

Technical report

CampEc-NET

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Draft

Authors: Merete Hofshagen with assistance from
Trudy Wassenaar
Anne Margrete Urdahl
Arie Havelaar
Jukka Ranta
Rowena Kosmider
Danica Grahek-Ogden

Participants

Project owner:

National Veterinary Institute, Norway

Steering group:

Merete Hofshagen (project manager), Trudy Wassenaar, Anne Margrete Urdahl, Arie Havelaar, Jukka Ranta, Rowena Kosmider,

Participating institutions and their contact persons:

University of the Basque Country

Dr. Aurora Fernandez Astorga

Federal Institute for Risk Assessment

Dr. Lothar Beutin

Molecular Microbiology and Genomics

Consultants

Dr. Trudy M. Wassenaar

University of Göttingen

Prof. Dr. Uwe Groß

University of Magdeburg

Dr. Steffen Backert

Technical University of Denmark, Food Institute

Head of Section Bjarke Bak Christensen

Finnish Food Safety Authority Evira

PhD Jukka Ranta

National Public Health Institute of Finland

PhD Markku Kuusi

MATIS

Director of department Viggó Thór

Marteinsson

Institute of Experimental Pathology, Keldur

Head of Department Eggert Gunnarsson

Icelandic Poultry Industry - Reykjagardur

Veterinarian Jarle Reiersen

Academic Medical Centre

MD, PhD Wim Ang

The Central Veterinary Institute of Wageningen

Dr. Dörte Döpfer

National Institute for Public Health and the Environment

Dr. Arie Havelaar

Utrecht University

Professor Jaap Wagenaar

Centre for Poultry Science, Animalia

Manager Kristian Hoel

National Veterinary Institute

Dr. Merete Hofshagen

Norwegian Institute of Public Health

Professor, PhD, MSc Georg Kapperud

The Norwegian School of Veterinary Science

Professor Yngvild Wasteson

Faculty of Veterinary Medicine, Portugal

DMV, MSc, PhD Maria João Fraqueza

Instituto nacional de Saúde Dr. Ricardo Jorge

Head of Unit Jorge Machado

National Veterinary Institute (SVA), Sweden

Dr. Anna Aspan

Liverpool University

Dr. Helen Clough

Veterinary Laboratories Agency

Dr. Rowena Kosmider

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Fact sheet

Title: Campylobacter and E. coli – a network project (CampEc-NET)

Project number: 60456

Authors:

Merete Hofshagen, National Veterinary Institute, PO Box 750 Sentrum, N-0106 Oslo, Norway

Trudy Wassenaar, Molecular Microbiology and Genomics Consultants, Tannenstrasse 7, 55576 Zotzenheim, Germany

Anne Margrete Urdahl, National Veterinary Institute, PO Box 750 Sentrum, N-0106 Oslo, Norway

Arie Havelaar, National Institute for Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands

Jukka Ranta, Finnish Food Safety Authority Evira, Mustialankatu 3, FI-00790 Helsinki, Finland

Rowena Kosmider, Veterinary Laboratories Agency, New Haw, Addlestone, KT15 3NB, Surrey, UK

Danica Grahek-Ogden, National Veterinary Institute, PO Box 750 Sentrum, N-0106 Oslo, Norway

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Abstract:

The zoonotic bacteria *Campylobacter* and Verocytotoxin-producing *Escherichia coli* (VTEC) have a major impact on public health. This project aimed at integrating veterinary, food and medical laboratory-based surveillance through a range of activities including comparison and harmonization of the methods used in laboratories across Europe, workshops addressing various topics and performing state of the art analyses and mapping of current research activities and centres of activity.

The project involved 25 institutions in 11 countries/regions. This contributed to building of the European Research Area (ERA) in the area of foodborne zoonoses.

Results

Many institutions have participated in the process of harmonizing data collection and sharing regarding *Campylobacter* and VTEC. Knowledge has been transferred between laboratories by visits to learn methods and exchange experiences. A state of the art review of the impact of acquired immunity on campylobacteriosis in humans has been provided and the possibility of mathematical modelling of this complex and dynamic system has been explored as has strategies to obtain further observational and experimental data. Case reports and also new modern computational techniques to assess and model food related risks in the area of zoonoses have been provided. Future research needs have been identified.

The results obtained in the project will be communicated to major stakeholders such as national food and health authorities, the meat and poultry industry and other target groups such as the European Food Safety Authority (EFSA), European Centre for Diseases Prevention and Control (ECDC), WHO, the European Commission and national research organizations within the SAFEFOODERA.

Executive summary

Main objectives:

The zoonotic bacteria *Campylobacter* and Verocytotoxin-producing *Escherichia coli* (VTEC) have a major impact on public health. Veterinary, medical and food laboratories perform laboratory based surveillance activities related to *Campylobacter* and VTEC, but the diagnostic methods used often differ. This project aimed at integrating veterinary, food and medical laboratory-based surveillance through a range of activities including comparison and harmonization of the methods used in laboratories across Europe.

The epidemiology of *Campylobacter* and VTEC is complex. Obvious knowledge gaps are our understanding of diversity at the species level and the role of the host determining disease outcome and the infective dose. This project focused on these questions, combining the collection of data with networking and coordination activities to optimally make use of European expertise in the relative fields. Added value was provided by sharing of expertise and insights of experts working on the two key organisms.

Moreover, an important objective of this project was to increase the knowledge of molecular epidemiology, host immunity and source attribution for *Campylobacter* and VTEC, enabling the development of recommendations on short and long term research needs with the ultimate goal of reducing these food-borne infections.

Method/implementation:

This project aimed at integrating veterinary, food and medical laboratory-based surveillance through a range of activities including comparison and harmonization of the methods used in laboratories across Europe, workshops addressing various topics and performing state of the art analyses and mapping of current research activities and centres of activity. The project involved 25 institutions in 11 countries/regions and this provided excellent opportunities to contribute to building of the European Research Area (ERA) in the area of foodborne zoonoses.

The project was divided into 5 working areas:

Work package 1: Molecular epidemiology of *Campylobacter*: Comparing European regions.

Work package 2: Molecular epidemiology of VTEC: Identification of risks that ubiquitous VTEC reservoirs represent for generating new human pathogenic VTEC.

Work package 3: Role of specific immunity in risk reduction of human infections during exposure to *Campylobacter*.

Work package 4: Development of methods for source attribution for *Campylobacter*.

Work package 5: Development of methods for source attribution for VTEC.

Work package 1 and 2 focussed on increasing the understanding of molecular epidemiology of VTEC and *Campylobacter*. Data comparison of *Campylobacter* and VTEC can be difficult between countries and even between laboratories within a country/region. In addition, the populations display variation in serotypes, pathogenicity factors and mobile genetic elements, reflecting that distribution of virulence properties is not stable in bacterial populations. Transfer of genetic material highly complicates the molecular epidemiology of both VTEC and *Campylobacter*. Comparison of isolates from various sources is important in order to understand which factors dictate carriage, expression and transmission of mobile genetic elements. For *Campylobacter*, specific questions to be addressed were regional differences in seasonal patterns and overall illness rates between Nordic, mid-European and Mediterranean countries.

For VTEC, one aimed at detecting horizontal gene transfer during a multi-laboratory screening effort by mutually implementing laboratory tests and exchanging experiences.

Work package 3 focussed on the role of host immunity in the outcome of disease for *Campylobacter*. Humans in both developed and developing countries are repeatedly exposed to *Campylobacter* from different sources, but their exposure levels differ. This exposure leads to relatively high disease incidence rates in young children. At older age, acquired immunity may protect from serious illness, but colonization without clinical symptoms or relatively mild symptoms may continue to occur. Antigenic differences between *Campylobacter* strains may result in only partial protection from previous exposures. Exposure levels probably even vary within a country as seasonality is frequently observed; moreover, exposure levels are likely to vary between European countries and this may have effects on partial protection levels of local populations.

Work packages 4 and 5 focussed on source attribution regarding *Campylobacter* and VTEC respectively. Probabilistic models and simulation tools are increasingly used in quantitative risk assessments on food safety and production. The applicability of such models needs to be assessed for better integration of the whole system knowledge and data sources (including expert opinions) over the production chain to the population level health effects. The Work packages did specifically build models for source attribution, and one developed novel statistical and mathematical models.

Work packages 4 and 5 had a close collaboration with joint workshops, a representative from Work package 2 attended one of these workshops. Work package 2 had close contact with WP26 of MedVetNet, CRL for VTEC and the Pathogenic E. coli Network (PEN) with regard to workshops and conferences, and Work package 5 had collaboration with WP28 of MedVetNet.

Results and conclusions:

Work packages 1 and 2 have contributed in the process of harmonizing data collection and sharing regarding *Campylobacter* and VTEC. This will aid in evaluating trends and sources for these zoonoses at the European level, improve recognition of national and regional differences, and thereby aid in risk assessments in the field. Both these Work packages have contributed in sharing knowledge by personnel from laboratories visiting other laboratories to learn methods and exchange experiences.

The molecular epidemiology of *Campylobacter jejuni* and *C. coli* (*Campylobacter* for short) is still an enigma that nearly two decades of typing investigations have not been able to solve. Technical improvements and standardization of methodology have resulted in reliable and portable methods, but the data have not brought the expected insights in source attribution, relative risk, or recognition of higher or lower virulent subpopulations, despite numerable efforts. The latest addition to the firmament of typing methods has been complete genome hybridization by micro-array analysis. This may be the way forward, but technical improvements are needed to improve the output, increase the throughput and reduce the costs. In addition, it is necessary to complete our understanding of the true genetic potential of the organism, by generating and interpreting additional complete genome sequences. The knowledge thus gathered could produce micro-arrays that sufficiently cover the genetic repertoire potentially present in individual isolates. In addition, insights in the genetic basis of the virulent life-style of the organism can be gained, provided sufficient genomes are sequenced from less virulent, related species that can serve as a control against which genomes of virulent species can be compared. Finally, it can be envisaged that the generation of complete genome sequences may be the ultimate fine-tuned typing method of the future, provided technical improvements and reduced costs continue their current trends.

Through a scientific workshop and a multi-laboratory approach, Work package 2 have concentrated on the molecular epidemiology of *E. coli* and the risk gene flow within this ubiquitous reservoir represents for

generating new human pathogenic VTEC strains. Variation in pathogenicity factors and mobile genetic elements of *E. coli* isolates from an in vivo transduction experiment was characterized and horizontal gene transfer studied as this is an important part of the explanation of the wide spectrum of virulence seen within VTEC, and is a key aspect to consider in the understanding of their ecology.

Work package 3 has provided a state of the art review of the impact of acquired immunity on campylobacteriosis in humans. Also, the possibility of mathematical modelling of this complex and dynamic system has been explored as has strategies to obtain further observational and experimental data. This has resulted in new project plans, including several European partners. A multi-centre project is currently being prepared based on the results of the WP. This project will be submitted to the FP7 People Programme (Marie Curie Initial Training Grants). Serological analysis aimed to explore differences in the humoral immune response in infected individuals in relation to the presence or absence of clinically overt disease, in relation to age or in relation to the level of exposure (related to professional activities). Epidemiological evidence suggests that prior challenge with *Campylobacter* can induce protective immunity in infected patients. In order to obtain reliable epidemiological data as to the role of *C. jejuni* in causing late-onset complications such as arthritis or Guillain-Barré Syndrome, we developed a highly specific and sensitive diagnostic tool for the epidemiological investigation of *C. jejuni*-associated diseases. In addition, we have optimized the protocol procedures and plate media for better isolation of the *Campylobacter* bacteria from chicken broilers, and collected a large number of isolates from Germany, USA and Puerto Rico. Binding of *Campylobacters* to the host fibronectin is mediated by the bacterial 37 kDa outer membrane protein CadF. Immunoblot analysis of 58 *C. jejuni* and *C. coli* isolates of human and animal origin showed that CadF is expressed in every of the strains. Infection assays revealed that *C. jejuni* bound and invaded INT-407 epithelial cells much more efficiently than *C. coli* and that this difference was considerably reduced in isogenic *cadF* mutants. These results demonstrate that CadF is an important pathogenicity factor. The difference between CadF of *C. jejuni* and *C. coli* may potentially be exploited to discriminate these species in food and clinical specimens.

Work packages 4 and 5 have provided case reports and also new modern computational techniques to assess and model food related risks in the area of zoonoses. This is a valuable aid in the process of protecting consumers, based on more comprehensive use of all data sources, from regular surveillance as well as cross sectional studies. To our knowledge, source attribution of human VTEC infections has never been attempted earlier.

In WP 4, general approaches for source attribution problem were identified: (1) the approach starting from reported human cases, attributing them all to source groups, (2) the approach starting from a (single) specific food production chain calculating the case burden for that, (3) observations from direct population wide 'experiments' in special situations (e.g. withdrawal of some foods during crisis, thus eliminating one pathway and observing the result), (4) more detailed studies of diagnosed patients. For a full source attribution, none of the above is sufficient alone. In countries with a long history of good quality reporting of human campylobacteriosis as well as the surveillance of food production chains, a statistical source attribution model is a possibility but needs to be backed up with assumptions and external data. Posterior probability of the source intensities given the observed total of cases provides the correct constraint for the sum of intensities and unites the source model with the epidemiological data, but this could be combined with more detailed knowledge of some pathways and/or more detailed typing results. Currently, regular systematic time series data in Nordic countries are often reported for *Campylobacter* spp, or for some *Campylobacter* species, whereas MLST methods, possibly applied to a random subsample, could be a way forward in the future. Therefore, modelling methods need to be developed to account for the biased, flawed or otherwise sparse and limited data so that the model could be applied timely as a part of regular reporting. A pilot model based on existing data was made which can be further amended with new data.

The results obtained in the project are being communicated to major stakeholders such as national food and health authorities, the meat and poultry industry and other target groups such as the European Food Safety Authority (EFSA), European Centre for Diseases Prevention and Control (ECDC) and WHO. The recommendations regarding future research needs and strategies are made available to the European Commission and to national research organizations within the SAFEFOODERA.

Recommendations:

The documents on future research needs should be used by funders of research such as the European Commission (DG RTD), ERA-nets and national research agencies when developing future research strategies on *Campylobacter* and Verocytotoxin-producing *Escherichia coli*.

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