Workpackage 4:
Annual Research Report

WP number	4
Title	Development of a linked molecular surveillance database system for food-borne infections (PulseNet Europe)
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Figure 4.1. Customized PulseNet Europe BioNumerics software.

Progress 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*, verocytoxin-producing *Escherichia coli* (VTEC) and *Listeria monocytogenes*. Participants 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.

A trial run of the PulseNet Europe central database has been performed. The database contains specific databases for *Salmonella*, VTEC and *Listeria monocytogenes* and is now ready for use. 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 knowledge and experience obtained during this project will be of value to other groups (collaborators from different institutes and countries) that are planning to share information on species, strains and different molecular and typing techniques.

Objectives

The overall aim is to establish a real-time linked surveillance database system to detect disease clusters and investigate outbreaks of *Salmonella*, VTEC and *Listeria*. This will involve:

- 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.

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 was used. Further, it is impractical 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.

PulseNet Europe is a multi-disciplinary network of food, public health, and veterinary laboratories dedicated to molecular surveillance of food-borne infections. PulseNet Europe combines high discriminatory molecular typing with a truly 'farm-to-fork' approach to the existing surveillance at an international level. It envisions that 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 (Figure 4.1) 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*, verocytoxin-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 to 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). A memorandum of understanding (MoU), which defines the rules for collaboration, allowing exchange of information and molecular typing data between PulseNet Europe partners, is ready for signature. An MoU with PulseNet USA has been drafted and the PulseNet Europe Co-ordinator participates in the PulseNet International Steering Committee Meeting thus ensuring continued involvement at the international level.

The PulseNet Europe homepage (www.pulsenet-europe.org/) has been established (Figure 4.3). It contains general information on PulseNet Europe, participants, organization structure, the PNE forum, links to other networks and documents such as protocols, a gel analysis manual (BioNumerics PNE manual) and a troubleshooting help function. A pilot database has been set up and a trial run has been performed to indicate initial problems in the central database; identified problems have been resolved. Customized BioNumerics software (Figure 4.1) with full compatibility with other PulseNet networks has been developed and a manual of the customized PulseNet BioNumerics software has been written.

The PulseNet Europe BioNumerics client–server software (a product by Applied Maths) is used and analysis and comparison of PFGE profiles are done electronically over internet. The software is customized through the use of scripts, which automate certain functions and also include the reference system created from the international S. Braenderup standard. The participants may also submit raw image files to the curators, who will analyse the images for them.

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, DFVF, 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.

A communication system, available at www.pulsenet-europe.org/pneforum, has been set up. 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, 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 95 registered users.

A European Quality Assurance System (EQAS) has been created to ensure the comparability of the data uploaded from different laboratories. Further, after certification, in order to maintain a high level of image quality, participants are requested by curators to run Proficiency Testing strains.

Because PulseNet Europe participants must follow the standardized protocols and analysis parameters, PulseNet Europe applied for, and was awarded, additional funding from Med-Vet-Net to organize a training course for 15 participants.

The EQAS results have separate on-line BioNumerics EQAS databases in the central database. The PulseNet Europe scripts for BioNumerics and instructions on how to use the scripts were distributed along with protocols, other instructions, guidelines, checklists and username and password for accessing the EQAS database. The EQAS consists of two parts. Firstly the participant performs PFGE and produces images of the gels with lanes containing EQAS strains (distributed to the partner). The second step involves BioNumerics analysis of the images. The curators compare the results by both an 'eye match' and a BioNumerics analysis. The results and grades of the evaluation are recorded along with comments.

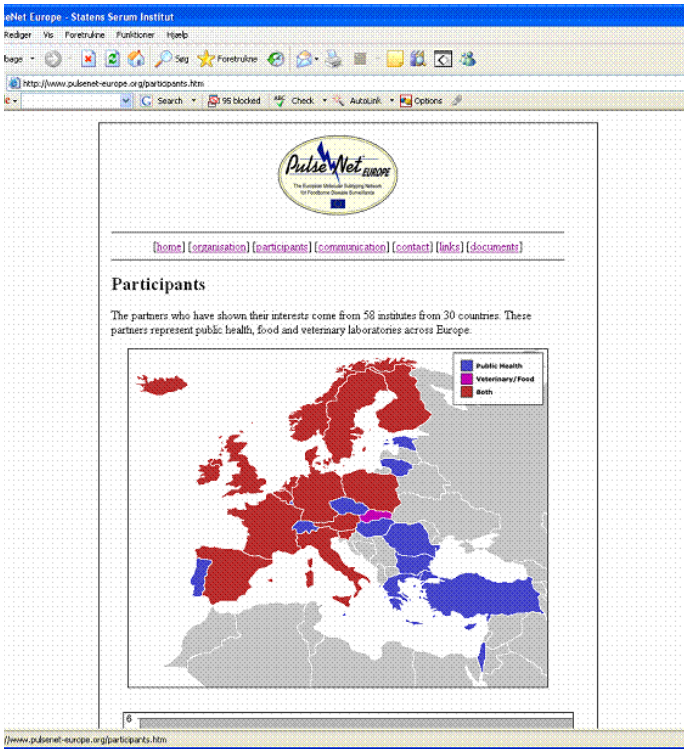

Figure 4.3 PulseNet website homepage.

The EQAS testing started in February 2006. Currently, certification to submit data to the central database using the customized scripts is awarded to 12 laboratories for *Salmonella*, 11 for VTEC and 12 for *Listeria*. The certification to submit data only via curators is awarded to 23, 4 and 23 participants, for *Salmomella,* VTEC and *Listeria*, respectively. The certification failed for 17 participants. Each of these laboratories received an evaluation of performance. The participants who failed have been provided with suggestions for improving their performance and offered training opportunities at their own cost.

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 participants came from Austria, Czech Republic, Denmark, Finland, Germany, Hungary, Ireland, Italy, Luxembourg, Netherlands, Poland, Romania, Spain 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 (Figure 4.1). The software is customized to automate certain functions; the scripts with the reference system were delivered to partners through the PNE forum. In addition, a manual of the customized PulseNet BioNumerics software has been made available.

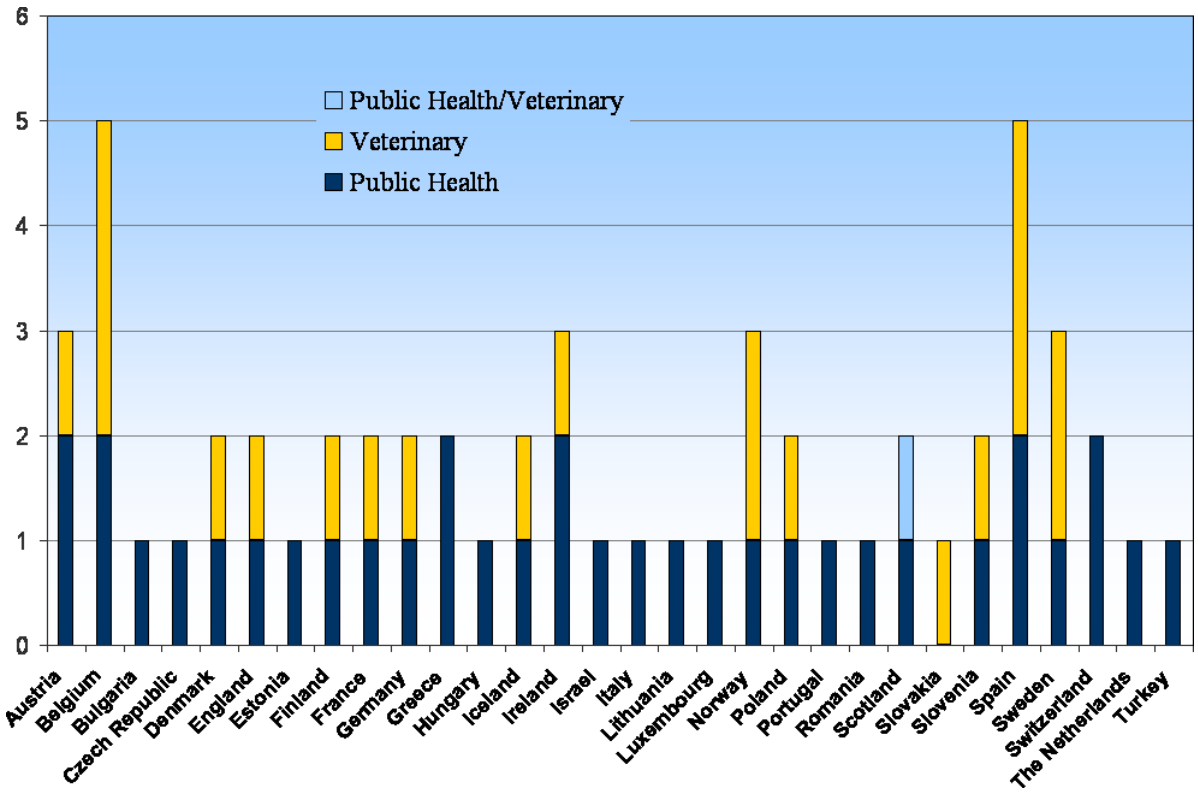

Figure 4.2. Countries and number of institutes involved in PulseNet Europe.