

SURVEILLANCE OF LISTERIA INFECTIONS IN EUROPE

H de Valk¹, C Jacquet², V Goulet¹, V Vaillant¹, A Perra¹, F Simon¹, JC Desenclos¹, P Martin²
on behalf of the *Listeria* surveillance feasibility study participants

In addition to the economic consequences and threats associated with outbreaks, listeriosis remains of great public health concern, as it has one of the highest case fatality rates of all the foodborne infections (20%-30%), and has common source epidemic potential. Changes in the way food is produced, distributed and stored have created the potential for diffuse and widespread outbreaks involving many countries.

In 2002, a survey was carried out to assess the need for and the feasibility of a European network on listeria infections in humans. Data on surveillance systems and laboratory methods were collected through two postal surveys sent to the national Centres for communicable disease surveillance and to the listeria reference laboratories. Surveillance systems for listeria infections were in operation in 16 out of the 17 countries surveyed, and 16 countries had a national reference laboratory (NRL). All countries based their case definition of listeriosis on the isolation of *Listeria monocytogenes*. Fourteen NRLs performed at least one typing method on human strains. At least 13 countries already carried out or expressed willingness to carry out characterisation of isolates by pulsed field gel electrophoresis (PFGE) of *L. monocytogenes* strains isolated from human cases following a standard protocol. The participants concluded that there was a clear added value to having a European surveillance network for listeria infections, particularly for outbreak detection and investigation, and that a surveillance network based on the existing national surveillance systems was feasible.

Euro Surveill 2005;10(10): 251-5

Published online October 2005

Key words: listeriosis, foodborne outbreaks, surveillance

Introduction

Listeria monocytogenes causes invasive illness, mainly in certain well-defined high-risk groups, including immunocompromised people, pregnant women and neonates. Listeriosis can, however, occur in otherwise healthy individuals, particularly in an outbreak setting. *L. monocytogenes* primarily causes abortion, septicaemia or infections of the central nervous system, with a case fatality ratio of 20%-30% [1]. It has only recently been recognised that foodborne transmission of *L. monocytogenes* can also cause a self-limiting acute gastroenteritis in immunocompetent persons [2]. The public health importance of listeriosis is not always recognised, particularly since listeriosis is a relatively rare disease compared with other common foodborne illnesses such as salmonellosis. Most countries within the European Union have an annual incidence between 2-10 reported cases per million population per year. However, because of its high case fatality rate, listeriosis ranks among the most frequent causes of death due to foodborne illness: it ranks second, after salmonellosis, in the United States (US) and France; and fourth in England and Wales [3-5].

Epidemiological investigations during the past 20 years have shown that listeriosis is a foodborne disease [6]. Discovery of *L. monocytogenes*, mainly in raw and ready-to-eat meat, poultry, seafood, and dairy products, has prompted numerous product recalls which have led to large financial losses for the food industry and numerous health scares. Effective prevention and control measures

exist, as documented in France and the US, where a threefold and a twofold reduction respectively in incidence over the past decade was attributed to increased regulatory activity, implementation of Hazard Analysis and Critical Control Points (HACCP) programmes throughout the food industry, and specific recommendations to high-risk groups [7,8]. However, several countries still have relatively high incidence, and many countries do not have a surveillance system that allows them to estimate incidence or evaluate incidence trends. Moreover, its common source epidemic potential presents a real threat and persists even in countries with a decreasing or low incidence. Changes in the way food is produced and distributed have further increased the potential for diffuse and widespread outbreaks involving many countries. Because these outbreaks can be dispersed with a limited number of cases in each country, they are likely to go undetected if information from these countries is not pooled. Improved surveillance, coordinated at a European level, combining rapid subtyping methods, cluster identification, and collaborative epidemiological investigation, can identify and halt these potentially large, outbreaks.

Because of the potential benefits of collaborative European surveillance described above, this project was initiated with the aim of defining the feasibility and scope of a European network on listeria infections, and to develop common methodologies for surveillance of listeriosis in Europe.

Methods

The project was coordinated by the Institut de Veille Sanitaire (InVS) and the French National Reference Centre for Listeria at the Institut Pasteur, assisted by an expert panel of microbiologists and epidemiologists from nine countries. Data for the inventory were collected through two postal surveys and, when necessary, completed through telephone interviews. One questionnaire, sent to epidemiologists in charge of surveillance of communicable diseases at the national level, collected information on surveillance systems, other data sources, information flow, case definitions, data collected, frequency of reporting and analysis, outbreak detection mechanisms, reported cases and outbreaks. A second questionnaire, sent to the national reference centre (NRL), collected information about their tasks as reference laboratory, the origin of isolates, identification and typing methods and practices, antibiotic resistance surveillance, and quality assurance and control. A third questionnaire was sent out to assess the acceptability, capacity and possibility that the NRL could routinely perform typing of *L. monocytogenes*, or at regular intervals, and with a specific common protocol. During a meeting with epidemiologists and microbiologists from each participating country, the results of the inventory were presented, different scenarios for European surveillance were discussed, and recommendations for a European listeriosis surveillance network were formulated.

Results

In total, 17 countries participated. This included 14 EU countries: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, the Netherlands, Portugal, Spain, Sweden, and the United Kingdom (England & Wales and Scotland only); and Norway, Iceland and Switzerland. We present the results of Scotland separately from England & Wales, but count England & Wales and Scotland as a single country within the United Kingdom (UK).

1 Institut de Veille Sanitaire, Saint-Maurice, France

2 Centre National de Référence des *Listeria*, Institut Pasteur, Paris

Surveillance systems

All countries except Portugal had at least one surveillance system for listeriosis, and 12 countries had more than one system. In several countries, notification of foodborne illness (e.g., Austria and Ireland) or foodborne illness outbreaks (e.g., Belgium, the Netherlands and France) was statutory, and in theory, listeria infections could be notified through these systems. In practice, however, listeriosis cases were not notified through these systems. In this inventory, therefore, we do not consider notification of foodborne illness and outbreaks to be the same thing as a surveillance system for listeriosis. Listeriosis was statutorily notifiable in 10 countries, four countries had universal voluntary reporting, 11 countries had listeria surveillance based on their NRL, two countries had sentinel surveillance, and five countries had syndrome based surveillance of infections of the central nervous system and blood stream infections that covered listeria infections among other infections.

In 15 countries, diagnostic laboratories were involved in reporting to at least one of the surveillance systems. In addition, physicians were involved in the reporting in 13 countries. In Italy, physicians were the only notifying partners.

Listeriosis surveillance data were available at the national level in 16 countries, either at the national surveillance centre (five countries), at the NRL (one country) or at both (10 countries). These data at the national level were available as single case reports in all countries. Data transmission to the national level was immediate or weekly in all countries with the exception of Italy, where it was done quarterly.

All countries based their case definition of listeriosis on the isolation of *L. monocytogenes*, with or without specific requirements regarding site of isolation and the presence of clinical symptoms. Two countries also considered the presence of serum antibodies as laboratory confirmation of a case, but in practice, only cases with an isolate were reported.

None of the countries had a specific definition for acute listeria gastroenteritis. Theoretically, in countries with a case definition based on the isolation of *L. monocytogenes* from any site, these patients should be reported. In practice, none of the countries had acute listeria gastroenteritis cases reported, although outbreaks of acute listeria gastroenteritis had occasionally been identified and reported to the national level: in Italy in 1993 and 1997, in Denmark in 1996, and in Belgium in 2001.

In general, countries with listeriosis surveillance collected at least basic demographic data (age/date of birth and sex), contact details for the reporting institute, laboratory confirmation (date of isolation of *L. monocytogenes* or date first positive specimen received in diagnostic laboratory), and the type of investigated material. Additional information such as principal diagnosis, associated pregnancy, outcome, and travel and food history, were available in between five to 10 countries.

National Reference Laboratories

All countries except for Ireland had an NRL. The tasks of these 16 NRLs were: microbiological surveillance (16 countries); detection of outbreaks (14 countries); provision of microbiological expertise (13 countries); research on listeria (12 countries); training (nine countries); and provision of reference material such as strains, sera, DNA profiles, protein extracts, phages, or guidelines for laboratory diagnosis (eight countries). Strains isolated from patients were sent to the NRL: in seven countries this was done systematically, and in eight countries this was done according to the will of the laboratory, or in specific situations such as outbreak or suspected outbreak settings. In Sweden and Switzerland, the sending of isolates to the NRL was statutory. In Spain, about half of the 16 autonomous communities sent their isolates to the NRL.

The NRLs also received information along with strains. This information concerned the site of isolation of the bacteria (13 countries), clinical data (11 countries), epidemiological data (10 countries), and strain characteristics (eight countries). In most countries (11 out of 17), the NRLs for human listeria also received listeria strains isolated from food, and in three countries, the NRLs received information on food strains.

Identification

Fifteen NRLs carried out identification of listeria strains. Only four countries performed a Gram stain and a catalase test. Biochemical characterisation was performed using API-*Listeria* in eight countries, API-*coryne* in one, while four countries used home made sugars. Nine countries looked for haemolysis, six for motility. Two countries also used polymerase chain reaction (PCR) for diagnosis, and one country also used an automated system of bacterial identification.

Characterisation of strains

Fourteen NRLs performed at least one typing method on human strains, either on an ongoing basis or at regular intervals. 13 NRLs routinely performed serotyping, either on an ongoing basis or at regular intervals. Seven countries used home made antisera, six used commercially available sera, and two used both. Thirteen countries had developed the capacity to perform DNA macrorestriction and pulsed field gel electrophoresis (PFGE) on human strains of *L. monocytogenes*, and performed it either routinely, for specific investigations or for ad hoc studies. All used the CHEF (contour-clamped homogeneous electric field) system for PFGE, and most used two enzymes, *AscI* and *ApaI*. Twelve countries said they would be willing to set up routine PFGE with image analysis, at least weekly or immediately after receiving a strain, in order to participate in a common surveillance system of human strains. Several countries, including one country not willing to carry out PFGE routinely, said they would be willing to send strains to another European laboratory to be typed by PFGE. Thirteen countries were willing to use a common standardised protocol for PFGE and to send profiles or strains to contribute to a European database. European surveillance including results of harmonised characterisation of isolates by PFGE of *L. monocytogenes* strains isolated from human cases could therefore cover at least 13 countries.

All countries who were performing or intended to perform PFGE said they would be willing to send PFGE profiles to a common European laboratory under the following conditions: access to common information (six countries), confidentiality (four), access restricted to participants only (one), and provided that strains were not distributed and profiles used only for the purpose of surveillance (one).

Antimicrobial susceptibility testing

Ten out of 17 laboratories (including Ireland) reported performing antimicrobial susceptibility testing. Three countries used the E test method for testing, and seven countries used agar dilution breakpoints. Two countries also used the Clinical and Laboratory Standards Institute (formerly NCCLS) method and one country also used a disk diffusion method. The antimicrobial agents tested varied between countries. Laboratories most frequently tested the susceptibility of listeria for gentamicin and trimethoprim-sulfamethoxazole (seven countries); ampicillin, tetracycline and erythromycin (six countries); ciprofloxacin (five countries); or chloramphenicol, streptomycin and vancomycin (four countries).

Quality control and quality assurance, accreditation

The NRLs in 14 countries reported having internal quality control for their identification procedures (nine countries) and/or typing procedures (nine countries).

Seven countries participated in an external quality control. Six of the seven countries used NEQAS from the Public Health Laboratory Service (PHLS) in the UK for identification procedures, and three also used another external quality control.

Seven NRLs were engaged in a quality assurance system, and five intended to be so in the near future. Six NRLs said that they were ISO/UE 17025 accredited and two more were accredited on an other standard: PHLS in the UK (Clinical Pathology Accreditation Ltd) and the NRL in the Netherlands (accredited by CCKL-test). One NRL is ISO 9001 certified.

Outbreak detection

Real-time reporting and analysis, high sensitivity, results of typing of strains available in real time for surveillance, and the existence of outbreak detection criteria or thresholds are all surveillance system characteristics that contribute to efficient outbreak detection. Eight countries have developed outbreak detection mechanisms and thresholds. Real time reporting and analysis characterised the surveillance systems of 15 and 11 countries respectively. The estimated or assumed sensitivity was reasonably high or high in at least 10 countries. For outbreak detection, 12 countries had results of strain typing available, routinely and on a real time or weekly basis: serotyping (12 countries), biotyping (four countries), ribotyping (three countries), PFGE analysis (six countries), and phagotyping (one country).

Reported listeria infections and outbreaks

The incidence of reported cases varied between 0.3 and 7.5 cases per million per year. The mean incidence of reported cases was 3.4 per million inhabitants (data from 16 countries, latest year available) [TABLE 1]. Five countries reported an incidence of more than four cases per million, and three of these five countries reported an

incidence of more than six per million population. These figures mostly reflect the sensitivity of the surveillance systems, as well as the incidence of the disease. However, few countries have formal evaluations or studies allowing estimation of sensitivity, geographical coverage and representativeness of their surveillance systems. In general, the surveillance systems described above covered, in principal, the entire country, except for Spain, where approximately half of the autonomous communities transmitted their data direct to the national level.

Between 1991 and 2002, a total of 19 outbreaks of invasive listeriosis were reported in nine different countries, with a total of 526 outbreak related cases) [TABLE 2]. While the number of reported outbreaks increased gradually over time, from seven outbreaks detected in the period 1992-1996 to 11 in the period 1997-2001, the mean number of cases related to these outbreaks decreased from 57 to 11 over the same period. This suggests more efficient outbreak detection, investigation and control. In addition, four outbreaks of acute listeria gastroenteritis were reported: two outbreaks in Italy in 1993 (18 cases) and 1997 (1566 cases); an outbreak in Denmark in 1996 (3 cases); and an outbreak in Belgium in 2001 (2 cases of acute gastroenteritis and one case of invasive listeriosis).

TABLE 1

Observed number of cases and incidence of listeriosis, by country, by surveillance system (latest year available), ListerNet

Country	Year	System	Observed cases	Observed incidence* (1 000 000)
Austria	2000	Reference laboratory	14	1.7
Belgium (Flandres)	1999	Statutory notification	26	4.4
Belgium	2000	Sentinel + reference laboratory	48	4.7
Denmark	2000	Syndromic surveillance (meningitis)	6	1.1
	2001	Statutory notification	38	7.2
	2001	Reference laboratory	38	7.2
England and Wales	2001	Universal voluntary reporting and reference laboratory	144	2.7
	2000	Reference laboratory	81	1.5
Finland	2001	Statutory notification	29	5.5
France	2001	Statutory notification+ reference laboratory	187	3.2
	2000	Syndromic surveillance (CNS+blood stream infections)	148	2.5
Germany	2001	Statutory notification	220	2.7
Greece	2001	Universal voluntary reporting	3	0.3
	2001	Syndromic surveillance (meningitis)	2	0.2
Iceland	2001	Statutory notification + NRL	0	0.0
Ireland	2001	Universal voluntary reporting	6	1.6
Italy	1999	Reference laboratory	11	0.2
	1999	Statutory notification	40	0.7
	2001	Syndromic surveillance (meningitis)	31	0.5
Netherlands	2001	Sentinel surveillance	17	1.1
	2000	Syndromic surveillance (meningitis)	26	1.7
Norway	2001	Statutory notification	17	3.8
	2000	Reference laboratory	11	2.5
Portugal		No surveillance		
Scotland	2001	Universal voluntary reporting	15	2.9
Spain	2000	Universal voluntary reporting	35	0.9
	2000	Reference laboratory	60	1.5
Sweden	2001	Statutory notification	67	7.5
	2001	Reference laboratory	12	1.4
Switzerland	2000	Statutory notification	54	7.4
	2000	Reference laboratory	46	6.3

* The observed incidence reflects both the real incidence and the sensitivity of the surveillance system. Therefore, data cannot be compared between countries without taking into account the differences in sensitivity of these surveillance systems

TABLE 2

Reported outbreaks of listeriosis and of *Listeria* gastroenteritis in Europe 1990-2002

Year	Country	Number of cases*	Transmission	Incriminated food	Potential international implication
1992	France	279	foodborne	Pork tongue in jelly (11)	Exported product
1992	Spain	24	foodborne	Unknown	
1992	Norway	6	foodborne	Sliced cold meat	
1993	France	38	foodborne	Rillettes (pork meat) (12)	Exported product
1993	Italy	18 gastroenteritis	foodborne	Rice salad (2)	
1994-95	Sweden	9	foodborne	Gravad trout (13)	
1995	France	36	foodborne	Cheese (raw cows' milk) (14)	
1995	Iceland	5	unidentified	Unidentified	
1996	Denmark	3 gastroenteritis	unidentified	Unidentified (15)	
1997	France	14	foodborne	Cheese (raw cows' milk)	Exported product
1997	Finland	5	foodborne	Cold-smoked rainbow trout (16)	
1997	Italy	1566 gastroenteritis	foodborne	Corn salad (17)	
1998-99	Finland	25	foodborne	Butter (18)	
1999	England and Wales	2	foodborne	Cheese/cheese salad/ sandwiches (19)	
1999	France	3	foodborne	Cheese (raw cow's milk)	Possible cases in Germany?
1999	France	10	foodborne	Rillettes (processed pork meat) (20)	Exported product
1999-00	Finland	10	foodborne	Vacuum-packed fish products (21)	Exported?
2000	France	32	foodborne	Pork tongue in jelly (20)	Exported ?
2000	Portugal	1	foodborne	Cheese	
2000	Spain	15	foodborne	Undetermined	
2001	Belgium	1 + 2 gastroenteritis	foodborne	Ice cream cake	Invasive illness of Belgian case diagnosed in France
2002	France	11	foodborne	Spreadable raw sausage (22)	Export to Germany, Belgium and Luxembourg

* Cases refer to invasive listeriosis unless otherwise specified

The incriminated food at the origin of the invasive listeriosis outbreaks was processed meat products (six outbreaks), cheese (five outbreaks), processed fish products (three outbreaks), butter (one outbreak) and undetermined (three outbreaks). The incriminated products for at least six of these outbreaks were known to have been exported, creating the potential for the occurrence of outbreak related cases in other countries. Moreover, cases related to one outbreak in one country were diagnosed in a neighbouring country.

The outbreaks of gastroenteritis were linked to the consumption of contaminated rice salad and corn salad respectively, while the Belgian outbreak of gastroenteritis and invasive listeriosis was linked to a contaminated ice cream cake. The origin of one outbreak of gastroenteritis remained undetermined.

Conclusions and recommendations

Based on the inventory, it appears that there is an appropriate basic infrastructure for a European surveillance network for listeria infections, and that the necessary harmonisation of methods is feasible considering the infrastructure already in place and the expressed willingness of countries to adapt or set up methodologies for European surveillance.

It was recommended by the representatives of the participating countries/the working group to set up a European network for the surveillance of listeria infections, with, as the main objectives, providing comparative data, monitoring trends of international importance, and rapidly detecting and investigating international outbreaks more efficiently. The network should also contribute to the strengthening of national surveillance in participating countries. In its initial phase the network should concentrate on surveillance of human

cases of listeria infection and not yet actively seek to collect data on food isolates. Once the network is well established and surveillance of human cases is operational, the possibilities of including data from food and animal surveillance should be studied.

Common case definitions should be agreed upon as well as a common minimum dataset, which could be further developed over time to include additional data (optimal dataset). Case definitions, in line with those developed by the Community Network (under decision N° 2002/253/EC, amended by Commission Decision 2003/534EC), and a minimum and optimal dataset, for which the collection is, at present, feasible for the majority of participating countries, were proposed [9].

Because of the wide disparity in listeria outbreaks, a common European database should include results of real time characterisation of strains to reinforce the ability to detect international outbreaks. The participants concluded that, at present, characterisation by both serotype and PFGE would be the most appropriate methods and the best option to meet the objectives of outbreak detection and trend analysis. The necessary harmonisation of microbiological methods and of the type of epidemiological data collected appears feasible considering the infrastructure already in place and the expressed willingness of countries to adapt or set up methodologies in the perspective of European surveillance.

The network should encourage individual countries to strengthen national surveillance of listeria infections, and should contribute to their strengthening by providing a model and specific tools for surveillance and investigations. Each country should set up a national database which combines laboratory data and data from the notification systems. Participating countries should be encouraged

to increase the sensitivity of the surveillance systems in order to reinforce the ability to detect national and international outbreaks. Countries can participate in a stepwise manner, contributing initially with the data they already have available, even if incomplete. With time, countries may wish to adapt their in-country data collection in order to cover all data fields in the database. For those countries where routine and ongoing typing of strains is difficult to carry out because of the low number of isolates, the possibility of having their strains typed in another country's NRL, should be investigated.

In addition to the harmonisation of epidemiological and microbiological methods and the creation of a common database, it was recommended that the network should develop outbreak detection algorithms and a protocol for collaborative investigation of international clusters and outbreaks. The network will need to develop principles of collaboration that should deal with access to the database by participants and by outsiders, confidentiality of country specific data, confidential and public domain reports, data protection requirements, as well as transmission to other programmes and projects. It was recommended to adapt the principles of collaboration of Enter-net to listeria [10].

Finally, the participants recommended that a project proposal be developed by the coordinators of the actual feasibility study. In May 2003, an application was submitted to the European Commission under the 2003 call for proposals in the programme of community action in the field of public health (2003-2008). Although the proposal was accepted, co-funding was not proposed by the commission until August 2004. By this time, the situation of the different partners of the project had evolved, and senior staff who committed themselves to contribute to the project had taken up other commitments. However, European investment in such a project remains a priority for the years to come. In particular, it would be important to assess how such a project could be integrated into other ongoing EU surveillance projects such as Enter-net.

Acknowledgements

The listeria surveillance feasibility study was co-financed by the European Commission, DG Sanco Agreement number SI2.326491 (2001CVG4-023).

Participants in the study are: **Austria:** Franz Allerberger, Bundesst. Bakt.-serol. Untersuchungsanstalt; Reinhild Strauss, FM for Social Security and Generations – **Belgium:** Francine Matthys, Epidemiology Section, Scientific Institute of Public Health - Louis Pasteur; Mark Yde, Bacteriology section, Scientific Institute of Public Health - Louis Pasteur – **Denmark:** Peter Gerner-Smidt, Statens Serum Institut; Brita Bruun, Department of Clinical Microbiology –; **England and Wales:** Mark Reacher, Jim McLauchlin and JW Smerdon, Public Health Laboratory Service Communicable Disease Surveillance Centre and Central Public Health Laboratory – **Finland:** Outi Lyytikäinen and Anja Siitonen, National Public Health Institute – **France:** Véronique Goulet, Institut de Veille Sanitaire; Paul Martin and Christine Jacquet, Institut Pasteur – **Germany:** Andrea Ammon and Helmut Tschaepé, Robert Koch-Institut; Herbert Hof, Institute for Medical Microbiology and Hygiene; Jochen Bockemühl, Institute for Hygiene – **Greece:** I Tselentis and Takis Panagiotopoulos, Hellenic Center for Infectious Diseases Control – **Iceland:** Gudrun Sigmundsdóttir, Directorate of Health – **Ireland:** Martin Cormican, University College Hospital, Galway; Paul McKeown, National Disease Surveillance Centre; Bartley Cryan, Cork University Hospital – **Italy:** Stefania Salmaso and Paolo Aureli, Istituto Superiore di Sanità – **The Netherlands:** Yvonne van Duynhoven and Wim Wannet, National Institute of Public Health – **Norway:** Line Vold and Jørgen Lassen, National Institute of Public Health – **Portugal:** Laura Brum and Jorge Machado, Instituto Nacional de Saude Dr Ricardo Jorge – **Scotland:** John M Cowden and Alison Smith-Palmer, Scottish Centre

for Infection and Environmental Health – **Spain:** Julio A Vasquez and Luisa P Sánchez Serrano, Instituto de Salud Carlos III – **Sweden:** Margareta Lofdahl, Birgitta Henriques Normark, Christina Johansson and Johan Giesecke, Swedish Institute for Infectious Disease Control – **Switzerland:** Hans Schmid, Swiss Federal Office of Public Health; Jacques Bille, Clinical Microbiology Laboratory University Hospital, Lausanne – and **Enter-net Surveillance hub:** Ian Fisher.

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