STECTRACK

Validation of methods for the detection of new emerging pathogenic *Escherichia coli*

DURATION OF THE PROJECT
01/01/2007 – 31/01/2009

BUDGET
345.293 €

KEYWORDS
STEC detection, validation, multiplex PCR, IMS

CONTEXT

Food safety is an important issue all around the world. Food borne Shiga-like toxin producing *Escherichia coli* or STEC infection is a serious problem in human healthcare. These extremely severe bacterial pathogens can cause a range of disease symptoms, such as diarrhea and hemolytic uremic syndrome (HUS). A routine detection method exists only for the sorbitol-negative serotype O157. However, other *E. coli* serotypes as O26, O103, O111, O145 and sorbitol-positive O157 are responsible for 80% of the human STEC infections and 23% of the HUS cases. These serotypes, also called non-O157 STEC, are regarded as emerging pathogens.
The development of a detection and isolation method for non-O157 STEC in this project will allow public authorities to compose a new preventive policy in order to improve food safety. It also emphasizes the concept of sustainable development, where the precautionary principle is crucial.

PROJECT DESCRIPTION

Objectives

This project aims at validating a detection and isolation methodology for non-O157 STEC and for sorbitol-positive O157 in food products, animal related farm samples and clinical human samples. A detection and isolation method for these serotypes has been developed during the previous SPSD II project (project numbers CP-02-581 and CP-43-582) and will be finally optimized and validated in this project.

Methodology

Enrichment procedures and isolation media that were developed during the SPSD II project will be optimized by the University of Ghent. The preparation of the selective isolation media will be simplified and the detection limit for STEC in feces will be improved. For the latter immunomagnetic separation (IMS) will be applied after selective enrichment and prior to plating on the selective media.
Multiplex PCR and Pulsenet Europe PFGE protocol will be implemented. A 25-amplicon multiplex PCR, designed by the UA-VIB (VIB8) will be used for strain characterization and serotype identification of a collection of human clinical strains available at the Belgian STEC Reference Center at the Brussels Academic Hospital (UZ Brussel). Furthermore a PFGE (pulsed field gel electrophoresis) protocol will be applied by ILVO–T&V. The patterns will be analysed, clustered and uploaded in the on-line PNE-database. This database facilitates early identification of common source outbreaks worldwide. Furthermore the UA-VIB will design multiplex PCRs on the level of serotypes and virulence genes. These multiplex PCRs will be optimized for routine screening of samples, as milk, cheese, meat and cattle feces by ILVO-T&V.
The optimized detection and isolation methods will be evaluated on human clinical samples by the UZ Brussel for feasibility for routine use in clinical laboratories.
Finally the whole isolation protocol of STEC will be validated in an in-house validation study, performed by the University of Ghent and ILVO-T&V. Different sample types will be inoculated at different levels with strains of the serotype: O26, O103, O111, O145 and O157 (sorbitol-positive). In a second stage the STEC isolation method will be validated during an interlaboratory study. This study will be coordinated by the University of Liege. The protocol of validation and the evaluation of the results will be based on the recommendations of ISO 16140.

INTERACTION BETWEEN DIFFERENT PARTNERS

The interactions between the different partners are outlined in detail in the methodology chapter.

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Link international programmes

There are no specific collaborations with other scientific programs. On the other hand in the interlaboratory study, laboratories from outside Belgium, involved in research of STEC, will participate. Including laboratories from outside Belgium will help to disseminate the existence of the new method on a large scale. The frequent contact with the international laboratories, also dealing with STEC detection limitations, allow us to discuss the methodology applied. The wide application can stimulate to use this isolation method as a basis for the development of a standard method for the isolation of specific STEC serotypes by international organisation as CEN and ISO.

EXPECTED RESULTS AND/OR PRODUCTS

First of all we expect that the enrichment procedures and isolation media will be optimized in such a way that for all different matrices a maximum detection limit of 1 CFU per gram sample will be reached. Secondly a collection of human clinical strains will be characterized and obtained PFGE patterns will be uploaded in an on-line PNE-database. Thirdly a routine detection method will be developed for screening of samples and a routine detection and isolation method for use on human feces samples in clinical laboratories. Finally an in-house validation study and an interlaboratory study will validate the STEC isolation method.

PARTNERS - ACTIVITIES

- Ghent University is experienced in conventional microbiological methods and in field research concerning meat contamination and slaughterhouse sampling.
- ILVO-T&V is experienced in molecular microbiology and bacterial taxonomic methods, including molecular typing tools. The institute has also a broad experience in the quality of milk and dairy products.
- UA-VIB8 is experienced in the development of molecular tools and has developed an algorithm for designing complex multiplex PCR assays.
- UZ Brussel (formerly AZ-VUB) is experienced in human clinical diagnosis and is functioning as STEC reference lab in Belgium. The partner has access to human clinical samples and has a collection of human clinical isolates from years.
- University of Liege is experienced in the validation of microbiological methods and has a broad experience in the organization of microbiological interlaboratory studies on national and international level.

CONTACT INFORMATION

Coordinator

Lieven De Zutter
Ghent University
Faculty of Veterinary Medicine
Department of Veterinary Public Health & Food Safety
Salisburylaan 133, B-9820 Merelbeke
Tel:+32 (0)9 264 74 51
Fax:+32 (0)9 264 74 91
lieven.dezutter@ugent.be
http://www.vvv.ugent.be/

Promoters

Lieve Herman, Marc Heyndrickx & Koen De Reu
Institute for Agriculture and Fisheries Research (ILVO)
Technology and Food Unit
Brusselsesteenweg 370, B-9090 Melle
Tel:+32 (0) 9 272 30 00
Fax:+32 (0)9 272 30 01
lieve.herman@ilvo.vlaanderen.be
http://www.ilvo.vlaanderen.be/TenV/

Jurgen Del-Favero
Applied Molecular Genomics Group
VIB Department of Molecular Genetics (UA-VIB)
University of Antwerp
Universiteitsplein 1 - Building V
B-2610 Antwerpen
Tel:+32 (0)3 265 10 32
Fax:+32 (0)3 265 10 12
jurgen.delfavero@ua.ac.be
http://www.molgen.ua.ac.be/ &
http://www.vibgeneticservicefacility.be/

Denis Piérard
Universitair Ziekenhuis Brussel (UZ Brussel, formerly Academisch Ziekenhuis)
Vrije Universiteit Brussel AZ-VUB)
Laboratory for Microbiology
Laarbeeklaan 101, B-1090 Brussels
Tel:+32 (0)2 477 50 02
Fax:+32 (0)2 477 50 15
denis.pierard@uzbrussel.be
http://www.uzbrussel.be/

Georges Daube
University of Liège
Faculty of Veterinary science
Sart-Tilman, B43b
B-4000 Liège
Tel:+32 (0)4 366 40 15
Fax:+32 (0)4 366 40 16
georges.daube@ulg.ac.be
http://www.mdaoa.be

Follow-up Committee

For the complete and most up-to-date composition of the Follow-up Committee, please consult our Federal Research Actions Database (FEDRA) by visiting http://www.belspo.be/fedra or http://www.belspo.be/ssd

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SSD SCIENCE FOR A SUSTAINABLE DEVELOPMENT

Belgian Science Policy • Wetenschapsstraat 8 Rue de la Science • B-1000 Brussels
Tel. +32 (0)2 238 34 11 • Fax +32 (0)2 230 59 12 • www.belspo.be/ssd
Contact: Christine Mathieu