Enteritidis phage type 6 in Austria, 2008. Wien. Klin. Wochenschr. (2009)121: 132-136.

Much P, **Pichler J**, Allerberger F. 2007: Lebensmittelbedingte Krankheitsausbrüche, Österreich 2006. Mitteilungen der Sanitätsverwaltung Oktober 2007: 25-31.

Much P, **Pichler J**, Luckner-Hornischer A, Pusker P, Trampler R, Lassnig H, Kornschober C and Allerberger F. 2007: When Epidemiological Evidence Should Suffice. Food Protect Trends, Vol. 27, No. 12: 967-970.

Much P, **Pichler J**, Allerberger F. 2007: Lebensmittelbedingte Krankheitsausbrüche, Österreich 2005. Wien. Klin. Wochenschr. 119/5: 150-157.

Schmid D, Gschiel E, Mann M, Huhulescu S, Ruppitsch W, Bohm G, **Pichler J**, Lederer I, Hoger G, Heuberger S, Allerberger F. 2006. Outbreak of acute gastroenteritis in an Austrian boarding school. Euro Surveill. 2007 Mar 1;12(3):224.

Brochures:

Vorgehen bei Gastroenterititis-Ausbrüchen durch Norovirus, June 2011

Reptiles and Salmonellosis in small children, March 2011

Pregnancy, Nutrition, Risk of infection, August 2008, reprint February 2009, available also in Turkish and Serbo-Croatian, October 2009

Bericht über Zoonosen und ihre Erreger in Österreich im Jahr 2010 (German version)
Bericht über Zoonosen und ihre Erreger in Österreich im Jahr 2009 (German version)
Report on Zoonoses and Zoonotic Agents in Austria in 2008 (German + English version)
Report on Zoonoses and Zoonotic Agents in Austria in 2007 (German + English version)
Report on Zoonoses and Zoonotic Agents in Austria in 2006 (German + English version)
Bericht über Zoonosen und ihre Erreger in Österreich im Jahr 2005 (German version)

Oral presentations:

Pichler J. Food safety & foodborne outbreaks in Austria and current state of the project 'Hygiene knowledge in kitchen staff in the City of Vienna'. Symposium 'An international research project to understand food handler knowledge' in Bolzano/Italy, June 2011.

Pichler J, Fretz R, Sagel U, Much P, Allerberger F. Food-borne listeriosis, Austria 2009-2010: grocery bills as a tool in outbreak investigation. ESCAIDE Conference in Lisbon/Portugal, November 2010. Presentation done by Prof. Allerberger.

Pichler J. Food-borne norovirus outbreak at a military base in Vienna, Austria 2008. 32nd Annual Meeting of the Austrian Society for Hygiene, Microbiology and Preventive Medicine (ÖGHMP) in Vienna/Austria, May 2010.

Poster presentations:

Pichler J, Fretz R, Much P et al. Two consecutive outbreaks of gastroenteritis due to infections with *Salmonella* Typhimurium DT193 in the Austrian Armed Forces, 2009. ECCMID Congress 2010 in Vienna/Austria, April 2010.

P. Much, **J. Pichler**, C. Lehner, H. Wildt, C. Kornschober, F. Allerberger. An Austrian-wide *Salmonella* Enteritidis PT 1 outbreak in 2007. KIT Congress 2008 in Innbruck/ Austria, February 2008.

Pichler J, Much P, Allerberger F. Foodborne outbreaks in Austria in 2006. ESCAIDE Conference in Stockholm/Sweden, October 2007.

Baseline studies:
Baseline survey on the prevalence of *Salmonella* spp. in herds of breeding pigs in the EU Member States from 1.1.2008 – 31.12.2008

Baseline survey on the prevalence and antimicrobial resistance of *Campylobacter* spp. in broiler flocks and on the prevalence of *Campylobacter* spp. and *Salmonella* spp. in broiler carcasses in the EU Member States from 1.1.2008 – 31.12.2008

Baseline survey on the prevalence of *Salmonella* in flocks of turkeys in the EU from 1.10.2006 – 30.09.2007

Baseline survey on the prevalence of *Salmonella* in slaughter pigs in the EU from 1.10.2006 – 30.09.2007

Vienna, December 2013 Mag. Juliane Pichler