

Workpackage 4: Final Research Report

WP number	4
Title	Development of a linked molecular surveillance database system for food-borne infections (PulseNet Europe)
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Summary

PulseNet Europe is a network of public health, veterinary and food laboratories for molecular surveillance of food-borne infections in Europe. The fingerprinting method used for this surveillance is pulsed-field gel electrophoresis (PFGE). The aim of PulseNet Europe is to establish a real-time linked surveillance database system for food, public health, and veterinary laboratories, to detect disease clusters and investigate outbreaks of *Salmonella*, verocytotoxin-producing *Escherichia coli* (VTEC) and *Listeria monocytogenes*. Participants currently represent 60 institutes from 30 countries.

A PulseNet Europe homepage – <http://www.pulsenet-europe.org/> – has been established and, in order to improve active exchange of information and communication between partners, a rapid communication system, called PNE forum – www.pulsenet-europe.org/pneforum/ – has been developed and implemented.

A group of six curators from different institutes was trained in 2005 and additional funding from Med-Vet-Net also enabled a training course for 15 PulseNet Europe participants in 2006.

A European Quality Assurance System (EQAS) has been created and certification started in order to ensure the comparability of the data that are uploaded from different laboratories.

The PulseNet Europe central database contains specific databases for *Salmonella*, VTEC and *Listeria monocytogenes*. Customized BioNumerics software has been developed with full compatibility with other PulseNet networks and a manual of the customized PulseNet BioNumerics software has been written for partners.

The molecular surveillance database system is ready, and ECDC has included PNE in their action plan for 2007, and the PNE steering committee and Med-Vet-Net have supported transfer of the databases to Stockholm.

Objectives

The overall aim was to establish a real-time linked surveillance database system to detect disease clusters and investigate outbreaks of *Salmonella*, VTEC and *Listeria*, including:

- establishment of real-time linked surveillance database system to detect disease clusters and investigate outbreaks of *Salmonella*, VTEC and *Listeria*
- establishment of a database curator system and training of the curators
- establishment of a rapid communication system
- establishment of a certification and proficiency testing programme and certification of participants.

Key Achievements

- A real-time linked surveillance database system to detect disease clusters of *Salmonella*, VTEC and *Listeria monocytogenes* for food, public health, and veterinary laboratories.
- A rapid communication system (PNE forum) which is also a link between food, public health, and veterinary laboratories in Europe.
- A customized web-based PulseNet Europe central database.
- A certification and proficiency testing programme and certification of participants.
- A memorandum of understanding between partners.

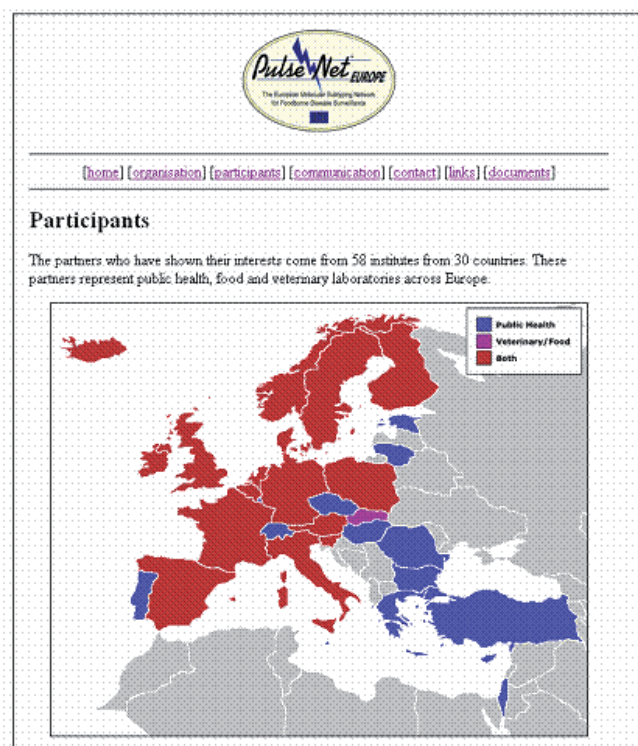


Figure 4.1 PulseNet website homepage.

Multinational outbreaks of food-borne infections have become frequent events with the increasing trade of raw, semi-processed and ready-to-eat foods between countries. Early detection and response to food-borne infections are therefore important both for public health and for trade of food products.

In Europe, for many years the problem has been that molecular typing results from different countries have not been comparable, even when the same method has been used. Further, it is impossible to exchange bacterial strains over long distances to perform the real-time typing necessary for outbreak detection and control. However, it is possible to compare locally obtained DNA fingerprints electronically in a central database, which enables a comparison of profiles of strains isolated in different countries in real time.

A customized web-based PulseNet Europe central database containing specific databases for

Salmonella, verocytotoxin-producing *Escherichia coli* and *Listeria monocytogenes* has been developed with full compatibility with other PulseNet networks.

PulseNet Europe is a multi-disciplinary network of food, public health, and veterinary laboratories dedicated to molecular surveillance of food-borne infections (Figure 4.1). PulseNet Europe combines high discriminatory molecular typing with a truly 'farm-to-fork' approach to the existing surveillance at an international level. Strains isolated from patients and food are locally typed by pulsed-field gel electrophoresis (PFGE) using a standardized protocol, and images of the PFGE-profiles are electronically submitted via the web to a central database to be compared with profiles submitted by other laboratories. Sophisticated image analysis software allows real-time comparison of profiles. The data are comparable internationally through PulseNet International (PulseNet Asia-Pacific region, PulseNet Canada, PulseNet Latin America

and PulseNet USA).

Partners have direct access to the PulseNet Europe central database containing specific databases for *Salmonella*, verocytotoxin-producing *Escherichia coli* (VTEC) and *Listeria monocytogenes*. A uniform nomenclature for PFGE-subtypes in all participating countries is ensured. Partners can check and compare their own data with the stored data when alerts of infection clusters are posted on the communication forum. The direct access to comparable typing data for isolates from human infections as well as food and animals will significantly improve the surveillance and trace-back of food-borne infections at the national, European and international level. Further, international clusters of food-borne infections that have too few cases to be recognized by national surveillance systems can be detected through the central PulseNet Europe surveillance system.

PulseNet Europe partners, submitting typing data for one or more of the pathogens under surveillance, represent public health, food, and veterinary laboratories across Europe. Partners to date represent 60 institutes from 30 countries including 12 institutes participating in Med-Vet-Net (Figure 4.2).

The quality of the PFGE gel images and correct band assignment are critical when data are uploaded to the central database for comparison. Six database curators from AFSSA, DTU HPA, IP, SSI and VLA have been trained and undertake quality control of the central database, perform uniform naming and confirm the PFGE profiles submitted by partners, and perform central cluster detection of all pathogens at regular intervals. The curators also organize training courses and are involved in troubleshooting for participant laboratories whenever requested.

Although Med-Vet-Net has a central website, the steering committee identified the need for a PulseNet Europe website for use by participants and to disseminate information to the scientific community. The PulseNet Europe homepage is: <http://www.pulsenet-europe.org> (Figure 4.1).

A communication system, available at www.pulsenet-europe.org/pneforum, has been set up (Figure 4.3). This enables active exchange of information and communication between partners from food, public health and veterinary laboratories. Through this forum, partners will be alerted by email when an international cluster is detected (Figure 4.3), and the images from clusters of PFGE profiles can be distributed by email. It is also a forum where discussions and questions can be asked concerning the PulseNet protocols and the database. Currently the PNE forum has 104 registered users.

A European Quality Assurance System (EQAS) system has been created to ensure the comparability of the data uploaded from different laboratories. Further, after certification, in order to

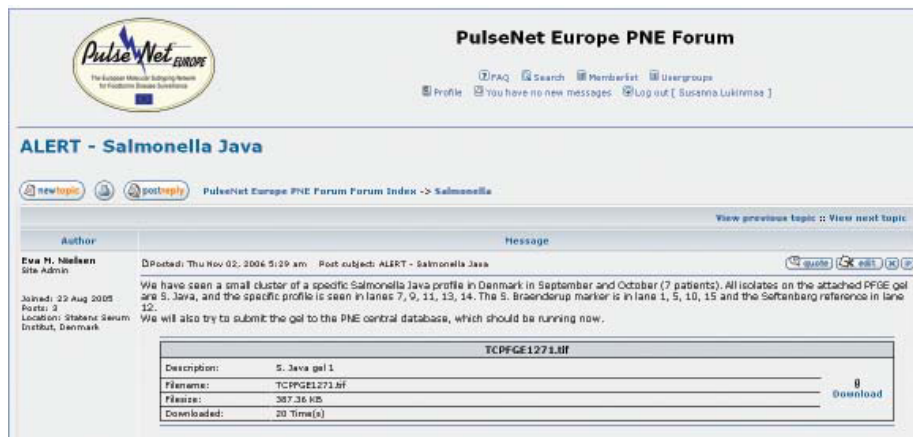


Figure 4.3 Customized PulseNet Europe BioNumerics software.

maintain a high level of image quality, participants are requested by curators to run proficiency testing strains.

The EQAS testing started at the end of February 2006 and the certification set was requested by 48 partners (42 requested *Salmonella*, 29 *Listeria* and 33 VTEC strains), however, testing has continued since with single requests and, currently, 44 *Salmonella*, 30 *Listeria* and 35 VTEC EQAS strains have been requested.

Certification to submit all *Salmonella*, *Listeria* and VTEC information (TIFF-file and analysed data) automatically direct to the PulseNet Europe central database using the BioNumerics with PulseNet Europe scripts (level A) has been received by 15, 11 and 12 participants, respectively. Certification to submit *Salmonella*, *Listeria* and VTEC TIFF-files and strain information to the central database by email only via curators (level B) has been received by 13, 3 and 13 participants, respectively.

Each participating laboratory received an evaluation of their performance, both of their

PFGE preparation level and the gel analysis as well as the certification papers if passed. Participants who failed were provided with suggestions for improving their TIFF-file and were offered an opportunity to visit and follow the procedure of preparing PFGE gels and analysing gel images at the curators' laboratory (DTU and SSI) at their own cost and asked to repeat the certification set and re-submit results to the curators.

Participants who failed the first testing and were provided with suggestions for improving their TIFF-file have now received level A certification by 1 participant for *Salmonella* and the level B certification by 1 for *Salmonella*.

With additional funding from Med-Vet-Net, 15 PulseNet Europe partners selected from food, public health, and veterinary laboratories were trained to perform PFGE and image analysis according to the defined PulseNet Europe rules, in Colindale, UK, in February 2006. The 15 participants came from Austria, Czech Republic, Denmark, Finland, Germany, Hungary, Ireland, Italy, Luxembourg, Netherlands, Poland, Romania, Spain

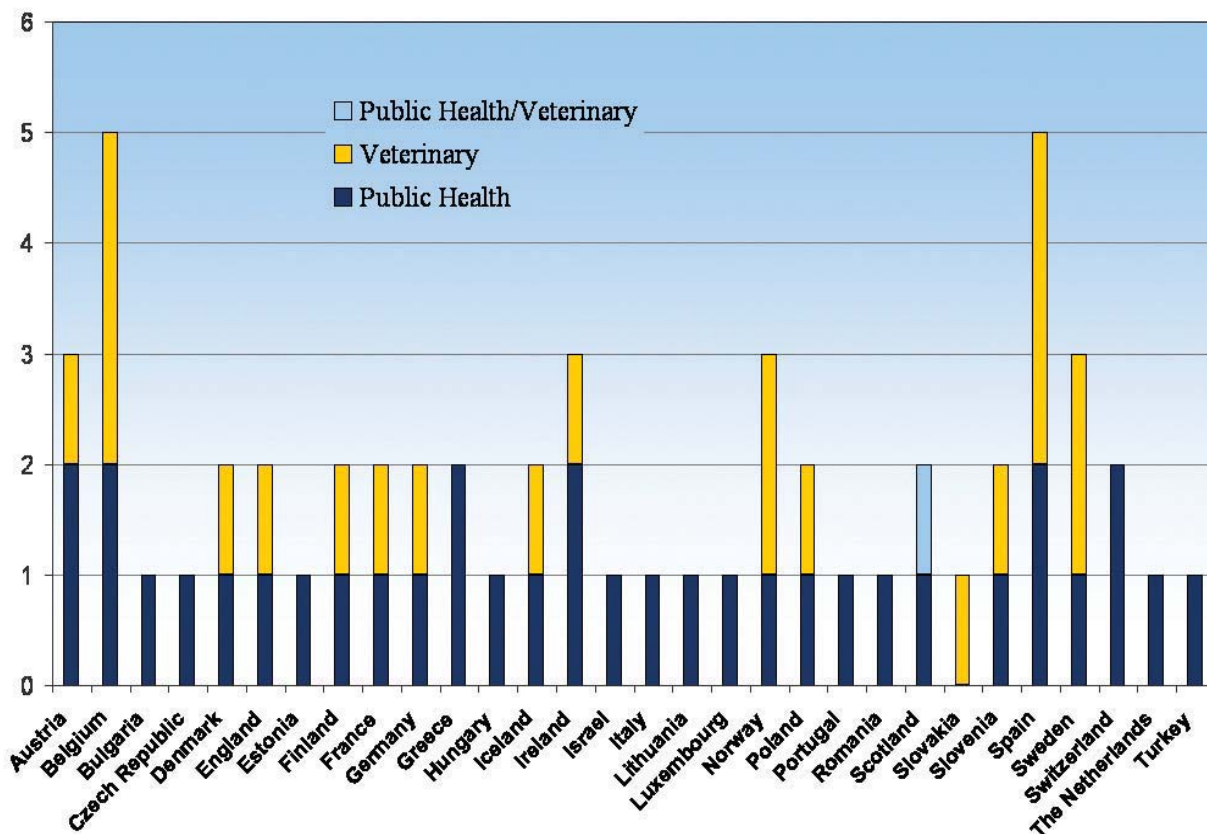


Figure 4.2 Countries and number of institutes involved in PulseNet Europe.

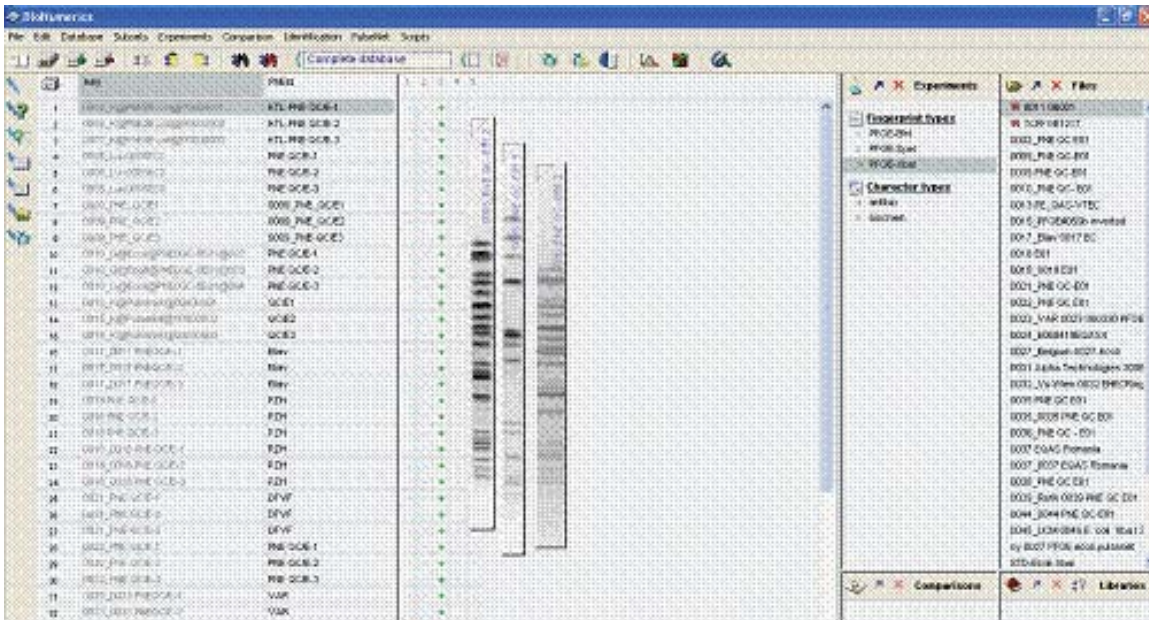


Figure 4.4 Customized PulseNet Europe BioNumerics software.

and Sweden. The course included both theory and hands-on laboratory practice on PulseNet standardized protocols and the PNE forum.

The central database, which is password-protected, currently contains specific databases for *Salmonella*, VTEC and *L. monocytogenes* with over 75 PulseNet Bionumerics scripts to assist partners in the project. The software is customized to automate certain functions; the scripts with the reference system created from the international S. Braenderup standard H9812 (Hunter SB et al. J Clin Microbiol 2005; 43:1045-50) were delivered to partners through the PNE forum. In addition, a manual of the customized PulseNet BioNumerics software has been made available.

Partners have direct access to the database and they can compare their PFGE data to the data that are stored in the database. Comparison can be made in two different ways: partners can download selected data from the central database to their own computer for temporary comparison (Figure 4.5A) or one selected profile can be compared in the central database and the most probable matches (Figure 4.5B) will be given.

A memorandum of understanding (MoU) between PulseNet Europe partners has been written and signed by partners to define the rules for collaboration allowing exchange of information and molecular typing data between PulseNet Europe partners. An MoU with PulseNet USA has also been drafted and is ready for signatures. This MoU is the foundation to the single MoU between all PulseNet Networks.

Passwords for the central database were distributed from the end of October 2006. Up until the project ended in November 2006, three alerts were posted on the PNE communication forum. The first alert was from the US concerning a spinach-associated *E. coli* O157 outbreak in the USA and was sent via the PulseNet Europe coordinator. Two other alerts concerned a *Salmonella* Java outbreak and a *S. Enteritidis* RDNC/untypeable outbreak. These two outbreaks were noticed at the national level and alerts were sent by partners.

The European Centre for Disease Prevention and Control (ECDC) has a mandate to detect, assess and respond to health threats including multinational food-borne outbreaks. PulseNet Europe supports rapid detection of outbreaks associated with food and animal sources and close links to ECDC were therefore built up, and ECDC has included PulseNet Europe in their 2007 action plan. The PulseNet Europe steering committee and Med-Vet-Net both support transfer of all ongoing PNE activities and databases to ECDC in Stockholm. ECDC will prepare a strategy on integration of the molecular databases into future surveillance. This transfer is also supported by the European Food Safety Authority (EFSA), which is important because PulseNet Europe has many partners from food and veterinary laboratories.

In conclusion, PulseNet Europe is a unique multi-disciplinary construction in the EU and will add highly discriminatory molecular typing and a truly 'farm-to-fork' approach to the existing surveillance. The direct access to comparable

typing data for isolates from human infections as well as food and animals will significantly improve the surveillance and trace-back of food-borne infections at national, European and international levels. Further, PulseNet Europe gives a valuable link between public health, food and veterinary laboratories.

The knowledge that has been gained on how to build customized BioNumerics databases with server access via the Internet could be valuable for other groups (collaborators from different institutes and countries) that are trying to create a platform for sharing information on species, strains and different molecular and typing techniques. Further the database structure and idea of the PulseNet Europe databases is very easy to modify for other pathogens, not only for food-borne ones. Furthermore, the spectrum of typing methods can be expanded easily.

According to the EQAS certification results, the PFGE protocol for *Listeria* caused problems for partners, therefore in the future, a separate training course on the PFGE protocol for *Listeria* would improve the capability of partners to perform *Listeria* PFGE.

During the period of transfer from Med-Vet-Net to ECDC the PulseNet Europe partnership will still be able to use the PNE forum for discussions and questions. Also in outbreak situations, alerts, with attached TIFF files, can still be posted to detect clusters.

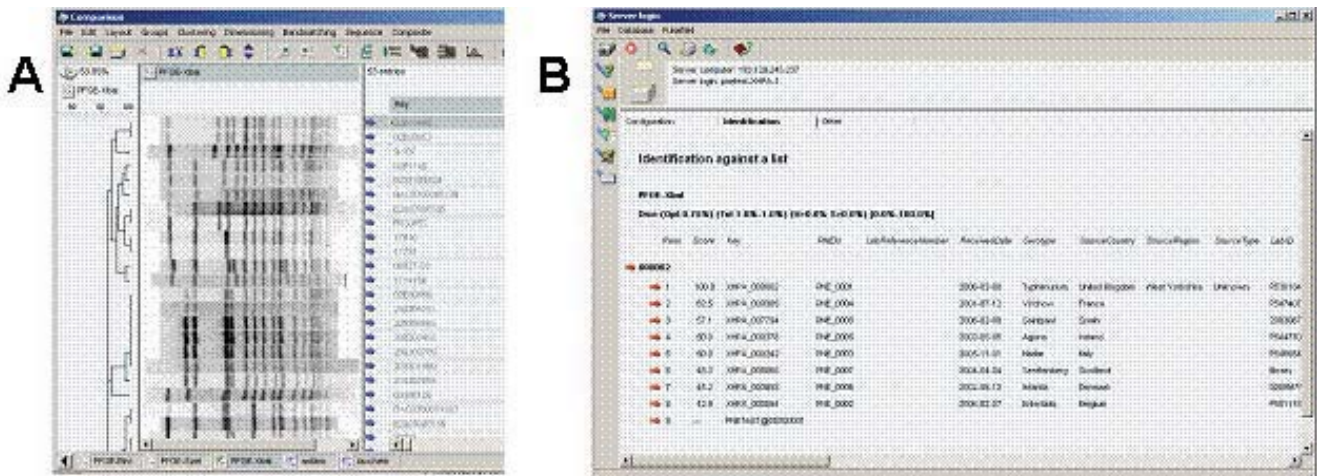


Figure 4.5 Examples of: (A) comparison of selected data uploaded from central database; and (B) comparison of one profile to the central database.