Proposal/Contract no.: 506122  •  Project acronym: Med-Vet-Net
Project full title: Network for the Prevention and Control of Zoonoses
NETWORK OF EXCELLENCE  •  PRIORITY 5 Food Safety and Quality
Start date of project: 1 September 2004  •  Duration: 5 years
Period covered: from 1 September 2008 to 31 October 2009
Project Co-ordinator’s Representative: Dr André Jestin
Project Director: Professor John Threlfall

We have attempted to ensure permission for reproductions of all images have been obtained and that all licenses are adhered to. If, however, you think there has been an error, please let us know at communications@medvetnet.org

Publicly available reports from Med-Vet-Net Workpackages can be obtained from: http://www.medvetnet.org/reports
This report is printed on forest managed and partly recycled paper. Printed by Fidelity Solutions, Ampthill, UK.
Preface

Med-Vet-Net’s achievements during the last fourteen months of the funded activities exceeded all expectations. The Network has gone from strength to strength with the many diverse activities reflected in a plethora of key performance indicators, but a major achievement has been the formation of the self-funded Med-Vet-Net Association.

In taking the unanimous decision to maintain the joint programming action and keep up the investment in the Network of Excellence, the heads of the scientific institutions involved in the collective venture that is Med-Vet-Net have ensured the Network’s long-term stability. And with the launch of the Med-Vet-Net Association, all can now proudly assert, in response to the challenge of zoonoses, not just “Yes, we can!” but “Yes, we do!”

We warmly thank the delegates from the European Commission and other networks who attended the Association’s formal launch in Brussels in October 2009. Their support is testament to the importance of the Med-Vet-Net Association, and their shared interest in the step we have taken in creating this new collaborative venture.

Initially the funds of the Med-Vet-Net Association will be limited and will contribute mainly towards establishing an administrative framework, training activities, a scientific meeting, and a website. But it is hoped the Association will grow strongly, generating funds wherever possible and increasing its membership and possibly its overall remit in response to new zoonotic disease challenges.

Once again, Med-Vet-Net’s three-day Annual Scientific Meeting, held this year in El Escorial, Spain, was an outstanding success with more than 200 delegates, including nearly 50 external participants. Sincere thanks are due to everyone who contributed to the organization of the meeting, and to the generous sponsors.

Training activities were a key aspect of the Network and our final year was no exception with three workshops, 15 short-term missions and five partially funded PhD students. Seven more scientists also completed various aspects of Med-Vet-Net’s Science Communication Internship.

Many of Med-Vet-Net’s scientific research efforts came to fruition in this final year and, by any criteria, the results are outstanding — clearly evidenced by the more than 150 peer-reviewed papers crediting Network activities and funding, with several more submitted. Med-Vet-Net scientists also presented the Network’s research to more than 2,000 people, including scientists, administrators and other key stakeholders, at 20 international conferences. And there have been increases in both the number and quality of databases, repositories and shared reagents, and joint quality control standards produced by various workpackages, many of which have been made available to external scientists and institutes, or will be in the near future.

The Special Interest Groups have also been enormous successes in their own right, increasing the coverage and impact of Med-Vet-Net not only in Europe, but worldwide.

Mention must also be made of the Network’s highly sophisticated communications. For those of us who interact daily with the Communications Unit, the efforts invested in ensuring the highest quality outputs are truly outstanding. Such outputs are clearly evidenced by Med-Vet-Net’s annual reports, which are widely distributed, and the stakeholder magazine showcasing Med-Vet-Net’s achievements that was launched in Brussels in October 2009.

Med-Vet-Net would not have succeeded without the continued interest and support of many people. The Governing Board, chaired by Valérie Baduel, has been a constant source of advice and support; the Co-ordinating Forum made sustained contributions to the scientific programme, ensuring that new areas of zoonoses were incorporated into various workpackages; the Administration Bureau — headed by the Co-ordinator’s Representative, André Jestin, and with invaluable support and financial input from Arnaud Callegari — has consistently provided financial details and projections, and contributed to both the day-to-day running and forward planning, including the work to secure Med-Vet-Net’s long-term viability; and last but not least, the sustained advice and support from Med-Vet-Net’s EU Officer, the EC Directorate-General for Health and Consumers, and EU organizations such as the European Food Safety Authority and the European Centre for Disease Prevention and Control.

What of the future? Hopefully, most of Med-Vet-Net’s activities will continue in the Med-Vet-Net Association — it is important for the Association and for the future of zoonoses research in Europe that activities do not stagnate. There are new challenges and new opportunities ahead, and new collaborations with new institutes, research groups and industry waiting to be forged. Nevertheless, Med-Vet-Net can be proud of its achievements over the past five years and the foundations it has laid for collaborative, inter-disciplinary research in food-borne zoonoses in Europe.
Contents

Executive Summary iv

Key Scientific Achievements of Year v

SECTION 1: PROJECT OBJECTIVES AND MAJOR ACHIEVEMENTS DURING THE REPORTING PERIOD 1

Project objectives .................................................. 1
Current relationship to the state-of-art ............................ 1
Summary of the objectives for the reporting period, work performed, contractors involved and major achievements ................................. 2
Key Performance Indicators ........................................ 5

SECTION 2: WORKPACKAGE PROGRESS FOR THE PERIOD 7

Workpackage 1: Annual Activity Report ......................... 8
Workpackage 2: Annual Activity Report .......................... 10
Workpackage 3: Annual Activity Report .......................... 13
Workpackage 6: Annual Research Report ........................ 16
Workpackage 21: Annual Research Report ...................... 18
Workpackage 22: Annual Research Report ...................... 21
Workpackage 23: Annual Research Report ...................... 23
Workpackage 24: Annual Research Report ...................... 25
Workpackage 25: Annual Research Report ...................... 28
Workpackage 26: Annual Research Report ...................... 31
Workpackage 27: Annual Research Report ...................... 34
Workpackage 28: Annual Research Report ...................... 37
Workpackage 29: Annual Research Report ...................... 40
Workpackage 30: Annual Research Report ...................... 45
Workpackage 31: Annual Research Report ...................... 48
Workpackage 32: Annual Research Report ...................... 52
Workpackage 33: Annual Research Report ...................... 55
Workpackage 34: Annual Research Report ...................... 58

SECTION 3: CONSORTIUM MANAGEMENT 61

Management issues and resolutions ............................ 61
Project timetable status ............................................ 62
Communication between partners, project meeting, possible co-operation with other projects/programmes .............................. 62
Co-operation with other projects/programmes .................. 62
FP7 and other proposals prepared during Year 5 with Med-Vet-Net partners actively involved ................................. 63

SECTION 4: OTHER ISSUES 64

Ethical Issues .......................................................... 64
Gender Issues .......................................................... 64

DELIVERABLES 65

MILESTONES 69

APPENDIX 1: PLAN FOR USE AND DISSEMINATION OF KNOWLEDGE 73

Section 1 — Exploitable knowledge and its use .................... 73
Section 2 — Dissemination of knowledge .......................... 73
Peer-reviewed publications ............................................ 73

ACRONYMS 78

MED-VET-Net Administration, Management and Communications 79

MED-VET-Net Partner Institutes 80
Executive Summary

Med-Vet-Net, a European Network of Excellence, has been primarily operated by and for scientists who, in the last fourteen months of the reporting period, belonged to 14 independent public health and veterinary institutes in 10 European countries. The Society for Applied Microbiology (SfAM) also supported the Network through the newly-appointed independent company, Science Communications Ltd. Med-Vet-Net’s fifth and final year has been highly productive with the many diverse activities reflected in a plethora of key performance indicators.

Over the past five years Med-Vet-Net developed a series of fully-integrated and interactive groups of scientists, including laboratory-based researchers, epidemiologists and risk assessors, all working towards the common objective of combating diseases in humans and animals throughout the European Union (EU). The Network’s main activities have been targeted at food-borne zoonotic diseases, although other non-food-related zoonoses, such as bat lyssaviruses, have been investigated through Special Interest Groups (SIGs) operating outside the scientific workpackages but with seed-corn funding provided by Med-Vet-Net.

In Year 5 the overall aims of Med-Vet-Net were to improve the understanding, prevention and control of zoonotic diseases in Europe through strategic and integrated high-quality collaborative research across the food chain; raise awareness among policy makers of zoonotic diseases, the general public and other stakeholders; and enhance the skills and knowledge base of European zoonotic disease researchers. A further objective was to take forward the concept of a ‘virtual institute’, thereby promoting the integration of veterinary and medical scientific activities within Europe in the field of food safety.

The tasks and responsibilities of the three overarching Workpackages remained unchanged during Year 5. Workpackage 1 was responsible for the Network’s administrative and financial aspects, while Workpackage 2 handled the overall project management. Activities included the reporting of deliverables and the non-financial annual report, as well as preparations for the scientific Joint Programme of Activities for the final year. Workpackage 3 continued to provide Med-Vet-Net’s knowledge hub and, through the Communications Unit, continued to produce regular newsletters with information about Network activities, profiles of key personnel and workpackages, and accounts of meetings held throughout the Network. Additionally in Year 5, the Communications Unit produced a very well-received glossy report of some 34 pages showcasing Med-Vet-Net’s research results and achievements.

The efforts to ensure the Network’s long-term sustainability have borne fruit, with the formation of the self-funded Med-Vet-Net Association, officially launched in Brussels on 6 October 2009. The Med-Vet-Net Association aims to increase, capitalize and disseminate scientific knowledge on zoonoses with a main emphasis on food-borne zoonoses. The Association’s statutes and internal regulation were registered on 1 June 2009 under the French Non-lucrative Association (1901) framework. It is noteworthy that all current scientific institutes have elected to join the Association as full members for an initial period of three years, on a self-funded basis.

Durable integration has also been addressed at the scientific level with Med-Vet-Net pursuing opportunities for funding within FP7 and other areas, with the primary objective of maintaining and expanding multi-disciplinary research teams by incorporating external research groups and institutes into new proposals. In that respect, at least 10 collaborative projects involving Med-Vet-Net workpackages and scientists have been developed or started in Year 5, all of which will continue when Med-Vet-Net has formally concluded. Preparatory meetings for such proposals were not funded by the EC contribution to the Network.
In December 2008 Med-Vet-Net was subject to an independent external review on the basis of its Year 4 activities, and rated as 'good to excellent'. That is, the project had “fully achieved its objectives and technical goals for the period and even exceeded expectations”.

Med-Vet-Net’s three-day Annual Scientific Meeting was held in El Escorial, Spain this year from 3–6 June 2009. Over 200 delegates, including 150 delegates from within the Network and over 70 external participants, attended. In total, seven lectures from international keynote speakers, 54 oral presentations and over 172 posters were presented during the meeting. Representatives of the current 15 scientific workpackages also presented the results and achievements of their respective workpackages.

During the reporting year three workshops were organized by Med-Vet-Net participants, and 15 short-term scientific missions were completed. Five PhD students were partially funded in four separate workpackages, with two PhDs awarded; one under the auspices of Workpackage 28 and another under Workpackage 31.

Med-Vet-Net’s scientific core is formed by the scientific workpackages, of which 15 were active during Year 5, including the new Workpackage 34 on the sources, control and prevention of Campylobacter in poultry. Communication and collaboration between the workpackages, as reported in Year 4 is vital, and has been actively promoted in the current reporting year. Collaborations between scientific workpackages have continued to develop and become more evident in terms of transparency and outcomes. Six workpackages sharing common interests have held joint meetings. — Workpackages 30 and 34 (Campylobacter), Workpackages 21 and 29 (antimicrobial drug resistance), and Workpackages 23 and 32 (prioritizing food-borne and zoonotic hazards at the European level/public health surveillance for food-borne infections: application of sero-epidemiology to validate the surveillance pyramid). Additionally, other workpackages have held joint meetings with SIGs. — Workpackages 26 and 33, and the Host-Pathogen Interactions SIG.

These meetings ensured a high level of interaction between participants and have had major benefits for the Network in terms of exchange of ideas, technologies and the exploitation of methods and analyses. In several instances such collaborations have resulted in either the submission, or publication of peer-reviewed papers with joint authorships from several different workpackages. An additional outcome of such inter-workpackage collaborations has been the informal linkage of scientists from different workpackages to develop and submit applications for funding, which will continue after Med-Vet-Net ceases.

Most notably, the research efforts of the scientific workpackages came to fruition in Year 5. By any criteria the results are outstanding.

Using a combination of laboratory-based methods, epidemiology and risk assessment of disease potential, Network scientists studied zoonotic organisms including bacteria, viruses and parasites. Of particular note was the continued development of diagnostic procedures for a wide range of zoonotic diseases, coupled with the consolidation and harmonization of procedures for identifying and subtying causative organisms, supported by the ongoing development of methods, repositories of strains and DNA, and dynamic, internet-accessible databases containing DNA sequences and genotype data linked to epidemiological information.

Some of the latter databases are already available for use outside Med-Vet-Net while others are expected to be accessible via the internet in the near future.

The workpackage targeted towards the development and use of geographic information systems, applied its activities to analysing the spatial distribution of human Q-fever cases and cattle farms in Denmark. Workpackages exploring various aspects of antimicrobial resistance combined their activities to study the occurrence and spread of newly emerging antimicrobial resistance determinants of considerable importance to human health. New methodologies, based on a real-time polymerase chain reaction (PCR) approach, were developed to detect zoonotic strains of Giardia while work on Q-fever diagnostics has culminated in the completion of a comparative ring trial for PCR detection of Coxiella burnetii, with guidelines and recommendations for the use of different methods being widely distributed. Studies on the factors contributing to the virulence and pathogenicity of Salmonella and VTEC have resulted in the development of virulotyping platforms for routine diagnostic use for Salmonella and VTEC across partner institutes in Europe, as well as the identification of possible virulence predictors for VTEC of significant public health impact. Such studies will undoubtedly contribute to the overall control of these important disease-causing organisms throughout the EU.

A new enzyme-linked immunosorbent assay method for the early detection of Trichinella infections in pigs has been developed coupled with the development of a gold standard for the molecular typing of Trichinella, and a range of laboratory-based, metagenomic and epidemiological studies have targeted Campylobacter, which remains the major cause of bacterial gastro-enteritis in many European countries. These projects have resulted in the development of a European consensus framework for risk assessment; enhanced knowledge about virulence-related properties in selected strains based on array analysis and identification of host-regulated factors that may result in the induction of some protection; and an evidence-based database of Campylobacter genes associated with colonization in chickens and their functions, together with a review of in vivo models of Campylobacter colonization in poultry, and recommendations for standardization and harmonization.

Work reflecting the current and future disease threat of ribonucleic acid (RNA) viruses in food-producing animals, particularly Hepatitis E virus (HEV), Anellovirus and Echoviruses circulating in pigs, and on Tick-borne encephalitis virus in relation to goat milk transmission, included cell culture methods and their functions, together with a review of antibody levels in pigs are a convenient substitute for population-based serum collections was assessed. In the prioritization of disease hazards, a modelling tool to reconstruct the surveillance pyramid was developed, a telephone survey was initiated, and a calculation tool to estimate the Disability Adjusted Life Years of infectious intestinal disease was developed and integrated into the modelling tool to reconstruct the surveillance pyramid. Finally, in the workpackage focused on identifying and prioritizing effective food safety interventions, a three-dimension Bayesian model for attributing human salmonellosis to specific sources and estimating the public health impact of different Salmonella subtypes was developed. The model can be applied to a variety of food-borne hazards and is appropriately tailored to agents that are a frequent cause of outbreaks.
Delegates at the Madrid conference meet during the break, for informal discussions about their work.

Presenting results in peer-reviewed journals continued to be afforded high priority by the Network, and is an internationally accepted recognition of scientific achievement. In Year 5, at least 78 peer-reviewed papers acknowledging Med-Vet-Net funding, were published or are in press, bringing the total activities credited to the Network to over 150. Several other papers are either in preparation or have been submitted for publication.

In Year 5, workpackage research and outputs, in terms of deliverables and milestones, continued to be maintained at a high level. Of the expected 142 deliverables, 132 (93%) were fully achieved, one was partially achieved (0.7%), three (2.1%) were achieved with modifications, two (1.4%) were withdrawn and only four (2.8%) failed. Of the 117 expected milestones, 111 were achieved (95.2%), one was partially achieved (0.8%), one (0.8%) was withdrawn and four (3.2%) failed.

These outputs are reflected in the list of Key Performance Indicators, as established by the Governing Board in Year 2. Of note are the number of different multi-site teams working together to achieve a range of objectives; the clear and transparent interactions between scientific workpackages and SIGs; increases in the numbers of databases, repositories and shared reagents; and joint quality control standards. Many of the databases and repositories are being made available to scientists and institutes outside Med-Vet-Net, or will be in the near future. Also of note is the growing international stature of Med-Vet-Net, evidenced by the number of interactions with organizations such as the European Centre for Disease Prevention and Control, the European Food Safety Authority, the American Centres for Disease Control and Prevention, and the World Health Organization; by invitations for Med-Vet-Net representation in other networks, programmes and projects within and out of the EU; and through the inclusion of Med-Vet-Net experts, in various capacities, on programmes such as the Co-ordination of European Research on Emerging and Major Infectious Diseases of Livestock's Delphi study; the EC infrastructure programme, Network Animal Disease Infrastructure Research; Networking Action 1; and the EU Disease Control Tools project.

Key Scientific Achievements of Year 5

WP6: Identification of the relationship, in five countries, between locality of VTEC patients and vicinity of cattle farms, and a study of the spatial distribution of human Q-fever cases and cattle farms in Denmark.

WP21: Elucidation of the clonal origin of isolates with ASSuT phenotype recently emerging in Italy and other European countries, and High Resolution Melting technique developed for characterization of Salmonella Genomic Island 1.

WP22: New real-time PCR methodologies to detect zoonotic strains of Giardia, and establishment of a dynamic internet-accessible database containing DNA sequences and genotype data linked to epidemiological data.

WP23: Telephone survey, based on the UK IID2-study, set-up in European Union countries; country-specific interim reports prepared for four participating countries; and development of a calculation tool to estimate the Disability Adjusted Life Years of infectious intestinal disease integrated into the modelling tool to reconstruct surveillance pyramid.

WP24: Launch of software tool Campylobacter Risk Assessment Framework (CRAF) 1.0 in February 2009 and CRAF 2.0 in August 2009.

WP25: Completion of human part ring trial for serological methods using CFT and ELISA, and isolation, characterization and comparison of new genotypes of Coxiella burnetii.

WP26: Virulotyping platforms for routine diagnostic use for Salmonella and Escherichia coli, and microarray comparison of VTEC seropathotypes B, C, and D, with type A (O157).

WP27: Harmonization of Trichinella infection control methods, and development of a gold standard for molecular Trichinella typing.

WP28: Development of three-dimension Bayesian model for attribution of human salmonellosis to specific sources and estimation of public health impact of different Salmonella subtypes, and a PhD, ‘Attributing human salmonellosis and campylobacteriosis to food, animal and environmental sources’, awarded.

WP29: Development of a standardized protocol for PCR detection of amrA, mntA and mntB, and first identification of mntC methylase in the EU.

WP30: Campylobacter strain set characterized and made publicly available, and standard operating procedure for pan-genome microarray analysis of Campylobacter developed.

WP31: Harmonization of HEV diagnostic procedures, control sera and MAbs to HEV and EMCV distributed, and a PhD, 'HEV detection and cell culture studies', awarded.

WP32: Cross-sectional serological surveys of antibody levels against Salmonella and Campylobacter in eight EU Member States completed; data analysed, sero-incidence estimates generated, and correlation with other available data on Salmonella and Campylobacter epidemiology in Europe investigated.

WP33: Observation that the host may down-regulate immune responses to Salmonella or Campylobacter during colonization or infection indicating a possible means of damage control by host.

WP34: Development of an evidence-based database of Campylobacter genes associated with colonization in chickens and their functions, review of in vivo models of Campylobacter colonization in poultry, and recommendations for standardization and harmonization produced.
Section 1: Project objectives and major achievements during the reporting period

Project objectives

The overall objective of Med-Vet-Net was to develop a network of excellence for the integration of veterinary, medical and food sciences, in the field of food safety, at the European level, to improve research on the prevention and control of zoonoses, including food-borne diseases, while taking into account the public health concerns of consumers and other stakeholders throughout the food chain.

In the project’s fifth year the following specific objectives were addressed:

Objective 1: To continue to develop and improve network management in response to the recommendations of the external review.

Objective 2: To develop and support strategies for the long-term objective of network sustainability through the Sustainability and Legal Officers subcommittees.

Objective 3: To develop and support strategies for durable integration, including support for the planning of integrated research by Med-Vet-Net partners extending after the projected duration of the project.

Objective 4: To maintain, consolidate and develop the high quality research programme consistent with the strategic science plan, institute aims and objectives, and EC requirements.

Objective 5: To promote collaborative activities between scientific Workpackages, thereby increasing both scientific flexibility and output.

Objective 6: To develop and expand collaborations with public health and food safety agencies in Europe, such as ECDC and EFSA.

Objective 7: To continue to develop the skills and expertise throughout the partnership with a programme of workshops, training courses, short-term missions, internships and studentships.

Objective 8: To continue to support platforms for the development, management and dissemination of knowledge in areas where critical mass is poor or a specific need is clear, by the provision and support of virtual Special Interest Groups.

Objective 9: To continue to expand the associate collaborative research network at the European level by involving external scientists in network research and integration activities, including Special Interest Groups, the Annual Scientific Meeting and scientific workshops.

Objective 10: To continue to enhance the development of stakeholders using the Spreading Excellence activities.

Objective 11: To continue development and expansion of international collaborations outwith the EU.

Current relationship to the state of art

From 1 September 2008 to 31 August 2009 Europe has continued to have been subjected to zoonotic infections of considerable magnitude and importance. Some of these have had major impact, not only in terms of the resultant diseases in both humans and animals, but also in relation to the deployment of staff to meet new challenges. The most obvious contender has been the global pandemic of swine flu influenza (H1N1) from March 2009. The direct zoonotic origins of the disease remain debatable; there is no evidence to suggest that date that avian influenza can be transmitted to humans through consumption of food, notably poultry and eggs.

Nevertheless, the deployment of staff for the laboratory-based diagnosis of the causative organism and for surveillance activities proved taxing for staff in many Med-Vet-Net institutes.

Although the overall incidence of salmonella infections in the EU appears to have declined since January 2008 national and international outbreaks of Salmonella have continued to cause major problems. Serovars include Typhimurium, Enteritidis, Give, Muenster and Stanley. Countries affected include most Northern European and Nordic countries, France, Bulgaria and Switzerland, and the vehicles of infection have included a wide variety of products, for example soft home-produced cheese and goats’ cheese as well as the usual ‘suspects’ of eggs and pork products.

Campylobacter has remained the most common cause of gastrointestinal disease in many European countries. Hopefully outputs from the various epidemiological and laboratory-based Med-Vet-Net workpackages (see below) will contribute to the long-term control of this organism and to its eventual elimination from poultry flocks.

There has been an outbreak of listeriosis in Spain coupled with increased number of infections in several European countries, predominantly in the elderly, and substantive outbreaks of cryptosporidiosis have been reported in the UK, Sweden and Finland. Diseases such as Q-fever and trichinellosis have continued to cause outbreaks of infection in several countries.

Outbreaks of Q-fever have continued to occur in The Netherlands, and also in several other countries in Northern Europe. An outbreak of trichinellosis in France in March 2009 was attributed to the consumption of warthog ham in an African country.

Contact with animals in the home and on petting farms has been an increasing cause of many infections, particularly of Salmonella and VTEC, often with serious consequences. In the UK a strain of Salmonella Typhimurium which has caused numerous infections throughout the country has been linked to imported frozen mice used to feed pet snakes. Similarly in that country possibly the largest ever outbreak of VTEC linked to a petting farm took place from August to September 2009, with many children hospitalized. A substantive VTEC outbreak, which occurred in The Netherlands in late 2008/early in 2009, has also been reported, in which infections were linked to the consumption of raw beef products.

In 2008/2009 resistance to critically-important antimicrobial drugs has been recognized as an increasing problem in almost all European countries. Although many of the problems are related to hospital-acquired infections with no obvious zoonotic link, organisms such as MRSA and Extended-Spectrum Beta-lactamase-producing (ESBL)-Escherichia coli have caused outbreaks of infection in both hospitals and the community. Although not proven, zoonotic reservoirs for at least some of the organisms are suspected.

The importance of viruses in food producing animals as potential source of viral zoonoses is now increasingly acknowledged and in 2008 a major outbreak of hepatitis E on an international...
cruise ship was epidemiologically-linked to the consumption of shellfish. Inhalation anthrax, although not a priority Med-Vet-Net disease has also been responsible for at least one death in 2008, linked to the use of imported animal skins for drum kits.

As stated in the annual reports for Years 3 and 4, it is vital that sustainable collaborations with key stakeholders are firmly established and that research into food-borne zoonoses is prioritized accordingly. Collaborations and interactions between workpackages within Med-Vet-Net have provided a sound basis for scientifically-applied fundamental research across national borders. It is hoped that these activities will be taken forward after the cessation of EU funding for the network, thereby facilitating rapid responses to ongoing and future challenges of zoonoses.

**Summary of the objectives for the reporting period, work performed, contractors involved and major achievements**

In its fifth year, Med-Vet-Net continued to build on the foundations laid to integrate research activities in the 15 public health and veterinary partner Institutes. The following objectives were addressed:

**Objective 1: To continue to develop and improve Network management in response to the recommendations of the external review**

The Network was subject to an external independent review on 12 December 2008 on the basis of its Year 4 activities. The project was rated as ‘good-to excellent’ — i.e. ‘the project has fully achieved its objectives and technical goals for the period and even exceeded expectations’. Recommendations in relation to Workpackages 6, 23, 28, 30 and 33 have been implemented. CRAW-2, an output from Workpackage 24, is being taken forward with collaborators in the USA, and the eventual outcomes may be applicable to organisms other than Campylobacter. Workpackage 30 has linked the results of pan-genomic analyses of Campylobacter with epidemiological information; and Workpackage 34 has been supported in achieving its stated objectives.

**Objective 2: To develop and support strategies for the long-term objective of network sustainability through the Sustainability and Legal Officers subcommittees.**

The efforts to ensure the long-term stability of the Network have borne fruit, with the formation of the ‘Med-Vet-Net Association’, which was officially launched in Brussels on 6 October 2009. Details of the Association are provided elsewhere in the report, but it is noteworthy that all present scientific institutes have elected to join the Association as full members for an initial period of three years, on a self-funded basis.

**Objective 3: To develop and support strategies for durable integration, including support for the planning of integrated research by Med-Vet-Net partners extending after the projected duration of the project.**

Durable integration has also been addressed at the scientific level, with Med-Vet-Net scientists continuing to pursue opportunities for funding within FP7 and other areas, with the primary objective of maintaining and expanding multi-disciplinary research teams, thereby incorporating research groups and institutes outwith the present network into proposals. In this respect several collaborative projects have ensued which will continue outside the EU-funded period of Med-Vet-Net. These are listed elsewhere in the report. It should be noted that preparatory meetings to prepare funding proposals were not funded by the EC contribution to Med-Vet-Net.

**Objective 4: To continue to maintain, consolidate, further develop and deliver a high quality research programme consistent with the strategic science plan, institute aims and objectives, and EC requirements.**

All Network partners have continued to be actively involved in the research programme under the leadership of the Health Protection Agency (HPA). During the final year, 15 scientific workpackages were ongoing — Workpackage 6, and Workpackages 21–34. In all, 22 research-associated meetings were held within the Network, involving over 300 scientists and including at least 100 external scientists. Three workpackages held joint meetings — evidence of the increased level interactions between different workpackages. Additionally, results from Med-Vet-Net scientific workpackages have been presented at more than 70 external meetings to an overall audience well over 1,500 people from within and outside Europe.

The presentation of results in peer-reviewed journals has continued to be afforded high priority and is an internationally-accepted recognition of scientific achievement. In Year 5 at least 78 peer-reviewed papers were published or are in press, with funding by Med-Vet-Net acknowledged, bringing the total credited to Network activities to over 150. Several other papers are either in advanced stages of preparation or have been submitted for publication.

**Objective 5: To promote collaborative activities between scientific Workpackages, thereby increasing both scientific flexibility and output.**

Collaborations between scientific workpackages have continued to develop and become more evident in terms of transparency and outcome during the fifth year. Six workpackages with common interests held joint meetings — Workpackages 30 and 34 (Campylobacter), Workpackages 21 and 29 (antimicrobial drug resistance), and Workpackages 23 and 32 (Prioritizing food-borne and zoonotic hazards at the EU level/Public health surveillance for food-borne infections: application of seroepidemiology to validate the surveillance pyramid).

Additionally, other workpackages have held joint meetings with Special Interest Groups (SIGs) — Workpackages 26 and 33 and the Host-Pathogen Interaction SIG group. These meetings ensured a high level of interactions between participants and had major benefits for the Network in terms of exchanging ideas, technologies and the exploitation of methods and analyses. In several instances such collaborations resulted in either the submission of, or publication of, peer-reviewed papers with joint authorships from several different workpackages. An additional outcome of such inter-workpackage collaborations was the informal linkage of scientists from different workpackages to develop and submit applications for funding.
A substantive VTEC outbreak, in which infections were linked to the consumption of raw beef products, was reported in The Netherlands in late 2008 to early 2009.

Objective 6: To develop and expand collaborations with public health and food safety agencies in Europe, such as ECDC and EFSA.

Raising awareness of Med-Vet-Net in the international context of food safety and zoonoses continued to be an important component of the Network during the final year. Although the ELUS-SAFEFood collaboration ceased in Year 4, a plethora of new international collaborations were initiated in Year 5. For example, an EU SAFEFoodERA project entitled, ‘The role of commensal microflora of animals in the transmission of extended spectrum β-lactamases’, was approved in February 2009 and commenced in September 2009. The project is a collaboration between five partners, four of which are from Med-Vet-Net — HPA, Veterinary Laboratories Agency (VLA), the German Federal Institute for Risk Assessment (BfR) and the Central Veterinary Institute (CVI) in The Netherlands.

The ECDC has shown great interest in the sero-epidemiological approach developed under Workpackage 32 and recently issued a call for tender for further development of this initiative. A joint initiative of industry and a wide range of stakeholders including the research community, regulators and users. It is actively encouraged and funded by the EC and provides a mechanism for focusing and prioritizing research that ultimately delivers new and improved vaccines, pharmaceuticals and diagnostic tests. Med-Vet-Net experts are involved in Workpackage 3 of DISCONTOLS, which is dedicated to the prioritization of animal disease, and the Med-Vet-Net Project Manager has been appointed to both the Executive Board and the Project Management Board of DISCONTOLS, ensuring ongoing representation within this important European initiative.

In addition to the above collaborations, Med-Vet-Net experts have contributed to the EMIDA ERA-NET programme’s Delphi study (Workpackage 4) that is developing a strategic trans-national animal health research agenda; and four Med-Vet-Net members are also members of the Network of Animal Diseases and Infectiology Research Facilities (NADIR), an EC infrastructure programme. NADIR’s Networking Action 1 (NA1) is, in part, aimed at developing an inventory of Europe’s animal experiment facilities and laboratories, and harmonizing their procedures and models. NA1 derived from Workpackage 1’s initiative to inventory the animal facilities within the Med-Vet-Net Network (see the Workpackage 1 report).

Other new international collaborations were also developed through the Special Interest Groups, and Med-Vet-Net scientists continued to support EFSA and ECDC through membership of panels, ad-hoc groups and other fora.

Objective 7: To continue to develop the skills and expertise throughout the partnership with a programme of workshops, training courses, short-term missions, internships and studentships.

Training activities remained an essential element and a high priority of the Network during Year 5. With the support of several other partners, the Technical University of Denmark (DTU) continued to lead this area of the Network’s activities. Applications for workshops and short-term secondments were channelled through DTU, and independently assessed by a small team.

In Year 5, three well-attended training courses were funded, which were separate to the courses independently organised within scientific workpackages were funded. These included: Bioinformatics for Laboratory Scientists; Deriving Disability Weights (DDW); and Development of a European consensus framework on risk assessment of Campylobacter in broiler meat. As well as participants from Med-Vet-Net, attendees at these workshops included scientists from numerous other European institutes as well as representatives from ECDC, EFSA, the USA, Japan and Canada. Together these workshops served to promote the activities of Med-Vet-Net in at least 30 countries around the world.

Short-term missions (STMs) and exchanges have continued to be well-subscribed, with 15 of 18 applications funded. Young scientists particularly benefited from the STMs, taking full advantage of the training opportunities offered and the opportunities to meet and work with established scientists in different institutes both within and outside Med-Vet-Net. The Network also continued to provide partial funding for five PhD students within four workpackages. One PhD entitled ‘Attributing human zoonotic infection with different animals, food and environmental sources’ was completed under the auspices of Workpackage 28. Another PhD entitled ‘HEV detection and cell culture studies’ was completed as part of Workpackage 31. Three further PhD students are now in their final year and it is hoped that their studies will be completed in the very near future.

Objective 8: To continue to support platforms for the development, management and dissemination of knowledge in areas where critical mass is poor or a specific need is clear, by the provision and support of virtual Special Interest Groups.

In Year 5, enhanced knowledge management within the Network continued within two Special Interest Groups (SIGs) — Wildlife-Related Emerging Diseases and Zoonoses (WirEDZ) and Host-Pathogen interactions, which was initiated in Year 3.

In December 2008, WirEDZ held a meeting in Budapest with 22 delegates from 13 countries attending. WirEDZ work in European countries, in particular those countries where systems and approaches to WirEDZ work was unknown, was discussed. By August 2009, 223 scientists from 35 countries had also registered on the SIG’s WILDLIST (www.medvetnet.org/wildlist). WirEDZ’s achievements have been instrumental in developing a basis for others to progress the foundation of wildlife disease reporting and collaborating networks across Europe.

The Host-Pathogen SIG continued to interact closely with Workpackage 33, and also facilitated numerous meetings, workshops and STMs within and outside the Network, such as a joint zoonoses meeting with the University of Surrey (UK) and North Carolina State University (USA), which was made possible through contacts generated through the SIG. In addition to providing a platform for networking in the area of host-pathogen interactions, the SIG also assisted the drafting of a number of EU and research council grant proposals.
Workpackage 34, a scientific workpackage targeted at the prevention and control of Campylobacter in broilers that originated from a SIG concept, commenced in September 2008. Since then the workpackage has developed an electronic communication system for debate on Campylobacter control throughout Europe.

Objective 9: To continue to expand the associate/collaborative research network at the European level by involving external scientists in network research and integration activities, including Special Interest Groups, the Annual Scientific Meeting and scientific workshops.

In Year 5 external scientists were involved in a range of Med-Vet-Net activities at an unprecedented level. For example, Dr. Ruff Lowman from Canada was a keynote speaker at the CRAFT 1.0 workshop held by Workpackage 24 at the BFR. The meeting, attended by 37 participants from 14 countries, including USA and Japan, was followed by a one-day CRAFT training course with 30 participants from 13 countries, including Canada, the USA and Japan. EFSA and the American Joint Institute for Food Safety and Applied Nutrition (JIFSAN) were also represented.

Twenty-two delegates from 13 countries attended the WiREDZ workshop in Budapest in February 2009, with delegates from the Republic of Ireland, Finland, Greece, Croatia, Russia, Lithuania, Romania, as well as Med-Vet-Net countries. Five external experts delivered presentations to the joint Workpackage 21 and 29 workshop in Paris in June 2009, and presentations were also made by external experts from the World Health Organization to the joint Workpackage 23 and 32 meeting in Poland in July. The Centers for Disease Control and Prevention (USA) among others have an active interest in Workpackage 23 and have participated in all meetings. Similar examples are provided in almost all workpackage reports for Year 5.

With regard to training, it is conservatively estimated that at least 40% of the more than 100 scientists who received training in Med-Vet-Net workshops in Year 5, were external to the Network. And lastly, at the Med-Vet-Net Annual Scientific Meeting in Madrid (June 2009), the number of presentations by international keynote speakers increased from six to seven, all of which were well received by delegates.

The Annual Scientific Meeting is a showcase for the Network’s activities and for developing collaborations with non-Med-Vet-Net scientists. In Year 5 this was held at the Euroforum Infantes conference centre in El Escorial, Spain, adjacent to the UNESCO World Heritage site of the El Escorial Monastery. Nearly 200 delegates, comprising 150 delegates from within the Network and 49 external participants attended. In total, seven lectures from international keynote speakers, 54 oral presentations and over 172 posters were presented to delegates, highlighting various Network activities and collaborative activities with scientists in external institutes worldwide.

Representatives from each of the ongoing 15 scientific workpackages also presented their respective results and achievements. All of the presentations, both oral and poster, were of a high quality, with several subsequently delivered at international meetings elsewhere.

Objective 10: To continue to enhance the development of stakeholders using the Spreading Excellence activities

A significant output this year was the stakeholder report ‘Building a European Community to Combat Zoonoses’, which provides an easy-to-read summary for non-scientific stakeholders. The report showcases the results and achievements of Med-Vet-Net’s workpackages and Special Interest Groups.

Med-Vet-Net News, the Network newsletter, was produced regularly throughout Year 5 and distributed as an e-bulletin with links to full articles on the public website. The newsletter reached more than 1,500 subscribers from a diverse range of sectors, both scientific and non-scientific, in 72 countries.

The Communications Unit also prepared magazine articles for publication in journals including Microbiologist (the magazine of the Society for Applied Microbiology), The Parliamentary Monitor, the EU-AgriNet website, and Vetro, the internal magazine of the VLA.

Objective 11: To continue development and expansion of international collaborations outwith the EU.

To maintain and further develop CRAFT, a proposal was submitted to the National Institute of Food and Agriculture of the US Department of Agriculture, by Food DTU and RIVM, together with JIFSAN, a partnership between the University of Maryland and United States Food and Drug Administration. With respect to Trichinella, a consortium established in 1998 will be expanded with the introduction of Med-Vet-Net as well as Chinese and Canadian partners. The Complutense University of Madrid is currently expanding Med-Vet-Net collaborations in antimicrobial resistance activities to include India. Four partners are already part of an FP7 project (VITAL 2008–2011) on Hepatitis E virus (HEV) Risk Assessment, and others are discussing participation in an ERA-NET proposal on HEV.
**Key Performance Indicators**

Table A1. Key Performance Indicators as designated by the Med-Vet-Net Governing Board.

<table>
<thead>
<tr>
<th>Key Performance Indicators</th>
<th>Sub-topic</th>
<th>Achievements</th>
</tr>
</thead>
</table>
| Durable Integration | Multi-site teams | - Four multi-site teams worked together to develop and implement sustainability plans.  
- Two multi-site teams worked together to specifically plan the day-to-day project management and longer-term development of the newly formed Med-Vet-Net Association.  
- Six multi-site teams organized and delivered training courses.  
- Five multi-site teams organized and delivered the 5th Annual Scientific Meeting (El Escorial, Spain, June 2009).  
- Two multi-site teams worked together on the Network’s governance and co-ordination.  
- Three multi-site teams worked together to ensure liaison at all times with appropriate European Commission (EC) officials, including the EC-appointed Med-Vet-Net Project Officer and Financial Officer.  
- Seven multi-site teams continued working together to ensure overall integration of the Network’s overarching and scientific activities.  
- Clear and transparent evidence of interactions between 12 of the existing scientific workpackages, including joint workshops and training courses, joint publications, and joint applications to new calls for funding. |
| Common databases | - Common databases, datasets strain collections and DNA repositories continued to be generated and improved upon in most of the current scientific workpackages.  
- One additional database and one strain inventory established in Year 5, bringing the total number in the Network to 21.  
- Dynamic internet-accessible database, containing DNA sequences and genotype data linked to epidemiological data, established for *Giardia*.  
- *Campylobacter* database compiled and extended with features of all strains from the previously established CampyNet (CNET) strain collection, including multi-locus sequence typing data. In parallel, a virtual database of strains from partner institutes was created with a public part linked to the CNET web-domain. This database is a *Campylobacter* reference collection of epidemiological and clinical interest.  
- Evidence-based database of *Campylobacter* genes associated with colonization in chickens and their functions.  
- Up-to-date database (Endnote-based) of publications on interventions for *Campylobacter* in poultry. |
| Multi-site funding | - Another six joint proposals that exploit Network integration have been submitted to FP7 or other funding bodies within EU, and three proposals have been submitted to other international funding bodies. |
| Joint training courses | - Three additional joint training courses in Year 5.  
- 120 scientists participated in multi-site training courses. |
| Short-term scientific missions | - Fifteen short-term missions organized involving secondment of staff to different institutions. |
| Joint quality control standards | - *Salmonella* strains of variable genetic background and antibiotic resistance profiles, and displaying variation in SGI1 make-up were collected, characterized and made available for reference purposes.  
- Plasmids, in which specific *Giardia* duodenalis sequences had been cloned, were produced and tested. These are now available as reference material.  
- Production and characterization of *Trichinella* reference material.  
- Production of standard reference positive and negative porcine sera for HEV antibody determination. |
| Outputs | External assessment | - EC referee’s report rated Year 4 activities as ‘good-to-excellent’ with the project having “fully achieved its objectives and technical goals for the period and even exceeded expectations”. |
| | Deliverables and milestones | - In Year 5, 132 of 142 (93%) deliverables and 111 of 117 (95.2%) milestones were achieved. |
| | Publications arising from Med-Vet-Net funded work | - 78 papers published or in press in peer-reviewed journals in Year 5.  
- Interactive *Salmonella* atlas to be published in 2010. |
<p>| | Presentations at international meetings | - Presentations of results from Med-Vet-Net scientific workpackages at approximately 70 external international meetings to a total audience in excess of 1,500 people. |</p>
<table>
<thead>
<tr>
<th>Key Performance Indicators</th>
<th>Sub-topic</th>
<th>Achievements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outputs (cont.)</td>
<td>Special Interest Groups</td>
<td>Two Special Interest Groups (SIGs) continued in Year 5: Host-Pathogen Interactions, and Wildlife-related Emerging Diseases and Zoonoses (WIREDZ). Evidence of international collaborations in both SIGs.</td>
</tr>
<tr>
<td>Scientific workpackages</td>
<td>- Fifteen scientific workpackages were operational in Year 5.</td>
<td></td>
</tr>
</tbody>
</table>
| Standardized procedures    | - New methodologies, based on a real-time PCR approach, to detect zoonotic strains of *Giardia*.  
- Release of *Campylobacter* Risk Assessment Framework (CRAF) software tool — CRAF 1.0 in February 2009 and CRAF 2.0 in August 2009.  
- Recommendations for best practice and harmonization of typing methods of *Coxiella burnetii*.  
- Development of a gold standard for molecular *Trichinella* typing.  
- Cell culture methods for swine HEV replication monitored for viral genome and antigen synthesis. |
| Repositories and shared reagents | - *Salmonella* strains of variable genetic background and antibiotic resistance profiles, and displaying variation in SGI1 available for reference purposes.  
- Plasmids, in which specific *Giardia duodenalis* sequences had been cloned, were produced and tested, and are now available as reference material.  
- Production and characterization of reference material for *Trichinella* identification and typing.  
- Control sera and monoclonal antibodies to HEV and EMCV for use between partners. |
| Development of new diagnostics or therapeutics | - Improved fingerprinting assay for characterization of SGI1 based on RT-PCR using SYBR-green and a new High Resolution Melting technique got SGI1 analysis.  
- Development of virulotyping platforms for routine diagnostic use for *Salmonella* and *Escherichia coli* across partner institutes in Europe. The array platform used for this purpose is commercially available under the brand name of IDENTBAC.  
- Development of a new iELISA for the early detection of *Trichinella* infections in pigs. |
| Skills improvements        | - Completion of three training courses involving over 100 scientists, approximately half of whom were external to the Network.  
- Fifteen short-term missions completed.  
- Seven scientists trained in science communication. |
| PhD studentships           | - Five PhD students fully or partially funded in four separate workpackages.  
- Award of PhD to student funded within Workpackage 28.  
- Award of PhD to student partially funded within Workpackage 31. |
| International meetings     | Med-Vet-Net’s 5th Annual Scientific Meeting was held at El Escorial, Spain, in June 2009 with 220 participants, of which 49 were external, from 18 countries. |
| Impact                     | Support for policymakers | - Information on calculation of DALYs of infectious intestinal disease and reconstruction of surveillance pyramid provided to WHO’s Food-borne Disease Burden Epidemiology Reference Group and the American Centers for Disease Control and Prevention (CDC).  
- Analysis of data from outbreak investigations for source attribution of human salmonellosis and campylobacteriosis in European countries submitted to the European Food Safety Authority (EFSA) and Member States.  
- Information on using outbreak data analysis for source attribution of human salmonellosis and campylobacteriosis in Europe distributed to EFSA, Member States and the European Commission.  
- On-going discussions with poultry producers on effectiveness of biosecurity measures for *Campylobacter* control in poultry.  
- Information and advice provided to EFSA’s BIOHAZ Panel working groups. |
| European/international reputation | - Delegates from six non-Med-Vet-Net countries including one non-European country attended the 5th Annual Scientific Meeting.  
- On-going collaboration with the European Centre for Disease Prevention and Control (ECDC) and the USA CDC on serodiagnosis methodology and its application (Workpackage 32).  
- Future extensions of CRAF 2.0 in a new project with the USA-based organization Foodrisk.org.  
- General presentations delivered to ECDC, EFSA & the EC Directorate-General for Health and Consumers  
- On-going scientific interactions with EFSA, ECDC, CDC, United States Department of Agriculture, WHO, Food and Agriculture Organization, Community Reference Laboratories, National Reference Laboratories, and on-going and new surveillance programmes within EU Member States.  
- Appointment of Med-Vet-Net Co-ordinators Representative to EPIZONE board.  
- Appointment of Med-Vet-Net Project Director to DISCONTOOLS Management Board. |
### Key Performance Indicators

<table>
<thead>
<tr>
<th>Sub-topic</th>
<th>Achievements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Impact (cont.)</strong></td>
<td>- Collaborations in Workpackages 6, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 and 34 with scientists from institutes external to Med-Vet-Net, including in the USA, Canada and Japan.</td>
</tr>
<tr>
<td></td>
<td>- Expansion of international activities in the Host-Pathogen Interactions SIG with North Carolina State University, and WIREDZ (223 scientists from 35 countries registered on WILDLIST as at 31 August 2009).</td>
</tr>
<tr>
<td></td>
<td>- Agreement for Med-Vet-Net to be represented in DISCONTOOLS.</td>
</tr>
<tr>
<td></td>
<td>- Continued collaborations with the EPIZONE and EADGENE Networks of Excellence.</td>
</tr>
<tr>
<td><strong>General public information</strong></td>
<td>- A report entitled 'Building a European Community to Combat Zoonoses' aimed at non-scientific stakeholders provides an easy-to-read summary of the achievements of the entire project.</td>
</tr>
<tr>
<td></td>
<td>- Account of Network activities published five times in Microbiologist, in <em>The Parliamentary Monitor</em> in June 2009, on EU-AgrinNet — FP6 Project Success Stories (October 2009), and in Vetro, the internal magazine of the Veterinary Laboratories Agency (November 2009).</td>
</tr>
<tr>
<td></td>
<td>- Public website updated with articles and profiles from Med-Vet-Net News, as well as Disease Fact Sheets.</td>
</tr>
<tr>
<td></td>
<td>- Regular newsletter, Med-Vet-Net News, distributed to over 1,500 subscribers in 72 countries.</td>
</tr>
<tr>
<td><strong>Sustainability activities</strong></td>
<td>- Agreement to form self-funded Med-Vet-Net Association following cessation of EU funding. All 14 scientific institutes have elected to become full members.</td>
</tr>
<tr>
<td></td>
<td>- Formal approval by EC of Science Communications Ltd as a third party (commenced 1 September 2008).</td>
</tr>
<tr>
<td></td>
<td>- Continuing involvement in the EU Project Managers Association in The Netherlands and the European Community Project Managers’ Association.</td>
</tr>
</tbody>
</table>

---

**Section 2: Workpackage progress for the period**

The Workpackage reports provide a detailed insight into the administrative and scientific activities of the Network in Year 5. Reports of the respective Workpackage activities have been compiled by the individual Workpackage Leaders, following consultation with, and input from, all Workpackage members. As such, these reports fully reflect the key results and achievements of the Workpackages, as envisaged by the Workpackage members. It is particularly rewarding to note the increasing evidence of interactions and collaborations between Workpackages, thereby enriching not only their outputs as evidenced by several joint scientific publications and reports, but also the scientific reputation of the Network as a whole.

The list of Workpackages, with reference to activity areas is given in Figure A1. (inside front cover).

Within this section Annual Activity Reports are provided for the Overarching Workpackages 1–3. For the scientific Workpackages 6 and 21 to 34 Annual Research Reports are presented with objectives and progress summaries.
Workpackage 1: Structuring the Virtual Institute

WP number: 1

Title: Structuring the Virtual Institute

WP Leader: André JESTIN

Name and Address: French Food Safety Agency (AFSSA)
27-37 Avenue du Général Leclerc
94700 Maisons-Alfort CEDEX
FRANCE

Project Start date: 1 September 2004

Project End date: 31 October 2009

Progress summary

During the fifth year of activities, Workpackage 1 continued to manage the administration and overall finances of the Network and play an interfacing role between the European Commission (EC) services and Med-Vet-Net as follows:

- Organization and implementation of the periodic Co-ordinating Forum and Governing Board meetings, as well as participation in the organization and implementation of the Annual Scientific Meeting, held in El Escorial, Spain, and organization of the handover meeting between the EC project and the Med-Vet-Net Association.
- Update of administrative and financial information on the website.
- Administrative, financial and legal support and assistance to Network partners.
- Implementation and management of administrative and financial procedures.
- Drafting of the financial annual report and the Joint Programme of Activities.
- Network budget management including budgeting of workpackages, and consolidation into a network budget, reflection on finance management to prepare the final settlement, and information, support and assistance to partners (workpackage leaders, scientists, financial officers of partner institutes).
- Payment of pre-financing to partner institutes and management of the cash flow among the Co-ordinator and the partner institutes.
- Regular contacts with the EC legal and financial services to request amendments to the contract when a partner’s situation changed, or to obtain clarification and advice on EC financial rules.

Of particular note in Year 5 was Workpackage 1’s role in achieving the Network’s sustainability after the end of EC funding by setting up an organization and attendees.

The objective was to ensure an effective and efficient administrative financial legal and logistical support for the Network, and to ensure delivery of all documentation and information required to the EC. This will be undertaken to provide the following sub-objectives:

- to provide logistical legal administrative and financial management for the Network
- to coordinate and undertake the final stage of the project
- to deliver required reports and documentation to the EC
- to develop, apply and improve the working procedures
- to provide effective administrative and financial support for the local organizers of the 5th Annual Scientific meeting in El Escorial, Spain, in June 2009.

During Med-Vet-Net’s final year, an additional key objective was to achieve the sustainability plans through the maintenance of the present Network and by continuing and developing integration activities.

A further objective for Year 5 was to expand collaborative activities within the European Union and, where possible, participate in strategic European structures working in the field of food safety and animal health, such as the European Food Safety Authority, the European Centre for Disease Prevention and Control, EMIDA ERA-NET, and the European Technology Platform for Global Animal Health.

Logistical, legal, administrative and financial network for the Network

The objective was to ensure an effective and timely management structure of the Network.

Core meetings

The Governing Board, Co-ordinating Forum chaired by the Project Manager, Co-ordinator’s Representative and Administration Bureau formed Med-Vet-Net’s management structure. These groups continued meeting to consider and agree integration policies, sustainability achievements and the distribution of funds to partners, monitor progress and achievement; and plan and manage activities and their related budgets. Selected key scientists from the Co-ordinating Forum and workpackage leaders were also involved in the EC reviewers’ meeting in December 2008. In addition, several meetings were held to discuss the set-up of the Med-Vet-Net Association. Recurrent but face-to-face when required.

Table 1.1 summarizes the meetings primarily focused on management of the virtual institute that took place during Med-Vet-Net’s fifth year.

<table>
<thead>
<tr>
<th>Date</th>
<th>Meeting group and attendees</th>
<th>Local organiser</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–3 October 2008</td>
<td>Co-ordinating Forum</td>
<td>ISS. Venue: ISS Headquarters, Rome, Italy</td>
<td>Half-yearly meeting</td>
</tr>
<tr>
<td>6–7 November 2008</td>
<td>Governing Board</td>
<td>VLA. Venue: VLA Weybridge, UK</td>
<td>Yearly meeting</td>
</tr>
<tr>
<td>12 December 2008</td>
<td>EC Reviewers meeting</td>
<td>EC Research Directorate-General, Brussels, Belgium</td>
<td>Yearly meeting</td>
</tr>
<tr>
<td>23–24 April 2009</td>
<td>Co-ordinating Forum</td>
<td>SC Ltd. Venue: SC Ltd Headquarters, Milton Keynes, UK</td>
<td>Half-yearly meeting</td>
</tr>
</tbody>
</table>

Legal actions

Two important amendments to the contract were requested during Year 5: a request for a two-month extension of the project, and a request for inclusion of a third party to one contractor as a ‘spin-off’ company. The two-month extension request was justified by the fact that the Med-Vet-Net project end date of 31 August 2009 was during the summer period. Due to a lack of time it was not possible to organize a meeting between the EC project and the newly formed Med-Vet-Net Association before the summer period, and such a meeting was required to ensure an effective handover and continuation of activities.

Table 1.1. Meetings held in Year 5 relating to the management of the virtual institute.
The inclusion of a ‘spin-off’ company as a third party to a contractor enabled communication work to be undertaken for Med-Vet-Net and other FP7 projects. This is an example of how Med-Vet-Net was able to profitably develop the skills of its members so that they could put into practice the experience gained through Med-Vet-Net into other EC projects.

Financial management
From September 2008 the Administration Bureau undertook a review of finances under the supervision of the Governing Board and Co-ordinating Forum. The purpose was to calculate the appropriate JPAS budget amount to allocate within the Network to ensure the total EC grant (€14.4 million) was spent at the end of the project, to manage the level of self-financing to be supported by Med-Vet-Net, and to adopt a method of sharing the self-financing.

As per previous years, an intermediary financial report assessing the level of budget spending and advising on the necessary corrective measures was prepared at the mid-point of Year 5 and presented to the Co-ordinating Forum at its meeting in April 2009.

An additional interim financial report was prepared after 12 months of work in Year 5, and presented at the final meeting of the EC project in October 2009 — two months before the project’s official end date when all partners, except the three involved in the final reporting stage, were no longer incurring costs for Med-Vet-Net. This interim report enabled an assessment of expenditure to be made for the last period financial report and any corrections to the management of the final settlement after the end of the project. That is, how the amount of self-financing among partners was to be shared.

Administration section on Med-Vet-Net website
The Administration Bureau’s web pages were regularly updated with administrative and financial information including the minutes of core meetings. This information was made available to the whole partnership through the relevant workplace areas on the private website.

Co-ordinate and undertake the final stage of the project
At the financial and administrative level, the last stage of the project involved making the necessary amendments to the procedures implemented within the Network so far, so that the project could be achieved in optimal conditions. This is illustrated by the replacement of the workpackage contract used to pay pre-financings to partners, with a Partner Agreement, thereby allowing cash flow remainders from previously achieved JPA1 and JPA2 workpackages to be taken into consideration. It is also illustrated by the work on the distribution of self-financing among partners.

At the sustainability level, in order to move to a new era of Med-Vet-Net, a handover meeting was organized in Brussels from 5–6 October 2009, involving 45 participants. The meeting aimed to prepare the finalization of the EC project; formally hand-over the EC project’s activities; define the Med-Vet-Net Association’s first actions; and introduce the Association to targeted stakeholders including various EC officials, and the general public by means of press participation.

Deliver required reports and documentation to the EC
The Year 4 reports and JPAS plans were delivered with a slight delay that was previously agreed by the EC. All corrections and clarifications requested by the EC were provided then until validation of the reports by the EC.

Develop, apply and improve working procedures
Workpackage 1 undertook an initiative to explore and inventory the common facilities, particularly animal experimental facilities, throughout Med-Vet-Net with the aim of achieving a real sharing among, and access for partners to animal experimental facilities provided within the Network. To reach this objective however, some compulsory preliminary steps would have been required, including access/sharing of procedures and harmonization of experimental models available throughout the partnership. Regrettably, due to security considerations, not all institutes were able to provide the necessary information and the initiative was discontinued. Additionally, this kind of initiative would have required going well beyond a simple inventory of available animal experimental facilities.

Simultaneously to the Workpackage 1 initiative, a new FP7 EC infrastructure programme, the Network of Animal Disease and Infectiology Research Facilities (NADIR), in which several Med-Vet-Net veterinary institutes would be participating, was implemented. Given the NADIR project’s similarities to the Med-Vet-Net partnership and its very similar approach and objectives, it was considered that Med-Vet-Net had provided the impetus for moves towards sharing animal experimental facilities on a European basis. Consequently, the planned deliverable (D1.08) and milestone (M1.20) relating to animal experimental facilities were withdrawn.

Provide effective administrative and financial support for the 5th Annual Scientific Meeting
The Administration Bureau also participated in the organization of Med-Vet-Net’s 5th Annual Scientific Meeting, held in El Escorial, Spain, together with the local organizers, the Complutense University of Madrid; the Communications Unit as meeting facilities support and communications provider; and the Project Management team as scientific organizer. The role of the Administration Bureau, which was successfully undertaken, was to ensure good administrative and financial preparation for this major Med-Vet-Net event. The more than 200 delegates considered the Network’s last Annual Scientific Meeting a huge success.

Establish a sustainable structure for the Network
The Network’s sustainable structure was achieved through the registration in June 2009 of the Med-Vet-Net Association on zoonoses research, which will aim to ‘co-ordinate, increase, capitalize, and disseminate scientific knowledge or research activities on zoonoses with main emphasis on ‘food-borne zoonoses’.

As planned, two sub-committees were created. The first comprised members of the Co-ordinating Forum and the Governing Board of the Network of Excellence, and set out to define the aims and means of action of the Association (that is, why create an Association and what to do). The second sub-committee was made up of interested Legal Officers of partner institutes who collaborated in drafting the legal documents of the Association, that is the statutes and internal regulations. Based on the successful achievements of these two sub-committees, Med-Vet-Net’s Governing Board adopted the proposed model for a new Med-Vet-Net Association. The next step was to obtain commitment from the partner institutes, which was also successfully achieved — 14 of the 15 partners of the EC project agreed to become full members of the Association and continue to undertake common work and seek ways to integrate research.

To ensure the achievements made during the life of the EC project continue, the structure of the Association is a ‘light’ version of the structure of the Network of Excellence, and retains the same key people who were involved in Med-Vet-Net.

The creation of the Med-Vet-Net Association was a major outcome of the Med-Vet-Net Network of Excellence, and its progress has been closely followed by the European Commission. The Association was formally presented to the EC at a meeting in July 2009 with the Head of Unit and scientific officers on whom Med-Vet-Net was dependent, and to stakeholders and the press at an official handover meeting in October 2009.
Workpackage 2: Annual Activity Report

WP number 2
Title Strategic Scientific Integration
WP Leader John THRELFA LL
Name and Address Health Protection Agency (HPA)
61 Colindale Street
London NW9 SEQ
UNITED KINGDOM
Project Start date 1 September 2004
Project End date 31 October 2009

Progress summary

In Year 5, Workpackage 2, under the leadership of Professor John Threlfall of the Health Protection Agency in the United Kingdom (UK), continued to undertake all scientific aspects of Med-Vet-Net (MVN) project management rather than the financial project coordination. During the reporting year, 18 workpackages (WPs) comprising three overarching and 15 scientific WPs were functional. The fifth year of activity produced a vast amount of research data culminating in at least 78 peer-reviewed scientific publications, various new collaborations were initiated, 132 deliverables were produced and 111 milestones achieved, and we continued to spread knowledge on zoonotic diseases, particularly food-borne, to a general audience, scientists, and other stakeholders.

Three workshops or training courses were organized by MVN participants and 15 short-term scientific missions were completed. The Annual Scientific Meeting was held in El Escorial, Spain, from 3–6 June 2009, and brought together over 220 delegates who enjoyed seven keynote lectures, 54 oral presentations and more than 170 posters, as well as ‘state-of-the-art’ presentations by representatives of all the scientific workpackages.

In December 2008, Med-Vet-Net’s Year 4 activities were reviewed by two independent experts appointed by the European Commission (EC) through MVN’s EC Project Officer. The Network was rated as ‘good to excellent’ having “fully achieved its objectives and technical goals for the period, and even exceeded expectations”. Separate to the EC evaluation, the Network’s achievements were also reviewed, together with seven other Networks of Excellence (NoEs), by consultancy company COWI, which was appointed by DG-Research to review NoEs within the EU food quality and safety programme. Med-Vet-Net rated highly in most of the aspects reviewed, particularly bringing together researchers with a common interest, training, development of common research methods, joint execution of research projects, sustainability, and electronic communication.

Research co-ordination

Workpackage 2 continued to undertake all scientific aspects of Med-Vet-Net (MVN) project management except financial co-ordination, under the overall direction of Professor John Threlfall of the UK’s Health Protection Agency.

Eighteen workpackages were functional during Year 5, including the three overarching and 15 scientific WPs. Overall, progress was excellent. Most deliverables were met (see page 11), 78 peer-reviewed papers were published or are in press with several more submitted or in preparation, and two versions of the Campylobacter Risk Assessment Framework (CRAF) software package were launched (CRAF 1.0 in February 2009 and CRAF 2.0 in August 2009). To maintain and further develop CRAF, the Danish National Food Institute and the Dutch National Institute for Public Health and the Environment, in collaboration with Foodrisk.org, have submitted a proposal to the American National Institute of Food and Agriculture, an agency within the United States Department of Agriculture.

Inter-workpackage collaborations increased during Year 5, the fruits of which are described in the individual workpackage reports. Additionally, three joint workshops were held in the fields of antimicrobial resistance, source attribution and prioritization of food-borne and zoonotic hazards, and Campylobacter. Representatives of all WPs presented brief summaries of their achievements in three open sessions at the Annual Scientific Meeting in June 2009, which were well received by delegates.

Development of research skills continued to be an area of high priority during the reporting year with three workshops/training courses organized by MVN participants, and a further 15 short-term missions (STMs) completed. In all, these activities resulted in training for over 100 scientists both within and external to the Network. Additionally in Year 5, there were 22 ‘internal’ research based workpackage meetings involving over 300 scientists of whom at least 100 were from outside Med-Vet-Net.

Results from MVN scientific workpackages were also disseminated at 70 external meetings to an overall audience of well over 1,500 people from within and outside Europe.

As in previous years, Workpackage 2 supported inter-network communications and collaborated closely with other EU-funded NoEs, in particular EPZONE, EADGENE and ERA-Net PathoGenOmicS. In respect of the latter NoE, Med-Vet-Net’s Project Director was invited to attend an ERA-Net meeting in May 2009 to present aspects of Med-Vet-Net and advise on actions taken by the Network to ensure its sustainability.

Workpackage 2 has had overall responsibility for the co-ordination, collation and delivery of the Periodic Activity Reports from all workpackages.

The document outlining the scientific planning of the Fifth Joint Programme of Activities for the period 1 September 2008 to 31 August 2009 was accepted by the MVN European Commission Project Officer in April 2009, and Med-Vet-Net’s Annual Report: 1 September 2007–31 August 2008,
was published and distributed in May 2009. An account of the Network’s activities, its achievements and potential applications was also included in ‘Emerging Epidemics Research: EU-funded projects 2002–2008’, published by the European Commission’s Directorate-General for Research, in September 2008.

Of the expected 142 deliverables due in Year 5, 132 (93%) were fully achieved, one partially achieved (0.7%), three (2.1%) were achieved with modifications, two (1.4%) were withdrawn and only four (2.8%) failed. Of the 117 expected milestones, 111 (95.2%) were fully achieved, one (0.8%) was partially achieved and four (2.8%) failed. Figure 2.1 illustrates the outcomes.

The scientific outputs of various workpackages has been reflected in the publication in this reporting year of at least 78 papers published or in press in peer-reviewed journals, bringing to more than 150 total number of publications credited to Network. Several other papers are either in preparation or have been submitted for publication.

Collaborations between scientific workpackages continued to develop and become more evident in terms of transparency and outcome during Year 5. Six workpackages with common interests held joint meetings — WPs 30 and 34 (Clostridium), WPs 21 and 29 (antimicrobial drug resistance), and WPs 23 and 32 (Prioritizing food-borne and zoonotic hazards at the EU level/public health surveillance for food-borne infections: application of seroepidemiology to validate the surveillance pyramid). Other workpackages also held joint meetings with the Special Interest Groups (SIGs), for example WPs 26 and 33 with the Host-Pathogen Interactions SIG. The joint meetings ensured a high level of interactions between participants and have had major benefits for the Network in terms of exchange of ideas, technologies and the exploitation of methods and analyses. Additionally, for several workpackages the free exchange of information systematically improved the outputs. For example, information from WP21 was passed to WP26 to facilitate the inclusion of genetic information on the arrays used for virulotyping of Campylobacter, and results from subsequent analyses undertaken by WP26 were fed back to WP21.

In several instances inter-workpackage collaborations have resulted in the publication of peer-reviewed papers with joint authorships; other papers with evidence of such collaborations are currently under review following submission. Another outcome of the workpackage collaborations has been the informal linkage of scientists from different WPs to develop and submit applications for funding, which will continue after Med-Vet-Net ceases.

Durable integration
A key Network activity has been ensuring its future sustainability after the cessation of EU

Table 2.1. Workshops held during Year 5.

<table>
<thead>
<tr>
<th>Workshop</th>
<th>Month and year</th>
<th>Organising partner and location</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioinformatics for Laboratory scientists</td>
<td>November 2008</td>
<td>HPA, London, UK</td>
<td>To improve awareness of new bioinformatic approaches and tools for microbial genome comparisons relevant to assay design and characterization, and to give practical experience in the use of these tools</td>
</tr>
<tr>
<td>Deriving Disability Weights (DDW)</td>
<td>November 2008</td>
<td>RIVM, The Netherlands</td>
<td>Provision of training in reconstructing the surveillance pyramid and assessing the burden of food-borne disease</td>
</tr>
<tr>
<td>Development of a European consensus framework for Campylobacter in broiler meat</td>
<td>February 2009</td>
<td>BFR, Berlin</td>
<td>Provision of training in working with the consensus framework for Campylobacter risk assessment</td>
</tr>
</tbody>
</table>
A comprehensive review of the meeting was presented in the September 2009 issue of Med-Vet-Net News, the Network newsletter prepared and distributed by the Communications Unit.

**Special Interest Groups**

In Year 5 enhanced knowledge management within Med-Vet-Net continued within two Special Interest Groups (SIGs) — Host-Pathogen Interactions, which was initiated in Year 3; and the Wildlife-related Emerging Diseases and Zoonoses (WiREDZ), initiated in Year 4. These SIGs have been encouraged to maintain and develop their collaborations with the long-term aim of attracting future funding in their specialist areas within FP7.

**Host-Pathogen Interactions**

The Host-Pathogens Interaction SIG continued to interact closely with Workpackage 33, and also facilitated numerous meetings, workshops and STMs within and outside Med-Vet-Net. Additionally, the contacts generated through the group facilitated a joint zoonoses meeting in June 2009 in collaboration with the University of Surrey and North Carolina State University. As well as providing a platform for networking in the area of host-pathogen interactions, the SIG assisted with the drafting of a number of EU and research council grant proposals.

**Wildlife-related Emerg Dngs & Zoonoses**

By August 2009, WiREDZ had encouraged 223 scientists from 35 countries to register on the WILDLIST (see www.medvetnet.org/wildlist). A SIG meeting held in Budapest in December 2008 attracted 22 delegates from 13 countries to discuss WiREDZ work in European countries.

In particular, the countries where systems and approaches to WiREDZ work are not currently known were discussed.

The achievements of the WiREDZ SIG have been instrumental in developing a basis for others to progress the foundation of wildlife disease reporting and collaborative networks across Europe.

**Campylobacter workpackage**

Workpackage 34, a new scientific workpackage targeted at the prevention and control of Campylobacter in broilers commenced in September 2008. Over the course of the past 12 months Workpackage 34, which emanated from the SIG concept, developed an electronic communication system for debate on Campylobacter control throughout Europe.

**Contributions to external meetings**

In addition to the activities outlined, Workpackage 2 contributed to three international symposia and five national and international seminars and workshops expounding Med-Vet-Net’s activities, its contribution to EU research on zoonoses, and the actions taken to ensure sustainability after the cessation of EU funding.

---

### Knowledge management

The 5th Med-Vet-Net Annual Scientific Meeting was held from 3–6 June 2009 at the Euroforum Infantes conference centre, located next to the El Escorial Monastery, a UNESCO World Heritage site near Madrid, Spain.

The meeting was locally organized by the Med-Vet-Net partner institutes in Spain — Complutense University Madrid and the Institute of Public Health Carlos III. Over 220 delegates attended the meeting, including scientists representing all of Med-Vet-Net’s partner institutes along with many external delegates, to share knowledge about controlling and preventing zoonotic diseases and to develop greater external, worldwide collaborations. In total, seven keynote lectures, 54 oral presentations and over 172 posters were presented to delegates. Representatives from each of the 15 scientific workpackages also presented their results and achievements.

The seven keynote lectures were given by: Brett Finlay — Pathogenic Escherichia coli: contribution of the pathogen, host and microbiota; Fernando Baquero — Ecogenetical dynamics of bacterial pathogens; Peter Teunis — Epidemiology and risk assessment: an unsettled union?; Peter Mertens — The epidemiology and spread of the Bluetongue virus across Europe: the impact of climate change, insect vectors and vaccination; Carmen Buchrieser — Biodiversity and evolution of pathogenic Listeria: a genomics view; Patrice Courvalin — Evolution and dissemination of glycopeptides resistance operons; and José Manuel Sánchez-Vizcaíno — Epidemiological tools for surveillance. Summations of the presentations have been kindly provided for the 2009 issue of Microbiologist and online at www.sfam.org.uk.

The Annual Scientific Meeting was regarded as an outstanding success that clearly showcased Med-Vet-Net’s achievements. The efforts of all involved in the smooth running of the conference as well as all those who participated was fully acknowledged by the Med-Vet-Net Project Director, together with the generous financial support provided by Biorad, Fort Dodge, Merial, Anaporc, Biomérieux, and the Spanish Ministry of Environmental, Rural and Marine Affairs.

**WiREDZ Special Interest Group has encouraged 223 scientists to register on the WILDLIST for future collaboration in wildlife zoonotic disease.**

---

**Figure 2.2. Short-term scientific missions awarded during Med-Vet-Net. In Year 5, 15 STMs were granted.**
Workpackage 3: Annual Activity Report

WP number 3
Title Spreading Excellence
WP Leader Peter SILLEY
Name and Address Society for Applied Microbiology (SfAM)
Bedford Heights
Brickhill Drive
Bedford MK41 7PH
UNITED KINGDOM
Project Start date 1 September 2004
Project End date 31 October 2009

Progress summary

Workpackage 3, administered by the Communications Unit, delivered and shared information about Med-Vet-Net both internally with the Network’s scientists, and with external stakeholders such as other researchers, policy makers, industry, farmers, food producers, and the general public.

During Year 5, the Communications Unit continued to produce a regular newsletter (Med-Vet-Net News) as an e-bulletin, distributed to over 1,500 people in 72 countries. Articles and profiles from Med-Vet-Net News were also placed on the public website providing continual change to the site.

Two modules of the Science Communication Internship were held in Year 5 resulting in two more interns completing the entire four modules following their participation in Module 3, and four new participants completing an offering of Module 1. In total, 21 scientists participated in at least one of the Med-Vet-Net science communication modules, and six completed the entire course.

The Communications Unit continued to support the Network by assisting with the promotion and organization of the Annual Scientific Meeting in El Escorial, Spain. The Unit also hosted the Co-ordinating Forum in Milton Keynes in April 2009, and supported Project Management with the production of the Annual Report and Final Scientific Report.

A significant output this year was the stakeholder report, ‘Building a European Community to Combat Zoonoses’, which provides an easy-to-read summary of the entire project’s achievements.

Spreading the excellence of the Network, which involved disseminating knowledge among Med-Vet-Net scientists and externally to relevant stakeholders, has been integral to the success and future durability of Med-Vet-Net. The Society for Applied Microbiology (SfAM), which oversees the Med-Vet-Net Communications Unit through a third-party agreement with the new company, Science Communications Ltd, has held responsibility for this dissemination task. In addition, a Website Support Group, based at Sweden’s National Veterinary Institute (SVA), developed and maintained website communications and provided database support for the Network.

The Communications Unit’s role was to use the most effective mechanisms to deliver and share Med-Vet-Net information through the web and media (television, radio and the press), with the general public and stakeholders including policy makers, industry, farmers, food producers, and other researchers.

Publications

E-bulletin/monthly newsletter

Med-Vet-Net News, the Network newsletter was produced regularly during Year 5 and distributed as an e-bulletin with links to the full articles on the public website. At 1 October 2009 there were 1,533 newsletter subscribers in 72 countries. Four newsletters (March 2009, June 2009, September 2009, and October 2009) were produced during the reporting year covering the following topics:

- Year 4 scientific achievements
- 5th Annual Scientific Meeting (El Escorial, Spain) — advertising, highlights, overview and pictorial summary
- Science Communications Internship
- communicating science with the public
- Workpackage 34 — the prevention and control of Campylobacter in broilers
- the Med-Vet-Net Association — establishment, launch and developments
- Workpackage 21 and 29 joint meeting in Paris
- WIREDZ final meeting
- Med-Vet-Net achievements report
- Med-Vet-Net people
- other general news and announcements
- external congress.

External articles

The Communications Unit continued to provide articles to magazines and trade journals when the scientific workpackages generated results and reached milestones. Over the past year, the Communications Unit contributed to the following publications:

- Med-Vet-Net News — e-bulletin and PDF
- Double-page spread in the September 2008, December 2008, March 2009, June 2009 and September 2009 issues of Microbiologist, the magazine of the Society for Applied Microbiology, which is distributed to 1,400 members in 75 countries
- The Parliamentary Monitor, Issue 289, May 2009 (United Kingdom)
- EU-AgriNet in October 2009 — FP6 Project Success Stories (see http://ec.europa.eu/research/ agriculture/success_med-vet-net_en.htm)
- Vetro, the internal magazine of the Veterinary Laboratories Agency, in November 2009.

Press releases and news coverage

Over the five years of Med-Vet-Net, the Communications Unit established a diverse and comprehensive list of contacts, including journalists, that has been maintained and expanded.
In Year 5, press releases to promote the 5th Annual Scientific Meeting in Madrid in June 2009 were prepared and distributed via the Alphagalileo website. A number of e-bulletins were also sent to people who showed an interest in the conference, and to registered delegates.

News coverage was also sought for the Med-Vet-Net handover meeting in Brussels on 6 October 2009. Press releases were posted to the online press sources, Alphagalileo and EurekaAlert, and individual science journalists were identified through contacts as well as through the MediaAtlas database.

Annual and final scientific reports

The Communications Unit assisted Med-Vet-Net Project Management with the production of the Network’s fifth annual report and the final scientific report to the European Commission. The Unit was responsible for the design, layout, image acquisition and copyediting of the reports. The reports are printed on 55% recycled, 45% from sustainable forest, chlorine-free paper, and distributed to key stakeholders via partner institutes, and to over 500 contacts from the Communications Unit’s Stakeholder Database.

Stakeholder report

A glossy, magazine-style 34-page report entitled ‘Building a European community to combat zoonoses’ was produced by the Communications Unit in 2009. The report showcases the results and achievements of Med-Vet-Net workpackages and special interest groups, and is divided into sections. The first section, ‘Med-Vet-Net: Why and How?’, explains the background of the project and how it worked, and is followed by sections on the specific zoonoses studied — bacteria, viruses, and parasites. The thematic areas of Epidemiology and Surveillance, Host-Microbe Interactions, Antimicrobial Resistance, Detection and Control, Risk Research and Special Interest Groups describe the scientific achievements in a less technical language, and the final sections on Building a Successful Network and Nurturing Scientific Learning describe initiatives in communication and training.

The report was officially launched at the formal announcement of the Med-Vet-Net Association in Brussels on 6 October 2009. It is available for download on the Med-Vet-Net public site and will be distributed in hard copy over the coming months by the individual institutes and the Communications Unit.

Websites

Both the public and private websites are managed using a content management system hosted on a server at the National Veterinary Institute (SVA) in Sweden. The public website (http://www.medvetnet.org) was regularly updated during the year with articles and profiles from Med-Vet-Net News, as well as information relating to the 5th Annual Scientific Meeting, the launch of the Med-Vet-Net Association and other relevant news prepared by the Communications Unit. A calendar was also maintained with relevant conferences and worldwide events.

Statistics recorded on the public website since its inception indicate that visits have continually increased over the five years of the project, peaking at 37,968 visits in May 2009 during the organization of the 5th Annual Scientific Meeting. The Med-Vet-Net website also hosts a number of online databases including: EBLU (Lyssavirus), CampyNet, the Stakeholders Database, and the WILDLIST. Discussions are underway to move WILDLIST (part of the special interest group, Wildlife-related Emerging Diseases and Zoonoses) to the new FP7 project, WildTech, for continued support.

The Med-Vet-Net Association is to consider options for its new website, and the transfer of files into an archive. In the meantime, the SVA will continue to maintain both the public and private websites on the existing server.

Other Web 2.0 and online resources

The WebEx online meeting tool continued to be used for Med-Vet-Net and associated meetings, particularly relating to the organization of the 5th Annual Scientific Meeting. The Moodle subsite was also utilized as a virtual learning environment, particularly for the Science Communication Internship.

RSS (Really Simple Syndication) feeds on the public site also enabled visitors to subscribe to the Med-Vet-Net newsletter and website news items.

Science Communication Internship

The Science Communication Internship was open to students, researchers and staff of Med-Vet-Net partner institutes with all accommodation, travel and associated expenses funded by the Network. External candidates from related zoonoses institutes outside of Med-Vet-Net were also invited to participate at their own cost. PhD students of Med-Vet-Net and partner institutes were encouraged to attend Module 1 of the internship as it was viewed that the communication skills gained would benefit their current research, and provide essential skills for communicating and disseminating research in their future careers.

The Communications Unit offered two modules of the Science Communication Internship in Year 5.

Module 3 — Engaging Stakeholders

Module 3 ran for two weeks from 4–14 November 2008 with two Med-Vet-Net interns from Spain and the United Kingdom (UK) and one external participant from Norway attending. With Module 3 under their belts, two of those interns had successfully completed all four modules of the internship.

This third module examined communications with scientists, decision-makers, government, industry, non-government organizations, museums, schools and the public. Interns gained skills in influencing, networking, writing proposals, and organizing conferences and events.

Module 1 — Science Communication: Why and How?

Module 1 was held for the third and final time from 4–15 April 2009 with four participants: two from Denmark, and one each from Poland and the UK. Activities in the Module 1 programme included an introduction to science and the media, digital photography, public speaking and presentation skills, e-communications, and writing skills.
Final status of modules and completion of full internship

The Science Communication Internship began in 2005 in Year 2 of Med-Vet-Net and finished with the final module in April 2009. In that time, a total of 21 scientists from eight partner institutes (and one external), representing 10 countries (Spain, UK, Poland, France, Italy, Denmark, the Netherlands, Norway, Brazil and India) participated in the internship.

Of the 21 participants, four interns completed all four modules and two interns completed the entire initial 12-week course. Figure 3.1 shows the breakdown of participants between Med-Vet-Net partner institutes.

At the conclusion of Med-Vet-Net:
- six interns completed the entire internship
- one intern completed three modules
- four interns completed two modules
- ten interns completed one module.

Meetings, conferences and networking

It was imperative that the Communications Unit established relationships widely within the Network and externally with all interest groups and stakeholders. This was achieved through attendance at various conferences, and meetings with interested groups and stakeholders.

Annual Scientific Meeting

The 5th Med-Vet-Net Annual Scientific Meeting was held at the Euroforum Infantes conference centre in El Escorial, Spain from 3–6 June 2009. As in previous years, the Communications Unit played an integral role in the organization of the conference and was responsible for administering the online registration and abstract submission systems; production of delegate information and packs, conference posters, abstract book, programme; and all marketing material. The Communications Unit also prepared press material such as media releases, organized conference photos, and assisted with set-up, room preparations and the registration/helpdesk during the event. The Unit was also instrumental in liaising with industry sponsors and exhibitors.

CommNet

CommNet is an informal network of communication managers in research consortia that receive EU funds in the field of Food Quality and Safety (Priority Area 5) in both FP6 and, more recently, FP7. The members of CommNet address common issues related to the design and implementation of communication activities within their projects, share best practice, and run in-house training during the biannual meetings. Members of the Communications Unit have been actively involved in CommNet since its inception.

CommNet organized a joint event ‘European Food Science Day’ on 18 November 2009, aimed at providing results from FP6 and FP7 projects in food quality and safety. Med-Vet-Net was unable to participate in this event as the Network officially concluded on 31 October 2009.
Workpackage 6: Annual Research Report

WP number 6
Title Use of geographical information systems (GIS) in epidemiological analyses of zoonotic diseases
WP Leader Steen ETHELBERG
Name and Address Statens Serum Institut (SSI) Artilleirejvej 5 2300 Copenhagen S DENMARK
Project Start date 1 September 2004
Project End date 31 October 2009

Progress summary
Workpackage 6 focused on the geographical analysis of important zoonotic disease agents in Europe using geographic information systems (GIS). During the past year, workpackage members completed a number of analyses on several different disease agents.

A computer tool to find persistent clustering of patients over time was developed and used to analyse the occurrence of clusters of Campylobacter in Denmark. Another project, focusing on 10 selected Salmonella serotypes, employed several different spatial analysis methods to analyse a large dataset of salmonellosis from several European countries. The project team overcame the common difficulty of comparing data between countries by analysing the proportional occurrence of each serotype within each district of each country, rather than comparing incidences. A number of outbreaks, including cross-border outbreaks, and other hotspot or cold spot signals were detected.

A third project examined the relationship between human cases of Verocytotoxin-producing Escherichia coli (VTEC) and the distribution of the bovine population. Separate analyses undertaken in five different countries demonstrated that, in all five, there was an increased risk for people living near cattle farms, suggesting that direct transmission from cattle to humans is a significant route of VTEC infection.

A fourth project investigated the geographical distribution of human Q-fever cases in Denmark between 2006 and 2008. In this investigation living in close proximity to cattle farms was found to be an important risk factor for acquiring infection.

Background
Geographic information systems (GIS) are used to construct maps and analyse spatial data. A GIS captures, stores, analyses, manages, and presents data that refers to, or is linked to, a geographic location. GIS applications are tools that allow users to analyse spatial information, create interactive queries, edit data, produce maps, and present the results of all these operations. It is a technology with many practical applications, such as in urban planning, marketing or logistics, and it is also used within a number of scientific disciplines. Increasingly, the term "GIS" also encompasses statistical analyses, which can be quite complex.

Epidemiologists often need to describe the distribution of their studied populations in space. To plot disease cases on a map has always been part of epidemiology, but with GIS this can be done much faster and in a more exploratory way than previously, helping to identify associations not otherwise easily noticed. In addition, spatial statistical analyses tools offer new possibilities for epidemiological studies.

Within the epidemiology of zoonotic diseases and food-borne infections field, GIS may be very helpful for basic surveillance and outbreak investigations, and may also be used to analyse the distribution of zoonotic agents and identify risk factors for zoonotic diseases. Advanced spatial-temporal analysis tools also enable various types of cluster analyses, as well as examinations of possible associations between different factors (for example an association between human disease and areas where a particular food item is consumed).

During the reporting year, Workpackage 6 focused on four different scientific projects that all involved geographical analyses on aspects of different types of zoonotic pathogens. The projects were defined to ensure they comprised elements of human and veterinary medicine, and had an international impact. Following is a brief description of each of the four completed projects:

• A cluster-analysis of campylobacteriosis in Denmark
• A geographical analysis of Q-fever cases in Europe.
• Cross-border analysis of the geographical distribution of salmonellosis and campylobacteriosis in Denmark

For basic surveillance and outbreak investigations, and may also be used to analyse the distribution of zoonotic agents and identify risk factors for zoonotic diseases. Advanced spatial-temporal analysis tools also enable various types of cluster analyses, as well as examinations of possible associations between different factors (for example an association between human disease and areas where a particular food item is consumed).

Objective
Promote the use of Geographical Information Systems (GIS) and build up capacity among workpackage members through conduction of small-scale scientific projects, as follows:
• perform a GIS analysis of human VTEC cases and bovine populations in Workpackage member countries
• perform a GIS analysis of the bovine population and human Q-fever, Campylobacter, enteropathogenic Escherichia coli and attaching and effacing E. coli cases in Denmark and if feasible, also in one or more other Workpackage member countries
• construct a dynamic salmonella atlas
• perform a spatio-temporal analysis of the distribution of human salmonella cases in Europe.

Key achievements to date
• Analysis of the occurrence of clusters of Campylobacter in Denmark.
• Cross-border analysis of the geographical distribution of Salmonella cases in Europe.
• Identification of the relationship between locality of VTEC patients and the vicinity of cattle farms in five countries.
• Identification of the relationship between locality of Q-fever patients and the vicinity of cattle farms in Denmark from 2006 to 2008.

Because campylobacteriosis is generally considered a mild disease, surveillance data reflect the likelihood with which doctors will request examination for the disease, in addition to the real occurrence. To address this question of possible examination bias, data for general diarrhoea (that is, all samples submitted for microbiological examination) were analysed in addition to the Campylobacter data. We obtained information on the FY-co-ordinates of the addresses of all people living in the Danish county of Funen, and located the two types of disease cases within this population for the period 1995–2003. We used a modified form of local Moran’s I to test if areas with similar incidence rates occurred next to each other in space (and time), and compared the observed clusters with simulated clusters. The analysis tool was found to reflect the distribution of the underlying population, infections from ‘environmental’ sources are likely to be geographically clustered and also persist over time. As such, this project aimed to develop a tool to find persistent clustering in historic surveillance data of campylobacteriosis in Denmark.
made using Netlogo software, which has good visualization features.

The results showed a significant persistent clustering of Campylobacter incidence rates in the western part of Funen. The underlying causes of the observed clustering are not known and will require further examination, but may be partially explained by an increased rate of stool sample submissions by physicians in the area. We also developed a tool that we demonstrated as suitable for analyses of geographical clusters, which may form a basis for further epidemiological examinations to cast light on the infection sources. This study was published in the International Journal of Health Geographics.

**Association between VTEC cases and cattle farms in five European countries**

This project concerned Verocytotoxin-producing *E. coli* (VTEC) which is a diarrheagenic intestinal pathogen receiving particular interest because of its capacity to induce the life-threatening condition, haemolytic uraemic syndrome (HUS). VTEC has a natural reservoir in ruminants but the route of transmission to humans has not been fully elucidated. This project examined whether the spatial pattern of human VTEC cases in five different European countries was related to the spatial location of cattle farms, which would suggest direct transmission to humans from live animals or their faeces.

The project used existing recorded data on the demographics, VTEC patients and cattle farms in England/Wales, The Netherlands, Italy, Sweden and Denmark. Because the geographical spread of VTEC cases and cattle farms varied, the analysis was performed according to geographical administrative units. The types of available data differed between the participating countries, each country conducting its own analyses. To exemplify those national differences, some countries had data on all types of VTEC, some on *O157*, while one country collected data only on HUS cases. Similarly, the degree to which data could be geo-coded varied from exact XY-co-ordinates on human cases and cattle in one country to aggregation on smaller or larger areas in others. Different types of GIS methods and spatial statistical analysis were used to select the data, including buffer analysis, Moran’s I, and Poisson and logistic regression models.

The national analyses all identified an association between patients living in proximity to cattle farms and the risk of VTEC (or HUS) infection. Additional aspects were examined in some of the analyses — some countries found that the risk was increased only in young children (who may, because of their behaviour, be more likely to be exposed to animal faeces than adults); and one country conducted a parallel analysis of the risk of Q-fever cases and farms was evaluated by calculating the incidence of disease (and of those that tested negative), for each buffer zone. The incidence of disease was much higher in the buffer zones located close to cattle farms compared to those living further than two kilometres from a cattle farm. Living close to cattle farms was thus found to be strongly associated with disease.

It should be noted that the people who were tested for the disease were primarily farmers or otherwise occupationally exposed. Ultimately, it was difficult to separate the effect of those biased diagnostics from any real increased risk. Further results are presented in Deliverable D06.12.

**An analysis of clusters for 10 serotypes of Salmonella in Europe**

In this project we performed a cluster analysis of reported human *Salmonella* cases in a number of European countries. The aim of the project was primarily to examine the feasibility of different established spatial statistical methods for undertaking such an analysis involving a very large dataset. Our secondary aim was to find epidemiologically meaningful clustering that might be useful for *Salmonella* experts.

We analysed the geographical clustering of 10 selected *Salmonella* serotypes from 2001 to 2006. The serotypes were selected either because they were the most frequent (the top five), or because they were implicated in major outbreaks in Europe during the study period. Data from the national surveillance systems of 25 European countries were aggregated into geographical districts. Most of the data were delivered via Internet but some countries, such as Germany, supplied data directly.

Because surveillance systems vary between countries, direct comparison of incidences will inevitably lead to a biased analysis. We overcame this bias using an alternative approach of analysing the annual proportion of cases with each serotype out of all reported cases in each geographical district. The cluster analysis was performed using *SatScan* and Gi-star spatial statistics. Global clustering and geographical mean analyses were also performed. The results were presented as a series of hotspot and cold spot maps, which are available in a report presented as Deliverable D06.13.

The maps identified significant clustering of the annual proportional distribution of all serotypes in all six years, ranging from hotspots involving few districts in a single country to one or more coherent hotspots involving multiple districts in up to seven different countries. Relatively identical clusters in size and shape were identified for *Salmonella* serotype Enteritidis, *S. Typhimurium*, *S. Infantis*, *S. Virchow*, *S. Napoli* and *S. Stanley* during the six years, while the annual pattern of *Salmonella* *Bovis*-morboficanicus, *S. Agona*, *S. Hadar* and *S. Oranienburg* varied as the result of temporal outbreaks. A relative risk exceeding 10 was found for *S. Bovis*-morboficanicus, *S. Infantis* and *S. Hadar* in individual years, while high relative risks ranging from 13 to 117 were found for large clusters of *S. Stanley* in Scandinavia and for *S. Napoli* in western France. With this analysis we located several notable clusters, but to determine the possible significance of such clusters subsequent epidemiological evaluation is required.

A structured cluster analysis has not previously been performed on a trans-national scale. The results reported here indicate that *Salmonella* data can be compared meaningfully between countries using proportional analysis, and that geographical analysis may prove to be a valuable tool in the international surveillance of salmonellosis in Europe.

**Workshops and meetings**

In December 2008, Dr. Adnan Younas from the Veterinary Laboratories Agency in the UK spent one week at the SS as part of the ongoing work on the interactive *Salmonella* Atlas. Workpackage 6 participants from RIVM hosted a two-day workshop for both workpackage members and invited experts. The workshop consisted of presentations of results from the different workpackage project groups, and sub-project group meetings. In addition, presentations on several other subjects within the field of geographic analyses and zoonotic diseases were provided. A Workpackage 6 meeting was also held during the 5th Med-Vet-Net Annual Scientific Meeting, in El Escorial, Spain in July 2009.

![Figure 6.2. Results from structured cluster analysis indicates that Salmonella data can be compared meaningfully between countries.](image)
Workpackage 21: Annual Research Report

WP number 21
Title Molecular epidemiology of Salmonella Genomic Island 1 (SGI1)
WP Leader Dik MEVIUS
Name and Address Central Veterinary Institute (CVI)
PO Box 2004
8203 AA Lelystad
THE NETHERLANDS
Project Start date 1 March 2006
Project End date 31 October 2009

Progress summary
Findings generated by Workpackage 21 have been presented at more than 10 international meetings, and an EU Safefood-ERA project (commencing in September 2009) has been granted at no additional cost to Med-Vet-Net.

In the final year, the focus of Workpackage 21 research was to complete all planned activities, thereby producing measurable outputs. The workpackage team investigated the mobilization of Salmonella Genomic Island 1 (SGI1), and assessed the contribution of helper plasmids, and is in the process of preparing two publications based on the strain collections obtained within the workpackage. The findings generated by Workpackage 21 have also been presented at 10 international meetings, and an EU Safefood-ERA project, commencing in September 2009, has been granted at no additional cost to Med-Vet-Net.

Virotyping of SGI1-positive strains, selection and characterization of MDR Enterobacteriaceae
Virotyping of SGI1-positive isolates has been completed and a publication is in preparation. The results have also been added to the virotyping database established by Workpackage 26.

Based on the analyses of SGI1-positive isolates undertaken in the previous year, and the training course on molecular analysis of multi-drug resistant (MDR) Enterobacteriaceae, each partner institute selected SGI1-positive isolates to analyse locally or in collaboration with workpackage partners.

For Salmonella serotype Newport, 50 strains were selected from more than 200 strains isolated in nine European countries in the period 2005–2008. Analyses on the presence and variants of SGI1 in these isolates have been completed. The results of these analyses are summarized in the following sections.

Mobilization studies of SGI1 in Hungarian S. Typhimurium DT104 strains, and search for new helper plasmids
SGI1 can be mobilized between chromosomes with the help of a mobilization or ‘helper’ plasmid. For mobilization and transmission of SGI1, the helper plasmid R55 (supplied by Dr. Axel Cloeckaert of the French National Institute for Agricultural Research) was introduced into 12 SGI1-positive Salmonella Typhimurium strains to investigate its mobilization properties. The results demonstrated a relatively high conjugation frequency, indicating the helper plasmid was highly functional for mobilization of SGI1 in the genetic backgrounds tested.

Next we investigated if self-constructed transposons and other conjugative plasmids could also facilitate mobilization, however, positive results were not obtained suggesting that no other helper (or trapping) plasmid systems can replace plasmid R55 for mobilization of SGI1.

Other plasmids resembling R55 were then tested for conjugation. These plasmids, belonging to the “IncA/C” group, were supplied by Dr. Fernando de la Cruz of the University of Cantabria, Spain. Their characteristics are presented in Table 21.1. The results show that only plasmid 3181TcA1 facilitates mobilization of SGI1, and some of its genes have been correlated to this property.

Excision of SGI1 and analysis of transferred SGI1 segments by sequencing and nested PCR of flanking regions
In order to demonstrate the activity of site-specific recombination systems in particular SGI1-positive isolates, the excision of SGI1 from the chromosome was examined by attB/PCR. Problems with attB detection using regular PCR were solved by a newly developed nested PCR (Figure 21.1) that detected the gene in 17 S. Typhimurium DT104 strains isolated in Hungary. These were tested again after 43 passages (representing about 340 generations) to assess attB stability. A PCR was set up to specifically detect excised SGI1 (using thdF and the cryptic retrop phase as primer targets). This test was positive, illustrating that the site-specific recombination system was active during passage in these isolates.

Pathogenic potential of SGI1 deletion mutants of S. Typhimurium in vivo and in vitro
The role of SGI1 in pathogenesis is not completely understood so a deletion mutant was constructed and tested for its pathogenic potential. Due to inherent antibiotic resistances of the strains used, a simple knockout procedure with positive selection could not be followed. Homologous recombination was therefore applied to three different strains resulting in the desired SGI1 deletion mutants. Excision was confirmed by PCR.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Host</th>
<th>Marker</th>
<th>Conjugation</th>
<th>attB(PCR)</th>
<th>SGI1 Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>3181TCA1</td>
<td>E. coli</td>
<td>ApCm/FioSMTc</td>
<td>+</td>
<td>+</td>
<td>+*</td>
</tr>
<tr>
<td>HRC047</td>
<td>E. coli</td>
<td>Ap</td>
<td>-</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>HRC049</td>
<td>E. coli</td>
<td>Ap</td>
<td>-</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>HRC050</td>
<td>Salmonella</td>
<td>Sul</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HRC052</td>
<td>Salmonella</td>
<td>Sul</td>
<td>nd</td>
<td>a†</td>
<td>+†</td>
</tr>
</tbody>
</table>

nd = non detectable due to absence of appropriate AR-markers for selection of transconjugants .
* detected by PCR specific for DRRSt.
† detected by PCR specific for attB.

Objectives
The major objective of Workpackage 21 was to study the distribution and characteristics of Salmonella Genomic Island 1 (SGI1) in enteric bacteria (Salmonella, Shigella, Escherichia coli, and others) in a large collection of animal and human isolates. The resultant strain collection and the electronic database storing specific genetic information provided a basis for future virotyping and risk assessment of isolates harbouring SGI1.

Key achievements to date
- Workpackages 21 and 29, which both deal with antimicrobial resistance, held their final joint meeting from 14–16 April in Paris. Each workpackage presented its results and partners discussed how to best disseminate the results to the scientific community.
- The complete nucleotide sequence of the quinolone resistance gene qnrB19 from ColE-derived plasmid pSG115 has been deposited in GenBank. A manuscript including all the information on this plasmid has been accepted for publication to the Journal of Antimicrobial Chemotherapy.
- Helper plasmid for SGI1 mobilization was identified and active mobilization of SGI1 was demonstrated during in vitro passage of Salmonella isolates.
- Salmonella strains of variable genetic background, antibiotic resistance profiles, and displaying variation in SGI1 make-up have been collected, and characterized. These are available for reference purposes. The clonal origin of a multi-resistant clone with ACCuSrt phenotype, recently emerging in Italy, has been elucidated.
- An improved fingerprinting assay has been developed for SGI1 characterization based on RT-PCR using SYBR-green and a High Resolution Melting technique.
- As a direct spin-off from Workpackage 21, a European SafeFood-Era project (scheduled to start in September 2009) was granted for 2009/10, at no cost to Med-Vet-Net.
and resistance determinants. All isolates were tested for antimicrobial susceptibility by broth microdilution (Clinical and Laboratory Standards Institute). Typing experiments were performed using XbaI-PFGE, plasmid analysis and Multi-Locus VNTR Analysis (MLVA). Characterization of virulence, resistance determinants and class 1 and 2 integrons was achieved by PCR/sequencing and DNA-Microarray analysis.

A wide heterogeneity of SG1-positive isolates has been observed. Apart from the R-genes typical for SG1, several other chromosomal or plasmid located resistance determinants were detected. All isolates harboured class 1 integrons with a variable region of different sizes: 730/ dfrA15, 1000/ aadA2, 1200/ blaOXA-1 1600/ dfrA1 — aadA1, 1600/ aac(3)-Ie — aadA7.

In the majority of the strains (84%), plasmids were present. All isolates were distributed in 26 XbaI-PFGE patterns. Of the 23 S. Typhimurium were assigned to 21 MLVA-types. The unconventional Salmonella Agona and Salmonella Derby were negative. These results demonstrated that several genetic events have contributed to the wide variability of SG1, aiding its vertical and horizontal spread.

**Analysis of multi-drug-resistant S. Typhimurium**

Salmonella Typhimurium represents a prevalent cause of food-borne gastroenteritis in Europe, and isolates frequently exhibit multi-drug resistance.

A resistance pattern that includes A, S, Su, and T (ASSuT) has recently emerged. The antimicrobial resistance (AMR) genotype and phenotype of 650 strains, collected by the French Food Safety Agency’s “Salmonella Network” from food, feed, animal health and environment in 2005, was examined. AMR phenotypes were assessed by disk diffusion method. Genotyping targeted SG1, gnr and blaTEM as these plasmid genes are considered essential to be harboured in ASSuT strains of S. Typhimurium. Figure 21.2 illustrates these results.

In an international comparative study, 127 human and porcine strains of serovar 4,[5],12:i:-, 4,[5],12:i:-, and 4,[5],12:i:- from France, Germany, Italy, The Netherlands, Spain, Poland and the United Kingdom were further subtyped by phage typing and variable-number tandem-repeat analysis (VNTR). PCR was performed to identify the flc and fliB flagellar genes, and the resistance genes encoding R-type ASSuT.

The results showed that 41% of phage-typed strains were DT193. VNTR analysis identified 53 profiles with six closely related profiles found in more than five strains. Together these represented 43% of strains, of which approximately half were DT193. The clusters contained strains of both human and porcine origin and were generally not country-specific. The most common VNTR profile was found in 13 isolates. PCR identified blaTEM (ampicillin resistance), strA (streptomycin resistance), sul2 (sulfonamides) and tetB (tetracyclines).

Resistance phenotype ASSuT was not associated with class 1 or class 2 integrons, and 4,[5],12:i:- isolates were generally fliB-negative.

**Characterization of a collection of ASSuT-resistant monophagic Salmonella Typhimurium strain variants**

A marked increase in the prevalence of monophagic Salmonella enterica, particularly serovar 4,[5],12:i:- strains of resistance type ASSuT, has been noted in food-borne infections, pigs and pork meat in several European countries in recent years. For example, in Italy the ASSuT phenotype is frequently encountered with S. Typhimurium, and recently emerged among strains of Salmonella 4,[5],12:i:-. The ASSuT phenotype is less commonly found. Between 2003 and 2006, 533 Italian strains isolated from human infections were characterized by PFGE. Among both the S. Typhimurium and S. 4,[5],12:i:- ASSuT strains, a predominant PFGE profile was recognized, while the STM ASSuT strains belonged to the different patterns. Bioinformatic cluster analysis showed that more than 90% of ASSuT and ACSsuT-resistant strains were included in two distinct clusters with a genetic homology of 73%, suggesting that the ASSuT-resistant strains belong to the same clonal lineage but is different from that of the ACSsuT strains. Phage typing showed that 23% of the ASSuT S. Typhimurium strains were not typeable, and 22% were U302. This study indicated that the tetra-resistant ASSuT strains of S. Typhimurium and S. 4,[5],12:i:- that are increasingly being isolated in Italy belong to the same clonal lineage, and that the S. 4,[5],12:i:- strains circulating in that country mainly derive from S. Typhimurium. Figure 21.2 illustrates these results.

In collaboration with Workpackage 33, the obtained SG1-deletion mutants were tested in vitro for invasiveness of Vero cells and in vivo for caecal colonization and organ (liver and spleen) invasion using an oral infection chicken model, and an oral inoculation of Balb/c mice using various bacterial doses. In each assay, isogenic and an oral inoculation of Balb/c mice using invasion using an oral infection chicken model, SGI1 deletion was only significant (p<0.01) colonized chickens as their parental strain. A SGI1-deletion mutants survived, which is a within 15 days post-inoculation, all the mice with three doses of the wild parent strains died with the SGI1 has a multi-drug resistance region

**Figure 21.2.** Dendrogram of 20 PFGE patterns found among tetra-resistant and penta-resistant Salmonella Typhimurium and S. 4,[5],12:i:- strains.
Studies on the plasmid-mediated fluoroquinolone resistance gene, qnrS1, in Salmonella

Fluoroquinolone (ciprofloxacin) resistance in Escherichia coli poultry isolates with MICs of ≥ 0.125 μg/ml has been attributed to qnrS1, which was associated with a Tn3-like transposon. Such a transposon was previously described in a Salmonella Infantis strain of animal origin, but the plasmid carrying this element resembled a plasmid previously identified in Salmonella Dublin. These observations suggest that genetic exchange can occur among Salmonella and E. coli, and, as such, animal reservoirs for the qnr genes might exist.

In a follow-up study, plasmids were characterized in qnr-positive Salmonella isolates from The Netherlands with the aim to identify and characterize plasmids carrying qnrS1, qnrB2 and qnrB19 genes. Plasmids from 33 qnr-positive Salmonella strains were transferred to E. coli and analysed by restriction, Southern blot hybridization, PCR, and sequencing of resistance determinants. They were also assigned to incompatibility groups by PCR-based replicon typing, including three additional PCR assays for the IncU, IncN and ColE groups. The collection included isolates from humans and one from poultry meat. An example of the findings is shown in Figure 21.3.

Five IncN plasmids carrying qnrS1, qnrB2 and qnrB19 genes were identified in Salmonella serovars Bredeney, Typhimurium PT507, Kentucky and Saintpaul. In addition, qnrS1 genes were located on three further plasmid types belonging to the ColE (in Salmonella Corvallis and Salmonella Montevideo) and IncN (in Salmonella Montevideo) groups.

Use of High Resolution Melting (HRM) technique for fingerprinting SGI1

Previously a gel-based PCR fingerprinting method was reported for SGI1, which made use of 96 PCR amplicons or markers. This assay was adapted to a SYBR Green melting curve analysis that monitors the decrease in fluorescence during the dissociation of dsDNA over small temperature increments. Although this technique was successful in detecting SGI1-markers in a fast real-time PCR, the analysis of each marker’s melting point (Tm) was not accurate enough to distinguish small sequence variations. The High Resolution Melting (HRM) technique was considered as a possible solution.

HRM is a rapid, closed-tube technique capable of detecting single nucleotide variation directly in PCR amplicons. HRM differs from normal melt curve analysis in using a fully saturating, intercalating dye such as SYTO9® or EVGreen® or LC Green®. These dyes, compared to SYBR Green, have the advantage of low toxicity and a higher concentration for complete saturation of dsDNA samples. This provides high fidelity fluorescent signals resulting in a greater melting sensitivity and higher resolution melting profiles. Fluorescence data is analysed with sophisticated normalization and comparison software, resulting in precise melting curves capable of accurately discriminating small sequence changes.

The SGI1 fingerprint was successfully adapted to the HRM assay. An example of 27 strains thus characterized is shown in Figure 21.5.

The HRM SGI1 fingerprint took 2.5 hours to perform and analyse, excluding DNA extraction time, and the melting points obtained were found reproducible between experiments. The SGI1 fingerprint profiles, including the main ‘gaps’ found for some isolates (e.g. a Salmonella Derby or S. Kentucky and Salmonella Newport) isolates correlated with previous results and HRM assays were of superior sensitivity (94% of successful amplifications) compared to the classical gel-based PCR assays (90% successful amplifications).

In contrast to the gel-based assay, which reported only presence or absence of markers, HRM fingerprints also detected sequence variations — information provided by the Tm of each marker. The main difference between the SYBR Green and HRM assays was in the precision and accuracy of the Tm.

We concluded that the adaptation of the gel-based fingerprint to HRM technology provides rapid and sensitive fingerprinting of SGI1. The detection of the SGI1 in bacterial isolates is important not only in tracing the source of the infection but also for the treatment of multi-drug resistant isolates. In addition to ‘gaps’ or high sequence divergence within the SGI1 backbone that could be demonstrated by classical gel-based methods, HRM is also able to accurately detect sequence variations in each marker across the SGI1, without the need for sequencing.
**Workpackage 22: Annual Research Report**

**WP number** 22  
**Title** Zoonotic Protozoa network (ZoopNet) — Cryptosporidium and Giardia  
**WP Leader** Simone M. CACCIO  
**Name and Address** Istituto Superiore di Sanità (ISS)  
Department of Infectious, Parasitic and Immunomediated Diseases  
Viale Regina Elena 299  
00161 Rome  
ITALY  
**Project Start date** 1 March 2006  
**Project End date** 31 October 2009

**Objectives**  
The main objectives of Workpackage 22 were to:  
- harmonize molecular methods useful to detect Cryptosporidium and Giardia, and to distinguish human from non-human pathogens  
- maintain and enlarge repositories of standards (nucleic acids, cysts/ooysts and cloned DNA fragments) for both Giardia and Cryptosporidium  
- develop online databases to store and analyse the data produced during the project  
- perform phylogenetic and spatial analyses of sequence and epidemiologic data from animal and human parasite isolates to identify sources of infection across Europe.

**Key achievements to date**  
- Epidemiologic and DNA sequence data have been collected from the European countries represented in Med-Vet-Net, as well as from external research groups, on a voluntary basis.  
- New methodologies, based on a real-time PCR approach, have been developed to detect zoonotic strains of Giardia.  
- Plasmids in which specific Giardia duodenalis sequences had been cloned have been produced and tested, and these are now available as reference material.  
- Repositories of Giardia and Cryptosporidium reference material have been established at The Istituto Superiore di Sanità (ISS) and the Danish National Veterinary Institute (Vet-DTU).  
- A dynamic internet-accessible database containing DNA sequences and genotype data linked to epidemiological data has been established. The databases are currently beta-versions but will be accessible via the internet in the near future.

**Progress summary**  
The goal of Workpackage 22 was to implement expertise on the detection and control of Cryptosporidium and Giardia at a European level. These protozoan parasites are major causes of diarrheal disease in humans and animals, and have also been recognized as the predominant causes of protozoan waterborne diseases. Historically, host species, site of development and morphology of oocysts and cysts have been used to determine Cryptosporidium and Giardia species. Molecular methods are nowadays considered as the most reliable detection tool for determining species and genotypes.

In the course of the project, harmonization of procedures for the detection of both parasites has been accomplished through a number of activities, including the selection of the most appropriate molecular methods for both parasites, validation studies to implement PCR-based detection methods in all participating laboratories (based either on the use of specific parasite DNA sequences cloned into plasmids or on known numbers of parasites, oocysts and cysts, present on microscope slides and in liquid suspensions), the collection and genotyping of parasite isolates of human and animal origin from Europe, and the development of dedicated web-based resources to collect, store and analyse molecular and epidemiologic data.

As a result of this approach, a common molecular platform has now been established in Europe, and comparable data have been generated to investigate, compare and contrast the epidemiology of these infections in various countries, and to study the role of zoonotic transmission.

**Background**  
Cryptosporidium and Giardia are intestinal protozoan parasites causing gastroenteritis and diarrhoea in humans and animals. Cryptosporidiosis and giardiasis represent major public health concerns in both developing and developed countries. The direct and indirect economic losses caused by these widespread parasitic infections are considerable. Both Cryptosporidium and Giardia were included in the World Health Organization’s “Neglected Diseases Initiative” illustrating their involvement in poverty-related issues. The literature clearly identifies a high prevalence of the pathogens in both humans and animals across European Union countries. Nevertheless, the infections are under-diagnosed in both public and veterinary health because the parasites’ presence is not routinely tested in clinical material, and the symptoms they cause are similar to those of other common infectious diseases. Consequently, there is a lack of insight into the actual disease burden. Furthermore, knowledge about the prevalence, pathogenicity and the relative contributions of risk factors of both pathogens is still fragmentary. As a consequence, potential control methods have not yet been formulated.

Only some species or genotypes of Cryptosporidium and Giardia cause infection in humans, whereas many other members of these two genera infect only animals. Especially in the case of Giardia, the zoonotic potential of its species infecting mammals, in particular Giardia duodenalis, is not established. Important aspects of the epidemiology under investigation include identifying the main sources of infection, unravelling the host range of different species/genotypes, and determining the relative roles of the different transmission routes.

The lack of useful morphological characteristics to reliably discriminate Cryptosporidium and Giardia species/genotypes precludes the identification of human versus non-human strains by microscopy. Thus, molecular characterization has been put forward to evaluate the genetic variability within Cryptosporidium and Giardia, assess the role of animals in the epidemiology of human infection, and develop tools for tracing sources of infection. Most of these studies have been based on the analysis of a single marker and on a limited number of isolates. Furthermore, the genetic variability and the usefulness of the different loci in identifying species/genotypes have not been systematically evaluated. The lack of standardized methods and reference materials hamper comparisons of observations from different studies. Thus, cooperation between animal and human health sectors is instrumental for the detection, surveillance and the identification of potential zoonotic strains.

The overall objective of Workpackage 22 was to improve the ability of European scientists to detect and identify species and genotypes of the protozoan parasites, Cryptosporidium and Giardia. The focus was on both human and animal pathogens with an emphasis on zoonotic agents and their potential link to food. To that end, the activities of ZoopNet focused on identifying a common detection platform after evaluating the expertise of each participating laboratory; setting up, improving and de novo development of laboratory methods, particularly molecular methods; and developing dedicated databases to obtain, store and disseminate epidemiologic and experimental data.

**Methodologies developed**  
Development of real-time PCR assays for the detection of zoonotic strains of Giardia  
The genotyping approach currently in use is based on the in vitro amplification of a specific DNA sequence followed by restriction enzyme or sequence analyses. The approach has inherent limitations, particularly in cases when more
than one pathogen is present in a given matrix (that is, faeces or water), and when quantitative aspects are important. These limitations may be overcome using real-time PCR (qPCR) technology, with, by allowing the continuous monitoring of the amplification reaction, is particularly useful to detect multiple pathogens (in a multiplex platform) and to infer quantitative aspects.

qPCR assays were developed to target the triose phosphate isomerase gene, the glutamate dehydrogenase gene and the open reading frame C4 of *G. duodenalis*. Primers were designed for each of these genes to allow the specific amplification of DNA of assemblage A or B, and to generate products distinguishable by their melting curves, their sequence, their size or their restriction patterns. The assays showed full specificity and a sensitivity down to the level of a single trophozoite. We applied the three qPCR assays to genomic DNA extracted directly from 30 human stools and to *Giardia* cysts purified by immune-magnetic capture from the same samples. A simultaneous detection of both assemblages was observed in 37–83% of the genomic DNA extracted from samples, according to the target gene, and the vast majority of these cases could be attributed to mixed infections, as shown by experiments on single cysts from the same samples. These assays may be used to detect *Giardia* cysts in samples of human animal origin as well as in water and food samples.

The methodology can be easily transferred to partners depending on the availability of qPCR equipment. A paper describing this approach has been submitted for publication.

**Results**

**Repositories and reference material**

The Istituto Superiore di Sanità (ISS) and the Danish National Veterinary Institute (Vet-DTU) were responsible for the maintenance of two repositories of standards for both *Giardia* and *Cryptosporidium* (described in Deliverable D22.12).

Faecal samples of human and animal origin were screened using indirect immunofluorescence to detect oocysts and cysts. Positive samples were further processed to identify the species and genotypes using molecular assays. Aliquots of nucleic acids have been stored, and these represent the main reference material. In some cases, immunomagnetic separation was used to process and then stain highly purified parasites (*Cryptosporidium* oocysts and *Giardia* cysts).

Plasmids containing specific fragments amplified from genomic DNAs representing all zoonotic pathogens have also been produced. In the past reporting year, ISS has generated plasmids containing a 530bp fragment of the triose phosphate isomerase (TPI) gene of *Giardia duodenalis*. The inserted sequences were amplified from genomic DNA of the different assemblages of *G. duodenalis*, including both zoonotic (assemblages A and B) and animal-specific (assemblages C, E and F) strains. The plasmids were sequenced to verify the accuracy of the cloned sequences, and have been stored at ISS to be used as reference material.

**Genotyping activities**

During its final year, ZoopNet generated a considerable amount of sequence data, particularly for *Giardia* (described in Deliverable D22.16). In some instances the ISS laboratory offered technical help for sequencing specific amplification products. The main purpose of the genotyping activity was to increase the amount of data from specific countries for which little information was available at the start of the project, or to focus on specific hosts (such as pets) with the aim of understanding their potential zoonotic role.

One important study, undertaken in collaboration with the UK’s Health Protection Agency, concerned the genetic characterization of approximately 110 isolates collected after a waterborne outbreak of giardiasis in a restaurant in Leeds. An unexpected genetic variability characterized the available (and typable) isolates, indicating multiple contamination sources or a mixed infection source that contaminated the water. It was found that assemblage B caused the outbreak. A publication is in preparation to describe the study’s main findings.

A number of *Cryptosporidium* sequences were also generated, mainly from humans and livestock (described in Deliverable D22.15). Of particular interest is the analysis of the GP60 gene, which encodes for glycoprotein expressed by the invasive stage of the parasite. Furthermore, GP60 shows a very high degree of polymorphism and thus may be a useful marker to trace infection sources or to estimate the role of animals in the epidemiology of human *cryptosporidiosis*.

Initially, extensive characterization of the data stored in the *Giardia* database (see Deliverable D22.17) was performed indicating that a multi-focus approach was necessary to explore transmission patterns, and that the current data did not support a significant role of animals in the epidemiology of human giardiasis. These findings indicate a complex structure of the population of *Giardia* parasites, certainly more complex than previously thought, and will inform future ZoopNet studies as well as research external to Med-Vet-Net.

As the databases will continue to be maintained and operated, a similar analysis will be performed for *Cryptosporidium* in the near future.

**Databases**

There were two main efforts to produce databases for Workpackage 22. The first effort concentrated on developing a static database to share updated scientific information on the biology, epidemiology, detection and control of *Giardia* (see Deliverable D22.02). It was merged with the *Cryptosporidium* database previously developed during Workpackage 12 and hosted by ISS.

The combined *Cryptosporidium* and *Giardia* database is essentially complete, although the current web address (http://progettiss.iiss.it/zoop) is still provisional — several electronic resources related to infectious agents, including the ZoopNet database, will soon be moved into a special section of the ISS website. The second effort involved improving the dynamic database where sequence and epidemiologic data for both pathogens can be stored and analysed (Deliverable D22.11). In collaboration with the Dutch National Institute for Public Health and the Environment (RIVM), a software program was developed for the implementation of two web-based molecular epidemiological databases — one for *Cryptosporidium* and one for *Giardia* isolates. These online databases are coupled to the field data and isolates obtained from Workpackage 22 and partner institutes, such as the University of Leuven, Belgium, enabling researchers from public and veterinary health institutions to compare the molecular and epidemiological data of their field isolates with the ones present in the database. The database covers four *Giardia* genetic markers (16S, BG, GDH, TPI) and five *Cryptosporidium* markers (18S, COWP, GP60, HSP70, MLT). A public typing tool is under development for the single markers, as it is currently unclear to what extent genetic recombination and mixed infections are present in individual field isolates.

The databases are currently being tested offline and will be accessible via the internet once the necessary security layers have been added.

**Workshops and meetings**

Sweden’s National Veterinary Institute (SVA) hosted the final Workpackage 22 meeting on November 19–20, 2008 in Uppsala. Participants included representatives from SVA, ISS, RIVM, Vet-DTU, Poland’s National Institute of Hygiene, the UK’s Veterinary Laboratories Agency, Denmark’s Statens Serum Institut, Sweden’s Institute if Public Health, and Germany’s Federal Institute for Risk Assessment. The meeting’s main discussions were on validation studies, genotyping activities, database development and joint publications. During the meeting, presentations were given by Prof. Staffan Svard, Uppsala University and Karolinska Institute, on different aspects of *Giardia*, and by Dr. Charlotte Silverhå, Swedish University of Agricultural Sciences, on *Cryptosporidium*.

ZoopNet also held an informal meeting in El Escorial, Spain, during the Med-Vet-Net annual conference (3–6 June, 2009).
Zoonoses constitute a major public health risk and generate emerging disease problems worldwide. Besides direct contact with animals, contaminated food has been recognized as the main source of transmission. Social and economic costs of zoonotic and food-borne diseases on national economies are significant. Although, ideally, the prevention and control of all zoonotic diseases would be desirable, effective and efficient policy-making on control, prevention and surveillance of those diseases requires a focus on the most relevant ones. A prioritizing approach is therefore necessary.

**Approaches**

Workpackage 23 aimed to develop strategies and models to provide stepwise and transparent approaches to estimate priorities in a number of zoonotic diseases at the European level. The model was based on the (actual or expected) disease burden and cost of illness.

Eight countries participated in Workpackage 23. Following discussions within the workpackage, the RIVM approach for estimating the burden and costs of food-borne pathogens in The Netherlands was chosen as the basis for the work.

The assessment of disease burden was based on Disability Adjusted Life Years (DALYs). The key requirement of DALY calculations is to have a reliable estimate on the true incidence of the disease of interest in the population. Therefore a tool was established to modulate the surveillance pyramid of infectious intestinal disease at the three levels: the general population, consultation with general practitioner, and hospitalization.

The model accounted for different health-care seeking behaviour between countries at every step of the surveillance pyramid, for example differences in submission of stool specimens, laboratory practices with regard to pathogen analysis, reporting of positive-tested patients with gastrointestinal illness to national authorities, and differences in the sensitivity of analytical methods. The incidence reconstruction tool was also amended with tables summarizing data based on the previous Dutch work, in order to calculate the burden of disease expressed in DALYs per pathogen and, by modification, per country.

There was consensus to continue collaborations between Workpackage 23 participants to improve the current preliminary estimates, which are based on small scale studies or expert opinions. Information from the ongoing telephone surveys on incidence of infectious intestinal disease in the population and health-care seeking behaviour will greatly reduce uncertainty in final outcomes. Due to the uncertainty in the current estimates, the reported incidence and DALY estimates are currently anonymised.


**Reconstruction of the incidence surveillance pyramid for infectious intestinal disease**

An important factor in the priority setting model was to estimate the incidence of gastrointestinal illness associated with seven selected pathogens: Campylobacter spp., Shiga toxin-producing Escherichia coli O157, Salmonella spp., Cryptosporidium spp., Yersinia enterocolitica, Shigella spp. and

**Workpackage 23: Annual Research Report**

<table>
<thead>
<tr>
<th>WP number</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>Prioritizing food-borne and zoonotic hazards at the EU level</td>
</tr>
<tr>
<td>WP Leader</td>
<td>Arie HAVELAAR</td>
</tr>
<tr>
<td>Name and Address</td>
<td>National Institute for Public Health and the Environment (RIVM) PO Box 1 3720 BA Bilthoven THE NETHERLANDS</td>
</tr>
<tr>
<td>Project Start date</td>
<td>1 March 2006</td>
</tr>
<tr>
<td>Project End date</td>
<td>31 October 2009</td>
</tr>
</tbody>
</table>

**Objectives**

Workpackage 23’s objectives were to:

- agree criteria and methods for priority setting of food-borne and zoonotic hazards, including emerging and re-emerging pathogens
- collect and evaluate existing data on the incidence, health outcomes and costs of food-borne and zoonotic illness
- produce a preliminary estimate of the current disease burden and cost of illness of (selected) food-borne and zoonotic pathogens in Europe
- identify the major uncertainties in the data used to produce these disease estimates
- recommend additional studies, and prepare projects for additional funding.

**Key achievements to date**

- In July 2009, a workshop on the ‘Disease burden and serosurveillance of food-borne pathogens in the EU’, was jointly organized with Workpackage 32.
- A training course in reconstructing the surveillance pyramid and assessing the burden of food-borne disease was held in November 2008.
- A modelling tool to reconstruct the surveillance pyramid was developed. The model estimates the number of intestinal disease infections based on three levels of surveillance data; general population, consultation with general practitioner, and hospitalization.
- A telephone survey, on the basis of the UK IID2-study (The Second Study of Infectious Intestinal Disease in the Community), was set up in 2008 with country-specific interim reports prepared for the four participating countries (Denmark, Germany, Poland and Italy).
- A calculation tool to estimate Disability Adjusted Life Years (DALYs) of infectious intestinal disease was developed and integrated into the modelling tool to reconstruct the surveillance pyramid. Preliminary results for seven bacterial pathogens in eight European countries have been obtained.
- For most countries the incidence estimates obtained on salmonellosis correlated well to the findings obtained by Workpackage 32 on seroprevalence in the population, whilst the modelled and seroprevalence data did not correlate for campylobacteriosis.
enteropathogenic/enterotoxigenic *E. coli*. Only two countries, The Netherlands and the UK, have performed population-based studies, but these date back to the 1990s. Other countries report data, based on laboratory surveillance, which is only a proportion of the total incidence in the community. Moreover, this proportion differs between distinct countries.

A model has been evaluated that reconstructs the surveillance pyramid, based on reported data. The model accounts for differences between countries in health-care seeking behaviour of patients, submission of stool specimens, laboratory practices with regard to pathogen analysis and reporting of positive tested patients with gastrointestinal disease to national authorities, and the sensitivity of analytical methods. The model also includes stochastic procedures to estimate uncertainty of the incidence. Because the data initially used were from relatively small-scale studies or derived from expert opinions, the estimates were produced with high uncertainty. To enable improved estimates, and to reduce uncertainty on the incidence of infectious gastroenteritis in European countries in future, two studies have been carried out (and some are still ongoing) in several participating countries: a telephone survey to assess the incidence rate of gastroenteritis and health-care seeking of patients (see Med-Vet-Net’s 2008 Annual Report), and a laboratory survey to assess diagnostic practices.

Due to the uncertainty in the current estimates however, the incidence and DALY estimates were reported on a confidential basis.

In Figure 23.1, the incidence reconstruction model for *Salmonella* is presented for eight anonymised countries (A to H). Substantial differences can be seen in the fraction of patients who visit general practitioners. This fraction is lowest in countries E and B (14% and 21% respectively), and highest in country H (60%). Hence, this factor alone already explains an approximate four-fold difference in the multiplier. This difference is propagated in the next steps of the pyramid. To understand the differences between countries in further steps, the slope of the connecting lines should be evaluated. The first two steps in the pyramid clearly have the largest impact on the final results.

**Estimating the burden of disease**

The assessment of the disease burden will be based on DALYs. This non-monetary method presumes perfect health (in absence of infections) for the entire life span and measures the loss of life years due to subsequent illness.

A key aspect of assessing the burden of disease is the incidence of gastrointestinal illness; therefore the data tables that were constructed in order to calculate DALYs were incorporated into the incidence reconstruction tool. This allowed the calculation of the burden of disease (in DALYs) per pathogen and per country.

**Workshops and meetings**

A training course and a meeting were successfully organized in the reporting year.

The second workshop, “Reconstructing the surveillance pyramid and assessing the burden of food-borne disease”, was held on 5–7 November 2008, in Bilthoven, The Netherlands. The workshop on data integration was organized as training course and allowed Workpackage 23 participants to get familiar with the newly developed tool to estimate the true incidence of infectious intestinal disease in the population at distinct levels of the surveillance pyramid. An important activity during the meeting was discussing and reaching consensus among the participating countries on key parameters used in the tool. Additionally, DALY calculation data tables were explained and discussed. Following the workshop, the participants applied the tool to their national data to reconstruct the surveillance pyramid and amend national parameters, where available and necessary.

The final Workpackage 23 meeting (‘Disease burden and serosurveillance of food-borne pathogens in the EU’) took place on 15–16 July 2009 in Warsaw, Poland, in the form of a workshop jointly organized with Workpackage 32. The results of incidence estimates of gastrointestinal disease in participating countries, derived using the surveillance pyramid reconstruction tool, were presented at the workshop along with the methods and status of the telephone surveys running in five countries, and the laboratory survey performed in Italy. Guests from the European Centre for Disease Prevention and Control, the World Health Organization, and the American Centers for Disease Control and Prevention also attended the meeting contributing to the discussion on Europe’s future research needs in the field and stressing the need for the work to continue.

An interesting finding of the meeting was that in most countries the Workpackage 23 incidence estimates on *Salmonella* gastrointestinal disease correlated well to the Workpackage 32 findings on *Salmonella* seroprevalence in the population, but this was not the case for *Campylobacter*.

Materials (database software developed in the Workpackage) will continue to be available to partners after the cessation of Med-Vet-Net.

As data collection (via the telephone survey) will extend beyond the life of Med-Vet-Net, participants have expressed an active interest in continuing their collaboration under the umbrella of the Med-Vet-Net Association.
### Workpackage 24: Annual Research Report

<table>
<thead>
<tr>
<th>WP number</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>Comparison of Campylobacter risk assessment models: Towards a European consensus model?</td>
</tr>
<tr>
<td>WP Leader</td>
<td>Maarten NAUTA</td>
</tr>
<tr>
<td>Name and Address</td>
<td>Technical University of Denmark National Food Institute (DTU) Department of Microbiology and Risk Assessment Mørkhøj Bygade 19 Building C 2860 Søborg DENMARK</td>
</tr>
<tr>
<td>Project Start date</td>
<td>1 March 2006</td>
</tr>
<tr>
<td>Project End date</td>
<td>31 October 2009</td>
</tr>
</tbody>
</table>

### Objectives
The objectives of Workpackage 24 were to:
- develop a European consensus framework for risk assessment of Campylobacter in broiler meat
- release a software tool (CRAF 2.0) as guidance to risk assessors
- provide guidance and training for those wishing or planning to make such risk assessments
- anticipate a request for a European risk assessment by the EU.

### Key achievements to date
- Final general meeting on Campylobacter risk assessment (Berlin, February 2009).
- Launch of CRAF 1.0 (Feb 2009) and CRAF 2.0 (Aug 2009).
- CRAF training course (Feb 2009).
- CRAF workshop (June 2009).

### Progress summary
Since previous Workpackage 24 work had revealed that the construction of a single, ready to use, European consensus risk model for Campylobacter in broiler meat was neither feasible nor desirable, the main focus of Workpackage 24 in its final year was the development of a consensus framework.

This framework has been implemented in a software tool, ‘CRAF’ (Campylobacter Risk Assessment Framework), that can be used as a dynamic risk assessment catalogue for risk assessors. CRAF can help those intending to do risk assessments of Campylobacter in broiler meat to understand, compare and combine the existing European models developed by the workpackage partners, as well as the model produced in New Zealand.

The first version of the tool was presented at the successful final meeting of Workpackage 24 in February 2009, which was followed by a training course involving participants from 13 countries. It was concluded that the tool is one-of-a-kind, and that it should be extended to more pathogens and other food commodities.

After the next version of CRAF 2.0 is completed (see www.rivm.nl/craf), some workpackage partners will plan future extensions as part of a new project with the USA-based online resource, FoodRisk.org.

Importantly, more fundamental research in quantitative risk assessment modelling methods is needed to develop more efficient, harmonized risk models to meet future challenges in the control of food-borne zoonoses.

### Background
Over the past decade, microbiological risk assessment has developed into an important tool for food safety control. It offers a structural, unified approach to complex problems, as well as a scientific basis for risk-management decisions. Accordingly, international bodies such as the World Trade Organization, the Codex Alimentarius, the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the European Food Safety Authority (EFSA) have promoted its use. Within Europe, experience in risk research has substantially increased. Several food chain risk assessments have been conducted in different countries for various food-pathogen combinations. The variety of applied methodologies, each of which has strengths and weaknesses, is driving the progress of quantitative microbiological risk assessment, but it also has disadvantages: comparison of risk assessments between countries is complicated when analysis tools differ, and for researchers entering the field it can be difficult to choose the most appropriate methodology.

Campylobacter risk assessments have also been conducted outside Europe. Representatives from three Med-Vet-Net partners (the United Kingdom’s Veterinary Laboratories Agency (VLA), the Danish National Food Institute (Food-DTU), and the Dutch National Institute for Public Health and the Environment (RIVM)) have collaborated with partners from the USA and Canada in the WHO–FAO drafting group on risk assessment of Campylobacter in broiler chickens.

This exchange of experience in the search for a consensus approach in a global perspective proved to be useful both for Workpackage 24 and for collaborations with non-European experts. Results from the New Zealand Campylobacter risk assessment have also now been integrated into the workpackage.

Workpackage 24 started in March 2006. A review paper outlining the results of the first phase of work was published in the International Journal of Food Microbiology in early 2009. After concluding that the construction of a user-friendly software tool to serve as the European consensus risk assessment model was neither feasible nor desirable, the workpackage identified a need for a flexible tool that could help those wanting to set up a new risk assessment for their own country (or region), and that might help countries where a risk-assessment model was available, to further develop it. During this final year of the workpackage, work has concentrated on the development of such a tool.

Workpackage 24’s aim was to identify the best aspects of the individual approaches in order to improve microbial risk assessments within Europe by combining expertise and comparing different approaches. This comparison involved the methods applied as well as the results obtained to provide insight into the differences, and their relative impacts on the risk management advice.

It is envisaged that Workpackage 24 will lead to a consensus approach to microbial risk assessment modelling that may guide others in Europe wishing to set up their own risk assessment. Such a harmonized approach may also facilitate risk management on a European scale.

Workpackage 24 focused on Campylobacter in broiler meat as a model organism. Campylobacter is the most commonly reported food-borne bacterial pathogen in Europe, and broiler meat is generally considered a major source of campylobacteriosis. A number of quantitative risk assessments on Campylobacter in the food chain, some of which have been developed by Med-Vet-Net partners, are currently available or in progress within Europe. The different risk assessments have been established more or less independently — for each assessment both country-specific and international data were collected, and new models were built for various stages of the food chain.

Campylobacter risk assessments have also been conducted outside Europe. Representatives from three Med-Vet-Net partners (the United Kingdom’s Veterinary Laboratories Agency (VLA), the Danish National Food Institute (Food-DTU), and the Dutch National Institute for Public Health and the Environment (RIVM)) have collaborated with partners from the USA and Canada in the WHO–FAO drafting group on risk assessment of Campylobacter in broiler chickens.

This exchange of experience in the search for a consensus approach in a global perspective proved to be useful both for Workpackage 24 and for collaborations with non-European experts. Results from the New Zealand Campylobacter risk assessment have also now been integrated into the workpackage.

Workpackage 24 started in March 2006. A review paper outlining the results of the first phase of work was published in the International Journal of Food Microbiology in early 2009. After concluding that the construction of a user-friendly software tool to serve as the European consensus risk assessment model was neither feasible nor desirable, the workpackage identified a need for a flexible tool that could help those wanting to set up a new risk assessment for their own country (or region), and that might help countries where a risk-assessment model was available, to further develop it. During this final year of the workpackage, work has concentrated on the development of such a tool.

Workpackage 24’s aim was to identify the best aspects of the individual approaches in order to improve microbial risk assessments within Europe by combining expertise and comparing different approaches. This comparison involved the methods applied as well as the results obtained to provide insight into the differences, and their relative impacts on the risk management advice.

It is envisaged that Workpackage 24 will lead to a consensus approach to microbial risk assessment modelling that may guide others in Europe wishing to set up their own risk assessment. Such a harmonized approach may also facilitate risk management on a European scale.

Workpackage 24 focused on Campylobacter in broiler meat as a model organism. Campylobacter is the most commonly reported food-borne bacterial pathogen in Europe, and broiler meat is generally considered a major source of campylobacteriosis. A number of quantitative risk assessments on Campylobacter in the food chain, some of which have been developed by Med-Vet-Net partners, are currently available or in progress within Europe. The different risk assessments have been established more or less independently — for each assessment both country-specific and international data were collected, and new models were built for various stages of the food chain.

Workpackage 24 started in March 2006. A review paper outlining the results of the first phase of work was published in the International Journal of Food Microbiology in early 2009. After concluding that the construction of a user-friendly software tool to serve as the European consensus risk assessment model was neither feasible nor desirable, the workpackage identified a need for a flexible tool that could help those wanting to set up a new risk assessment for their own country (or region), and that might help countries where a risk-assessment model was available, to further develop it. During this final year of the workpackage, work has concentrated on the development of such a tool.
Approaches

The development of a consensus framework for risk assessment of Campylobacter in broiler meat started in 2008. The framework is implemented as a software tool called ‘CRAF’ (Campylobacter Risk Assessment Framework). CRAF contains detailed information on the Campylobacter risk assessment models made by the Med-Vet-Net partners from the United Kingdom (VLA), Denmark (Food-DTU), The Netherlands (RIVM) and Germany (Federal Institute for Risk Assessment, BfR). Additionally, in response to a request by the New Zealand Food Safety Authority, the Campylobacter risk assessment model built in New Zealand was incorporated into CRAF.

CRAF has been built as a dynamic model catalogue based on the available risk assessment models. It guides the user through the models without offering the software to run the models in order to protect the ownership rights of the developers. Hence, the consensus framework is not a ready-to-use software tool for risk assessment, but instead provides a useful overview of the available models. The core workpackage team guided development of the consensus framework with other workpackage partners providing advice as required. Special attention was given to the correct incorporation of data and adequate links between the modules provided.

For the construction of CRAF, specialists in information analysis and functional design at RIVM were consulted to structure the information that was to be provided in the framework. Next, a data model incorporating the relations between the different types of data was built. This is schematically represented in Figure 24.1. The data model was then implemented in a web application to allow user-friendly access to the data. A default Excel spreadsheet was constructed to facilitate standardized input of the data required for a comparative overview of the available models. This spreadsheet was subsequently sent to the risk assessors responsible for the models and the information was entered into CRAF by the RIVM developers. CRAF was built as a JAVA EE web application that does not depend on client-side facilities other than a web browser. CRAF employs a postgresql-8.1.407.jdbc3 driver to connect to the postgresql database. The application will work with a range of databases, requiring only minor changes in the build configuration. CRAF has been tested successfully on both Windows and Linux platforms.

Results: CRAF

Two consecutive versions of CRAF have been made publicly available. In February 2009, CRAF 1.0 was launched during the Workpackage 24 final meeting. CRAF 2.0 was made available on the internet on 31 August 2009 (see www.rivm.nl/craf).

CRAF is built up in the same modular structure that it summarizes and compares. In every module the changes in prevalence and concentrations of infected and contaminated birds and products are described mathematically. For five different risk assessment models, CRAF provides information on the modules for the farm, transport, industrial processing (divided into scaling, defleshing, evisceration, washing and chilling), cutting, retail, preparation and dose response. It offers a dynamic interface for the user who can obtain either an overview of the available models or get detailed information on the nature of the implemented distributions in terms of variability and uncertainty; a list of model parameters; an overview of model equations; and references and, if available, links to the original report with a full description of the models. Selecting inputs, outputs or model parameters gives a new screen with additional detailed information on those specific items.

The “compose chain” option aims to facilitate the construction of “new” models, built up as a novel combination of modules from different existing models with special reference to the unit that the prevalence and concentrations refer to, and the

Apart from providing a glossary of terms and general background information on the risk assessment models, the main functionalities of CRAF are the options to “compare modules” and “compose chain”. An example is shown in Figure 24.2 of the first screen displayed after choosing “compare modules”. It allows the user to select the models of interest for more detailed comparison. These details include a description of the modules; tables of inputs and outputs of the models with special reference to the unit that the prevalence and concentrations refer to, and the nature of the implemented distributions in terms of variability and uncertainty; a list of model parameters; an overview of model equations; and references and, if available, links to the original report with a full description of the models. Selecting inputs, outputs or model parameters gives a new screen with additional detailed information on those specific items.

The “compose chain” option aims to facilitate the construction of “new” models, built up as a novel combination of modules from different existing models with special reference to the unit that the prevalence and concentrations refer to, and the

Apart from providing a glossary of terms and general background information on the risk assessment models, the main functionalities of CRAF are the options to “compare modules” and “compose chain”. An example is shown in Figure 24.2 of the first screen displayed after choosing “compare modules”. It allows the user to select the models of interest for more detailed comparison. These details include a description of the modules; tables of inputs and outputs of the models with special reference to the unit that the prevalence and concentrations refer to, and the nature of the implemented distributions in terms of variability and uncertainty; a list of model parameters; an overview of model equations; and references and, if available, links to the original report with a full description of the models. Selecting inputs, outputs or model parameters gives a new screen with additional detailed information on those specific items.

The “compose chain” option aims to facilitate the construction of “new” models, built up as a novel combination of modules from different existing models with special reference to the unit that the prevalence and concentrations refer to, and the nature of the implemented distributions in terms of variability and uncertainty; a list of model parameters; an overview of model equations; and references and, if available, links to the original report with a full description of the models. Selecting inputs, outputs or model parameters gives a new screen with additional detailed information on those specific items.
models. The first screen encountered when choosing this option is shown in Figure 24.3.

After selecting different modules for each relevant stage in the food chain, the user gets an overview of the inputs and outputs and their characteristics. This provides insight into the feasibility of combinations of different modules from different risk assessments, and shows where additional assumptions or data collection will be needed to construct such a model.

Following the meeting in February 2009 during which CRAFT 1.0 was demonstrated, users and participants offered several suggestions for improvements. Based on that constructive criticism, Workpackage 24’s final available funds were used to re-develop the software into CRAFT 2.0, which has improved user friendliness and strengthened the consistency in representation of the models.

CRAFT 2.0 is now ready for use by interested risk assessors. It is built in a way that can be easily updated when new information from existing models becomes available, or when new models are added. Hosting on the RIVM server is guaranteed until the end of 2009.

**Workshops and meetings**

In Berlin in February 2009, BfR hosted an important Workpackage 24 meeting comprising a half-day workshop partner meeting and a full-day symposium on Campylobacter risk assessment developments at which Dr. Ruff Lowman of the Canadian Food Inspection Agency was a keynote speaker.

During the meeting, attended by 37 participants from 14 countries including Japan and the USA, discussions focused on the impact of the recent developments related to Campylobacter control in Iceland, and the role of risk assessment. There were four additional oral presentations on recent Campylobacter risk assessment activities, and the future of European Campylobacter risk assessment was also discussed with special focus on the sustainability of the work undertaken in Workpackage 24. The meeting closed with the launch of CRAFT 1.0.

A one-day CRAFT training course followed the February meeting. Thirty participants, including five trainers, from 13 countries including Canada, USA and Japan, attended. EFSA and the American Joint Institute for Food Safety and Applied Nutrition (JIFSAN), a partnership between the University of Maryland and the US Food and Drug Administration, were also represented.

To further promote the use of CRAFT, a short workshop was organized at Med-Vet-Net’s 5th Annual Scientific Meeting in El Escorial, Spain (June 2009). Ten participants attended.

**Conclusions and future perspectives**

With the finalization of CRAFT 2.0, its uploading to the internet, and the organization of a training course and workshop, Workpackage 24 has achieved most of its major objectives as well as completing its deliverables and reaching its milestones.

Several efforts have been made to achieve the objective “anticipation of a request for a European risk assessment by the EU.” EFSA representatives were invited to, and subsequently participated in, the workpackage’s final meeting and the CRAFT training course. The workpackage leader based his invited lecture at the EFSA scientific colloquium, “Assessing health benefits of controlling Campylobacter in the food chain” in Rome, December 2008, primarily on the outcomes of Workpackage 24. The workpackage leader and two other workpackage participants have also joined the EFSA working group on Campylobacter control established in autumn 2008. The working group will be guiding EFSA’s quantitative microbiological risk assessment of Campylobacter in the broiler meat chain, for which the call for tender has been posted (CFT/EFSA/BIOHAZ/2009/01).

Of particular concern is CRAFT’s future hosting, maintenance and further development following Med-Vet-Net. EFSA has been contacted to take over the European consensus framework for Campylobacter risk assessment as a tool to support the development of quantitative microbiological risk assessment in Europe. At the time of writing, a response had yet to be received. In contrast, interest expressed by the United States is promising.

A research proposal has been prepared together with FoodRisk.org, an integrated online resource for food safety and risk analysis, which is supported and managed by JIFSAN. The proposal has been submitted to the Cooperative State Research, Education, and Extension Service of the US Department of Agriculture. It aims to further improve CRAFT and to develop a publicly available online, integrated catalogue on risk assessment to compare existing risk assessments in detail. It will encompass not only Campylobacter in broiler meat, but also other food-pathogen combinations.

An important conclusion of the Workpackage 24 activities is that quantitative risk assessment is still developing. To harmonize and improve existing methods and to reach the objective of efficient, fast and fit-for-purpose risk assessment, more fundamental research into quantitative microbiological risk assessment methods is necessary. This is essential to warrant the future of quantitative microbial risk assessment as a widely used tool to effectively support the control of food-borne zoonosis in a food chain approach.

Given the complexity of the problems faced, it is crucial to bring expertise together and continue the European collaboration established in Workpackage 24.

---

**Figure 24.3. The “compose chain” screen in CRAFT.**

The CRAFT 2.0 software tool is a guide for risk assessors.
Workpackage 25: Annual Research Report

WP number 25
Title Development and application of improved diagnostics for Q-fever
WP Leader Richard THIERY
Name and Address French Food Safety Agency (AFSSA) – Sophia Antipolis 105 route des Chappes BP111, F-06902 SOPHIA ANTIPOLIS FRANCE
Project Start date 1 March 2006
Project End date 31 October 2009

Objectives
The overall objectives during the final year of Workpackage 25 were to compare available typing methods and develop a new approach through:
• the isolation, characterization and comparison of new strains
• the organization of a ring trial on genotyping of Coxiella burnetii (Multi-Spacer Sequence typing (MST) and Multiple Loci Variable number of tandem repeats (VNTRs))
• the development of genotyping using microarray

Key achievements to date
• Completion of a comparative ring trial for PCR detection using Q-fever positive veterinary abortion samples.
• Organization of a typing ring test for MST and VNTR assays.
• Use of VNTR typing on field samples.
• Isolation, characterization and comparison of new genotypes of C. burnetii.

The recent increase in Q-Fever could be partly due to increased visits to petting farms.

Background
Q-fever is a zoonosis caused by the intracellular bacterium C. burnetii. Q-fever is endemic throughout the world with the exception of New Zealand, and it has been described in almost every European country. Nevertheless, the situation of Q-fever in the different European countries is not easy to compare due to considerable variation in the surveillance systems across the EU member states.

Implementation, development and standardization of detection and genotyping methods is crucial for the prevention and control of this zoonosis, and for epidemiological analyses, as exemplified by the outbreaks that recently occurred in The Netherlands. Such outbreaks, as well as possibly better reporting, are responsible for an increase from 168 reported human cases in 2007 to over 1,000 cases in 2008, and already close to 1,500 cases in 2009 (to August).

The intracellular bacterium Coxiella burnetii is the causative agent of Q-fever, a worldwide-occurring zoonosis. Q-fever is endemic throughout European countries but its epidemiology is not well known because of a considerable variation in the surveillance systems across European Union (EU) member states.

Implementation, development and standardization of detection and genotyping methods is crucial for the prevention and control of this zoonosis, and for epidemiological analyses, as exemplified by the outbreaks that recently occurred in The Netherlands. Such outbreaks, as well as possibly better reporting, are responsible for an increase from 168 reported human cases in 2007 to over 1,000 cases in 2008, and already close to 1,500 cases in 2009 (to August).

During the final year of Workpackage 25, a comparative analysis of the serological methods (by CF, ELISA and indirect immunofluorescent antibody (IFA)) that are available for the detection of antibodies against C. burnetii was performed using human sera arising from a range of clinical presentations. Moreover, the genotyping ring trial, organized within the workpackage, identified some difficulties encountered when VNTR and MST assays are used without a standardized scheme for results interpretation. As a result, several recommendations have been formulated for best practice and for harmonization of typing methods of C. burnetii.

Besides the comparison of detection and typing methods, different partners provided significant input by isolation and subsequent sharing of C. burnetii strains, as demonstrated by the new genotypes obtained from France, The Netherlands and Poland.

C. burnetii has a wide host range and can be found in a large number of mammalian species, birds and arthropods, such as ticks. Various host species may play a role in the dissemination or maintenance of the disease as pathogen-carriers or as vectors, but their role in transmission of the disease has not been established with certainty. Nevertheless, domestic ruminants (cattle, sheep and goats) appear to be the principal reservoir of C. burnetii and their implication in disease transmission is central for humans. In these animals, similarly to humans, the infection by C. burnetii is often asymptomatic, but in pregnant females it may cause abortion and reproductive disorders, weakened offspring, metritis and infertility. Moreover, the bacteria are present in very high numbers in birth products and are also shed intermittently in the milk, faeces and urine of infected animals.

C. burnetii is extremely infectious and resistant in the environment due to the formation of "spore-like" forms. As the bacteria are excreted in the environment, the disease is transmitted to humans mainly by inhalation of infected aerosols or dust. Occupational exposure to sheep and goats is reported in most Q-fever cases.

The recent increase in Q-fever could be partly due to changes in human behaviour, such as increased interest in camping on farms or visits to petting farms. Climate changes cannot be excluded in the apparent shift north in European countries. Oral transmission seems less common as lambing and sheep shearing, can lead to environmental contamination.

Even though it’s historically not perceived as an important public health threat in the medical or veterinary communities, C. burnetii can cause debilitating infections and may result in potentially fatal chronic infections among humans. It is also considered a potential agent of bioterrorism because of its accessibility, low infectious dose, resistance to environmental degradation, and aerosol route of transmission. Moreover, outbreaks in urban or residential areas have been recently reported in Bulgaria, The Netherlands and Germany, involving large numbers of human cases that were linked to relatively small ruminant flocks.

These recent large outbreaks highlight how zoonoses, such as Q-fever, may also represent a public health threat for urban populations, and emphasise the need for strengthening control measures against this emerging problem. Therefore, the implementation, development and standardization of detection and genotyping methods are crucial for the prevention and control of this zoonosis.

The recent increase in Q-Fever could be partly due to increased visits to petting farms. but consumption of contaminated raw milk and dairy-products are recognized sources.
Advances in the technical capabilities across the public health and veterinary institutes responsible for surveillance are required to progress detection, diagnosis and typing, and to allow comparability of European data. Data from livestock as well as from human disease will help to identify infection foci and/or identify investigations to elucidate the likely source of infection.

Results

Comparison of serological methods

An inter-laboratory ring trial was conducted to compare the sensitivity and specificity of available tests for the detection of antibodies against C. burnetii in human sera. The organizing laboratory (National Institute of Hygiene, Poland) distributed 27 blood samples obtained from patients with acute or chronic Q-fever, and 10 potentially cross-reactive sera from patients infected with other pathogens to five other workpackage partners. The assessed methods were CFT, IFA and ELISA. The results obtained showed that the specificity and sensitivity of the ELISA and IFA methods were comparable; in contrast CFT displayed a lower sensitivity especially in early acute infection.

These results were notably similar to results obtained with veterinary samples. During this relatively small ring trial, the need for standardization of available commercial IFA tests was recognised, as used antigens and cut-off values varied such that without standardizing the results weren’t comparable. Since the diagnosis of Q-fever in human medicine relies mainly on serological evidence, and the IFA is currently the reference method for serodiagnosis of Q-fever, the need for standardization is of immediate importance.

Comparison of PCR diagnostic methods using field samples

Following discussions within Workpackage 25, the Veterinary Laboratories Agency organized a small ring trial assessing different Coxiella burnetii real-time PCR assays currently in use in different partner institutes. An earlier PCR ring trial, organized by the French Food Safety Agency in 2007, used purified DNA from the reference strain C. burnetii Nine Mile (NM) to spike different matrices (milk, placenta and phosphate-buffered saline).

In this second ring trial it was decided to use a panel of 10 different DNA extracts from Q-fever positive veterinary abortion samples. The DNA extracts were distributed to seven laboratories including the Health Protection Agency (United Kingdom), the Federal Institute for Risk Assessment (Germany), the Central Veterinary Institute and the National Institute for Public Health and the Environment (The Netherlands), and Sweden’s National Veterinary Institute, which tested the extracts using in-house assays. The results showed excellent agreement between the results obtained with the different assays. In addition, it was observed, as expected, that assays targeting the IS1111 repeat element provided increased sensitivity over assays targeting single copy genes. Additionally, the conclusions derived from the first ring trial were confirmed, highlighting the robustness of the PCR detection procedures used in the different laboratories.

Some of the results from this study were presented at the World Association of Veterinary Laboratory Diagnosticians conference in Madrid in June 2009.

Molecular typing of C. burnetii

Several genotyping techniques have been applied to analyse the genetic diversity of C. burnetii strains, and until recently, pulsed field gel electrophoresis (PFGE) was considered the most discriminatory method. However, the need for bacterial culture and highly purified DNA make the method time-consuming while interpretation of banding patterns can be difficult and subjective and the results are not easily portable.

Recently, alternative PCR-based genotyping approaches have been developed, such as Multi-Spacer Sequence typing (MST) and Multiple Loci Variable number of tandem repeats Analysis (MLVA), which compensate some of the limitations of PFGE. These methods promised to be much more discriminatory than PFGE, and they could be easily implemented in laboratories and produced data that could easily be compared without the need to exchange live strains or other biological material.

Two complementary MLVA schemes for C. burnetii genotyping have been developed. Until now the available MLVA data of C. burnetii were derived from a small number of isolated strains, especially those that were used for development of the method. The use of MLVA is rapidly increasing, including within the Med-Vet-Net partnership where, for instance, it was used, along with MST, to type six strains isolated from Polish cattle and people. This small study identified two MST sequence types and four MLVA types. MLVA was also used to analyse the geographical distribution of 146 C. burnetii samples from aborting goats involved in the two recent human outbreaks in The Netherlands. From this study 11 MLVA types were identified with one type being predominantly present in the southern part of the country (131/146 of the samples). In some flocks the presence of two or three different MLVA types was observed. Finally, MLVA and MST were used in the investigation of C. burnetii genotypes circulating in France, using clinical field samples. In a recent investigation, the MST analysis of 26 samples (from three goat flocks in the Centre-Ouest region of France) was identical but the isolates could be differentiated into four groups using the two different methods of VNTR. As a result of this study we recommend the use of the two VNTR panels simultaneously to achieve optimal discrimination of strains. It was observed that these strains are different from samples originating from farms in the south-east of France, which demonstrates that this method is useful for epidemiological studies. Applying these tests to larger numbers of samples is required to better understand how different genotypes spread between animal populations.

From the French and Dutch studies we conclude that MLVA and MST are feasible and allow a rapid characterization of strains without resorting to isolation of the bacterium because clinical material can be used. This is highly relevant for the study of C. burnetii, which is an obligatory intracellular bacterium that requires handling in a Biosafety Level 3 confinement laboratory.

Following discussions during the workshop on PCR-based detection and typing C. burnetii, a typing ring test was set up. Seven institutes participated in the trial that compared MLVA and MST. All participating laboratories were provided with identical samples consisting of DNA extractions obtained from five separate cultures of the reference C. burnetii NM strain. The samples were blindly coded and the participants were asked to perform the typing in the way they were accustomed to in their laboratories. An in silico analysis was used by the organizing laboratory to interpret the results.

To briefly summarize the MST results: we observed that the data obtained were robust but only two labs used this method and the tested panel was of limited diversity thus restricting the evaluation power of the trial.

The MLVA typing results from the ring trial are presented in Table 25.1 and Table 25.2. The differences found between the laboratories are

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Sample A to E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab I</td>
<td>4, 7, 8, 8*</td>
</tr>
<tr>
<td>Lab II</td>
<td>4, 7, un, un</td>
</tr>
<tr>
<td>Lab III</td>
<td>un, 7, un, 8</td>
</tr>
<tr>
<td>Lab VI</td>
<td>un, un, un, 8</td>
</tr>
<tr>
<td>Lab VII</td>
<td>4, 7, 8, 8*</td>
</tr>
</tbody>
</table>

* The presence of double peaks or bands using sample B was observed.
highlighted. Using ‘MLVA panel I’, shown in Table 25.1, few variations were observed between the five samples and the five laboratories. Some loci were novel to a particular laboratory and could not, therefore, be assigned. Sample ms36 displayed the most variation between labs. For ‘MLVA panel II’ (Table 25.2) a higher degree of variation was observed.

Compared to ‘MLVA panel I’, the results of ‘MLVA panel II’ (Table 25.2) were less consistent since more variation was detected between laboratories — with the exception of ms31 which was correctly identified by all labs. In some cases, two profiles were observed for a single sample, whereby the number of repeats appeared to differ.

The reported inconsistencies were mostly linked to the different definitions or nomenclature used in the labs, and even some published data were inconsistent, leading to misinterpretation of the results. For instance, the results reported for ms36 (Table 25.1) were all of the correct size but in four labs they were reported differently due to nomenclature differences. Similarly, for ms24 (Table 25.2) 27 repeats were reported by labs I-III and VII, and only nine by labs IV and V, but in fact both results are correct as they refer to different repeat units: when reporting 27 repeats it refers to a motif of 7pb which produce nine repeats of 21bp. Clearly, such nomenclature needs to be standardized.

Referring to published genome sequences, however, may not solve the problem: in silico analysis would clearly identify the number of repeats present in the sequence, but as reference strains are passaged in multiple laboratories, mutations may have occurred that changed the numbers of repeats.

To conclude, two different MLVA typing schemes are equally suitable for typing C. burnetii isolates and are in use by several Workpackage 25 members, although only one laboratory uses both MLVA panels. Despite PCR conditions differing between laboratories, the major difficulties are with different product analysis, size estimation and nomenclature. The ring trial highlighted the difficulties of using VNTR-based methods without a standardized scheme for results interpretation.

It is extremely important to reach consensus on which panel to use for typing of C. burnetii and which nomenclature. For instance, the production of stable control DNA could help to accurately size amplicons and thus facilitate the interpretation. This could be achieved by plasmid cloning of VNTRs amplicons, choosing well-defined repeats confirmed by sequencing, and using these as controls.

Comparative genome hybridization of C. burnetii

The availability of a genomic sequence of the reference strain C. burnetii NM, allowed the development of microarrays for use in comparative genome hybridization (CGH). CGH is being used for the analysis of a growing number of pathogenic bacteria — comparing strains of the same species that differ in virulence potential, environmental origin, animal host and so on. The method allows a rapid assessment of variation between strains of the same species that result from differences in presence, absence or divergence of genes. CGH could, therefore, identify novel genetic markers suitable for epidemiological studies of C. burnetii provided such markers were present in the reference strain.

During the final year of Workpackage 25, genomic DNA from a set of 15 C. burnetii isolates was compared by CGH. Microarrays were designed in partnership with the National Center for Scientific Research (CNRS) Sophia-Antipolis. All 2,016 open reading frames of the reference genome were covered by carefully designed 60-mer probes. Due to unexpected experimental problems, the slides could not be spotted so this task could not be finalized within the given time frame.

Table 25.2. C. burnetii typing results using MLVA panel II (Arricau-Bouvery et al., 2006 and Svraka et al., 2006).

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Sample A to E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab I</td>
<td>8* 27 4 4 6 5 5*</td>
</tr>
<tr>
<td>Lab II</td>
<td>8 27 4 4 6 5 5</td>
</tr>
<tr>
<td>Lab III</td>
<td>un 27 un 5 8* 5* 4</td>
</tr>
<tr>
<td>Lab IV</td>
<td>9 9 4 5 6 5 5</td>
</tr>
<tr>
<td>Lab V</td>
<td>9 9 5 4 7 5 5</td>
</tr>
<tr>
<td>Lab VI</td>
<td>un un 4 un un un un</td>
</tr>
<tr>
<td>Lab VII</td>
<td>9 27 4 4 6 nr 1</td>
</tr>
</tbody>
</table>

un: unused locus in this laboratory; nr: result not readable in this lab.
* The presence of double peaks or bands using sample B was observed.

Domestic ruminants appear to be the principal reservoir of C. Burnetii.
**Workpackage 26: Annual Research Report**

<table>
<thead>
<tr>
<th>WP number</th>
<th>26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>Virulotyping of new and emerging Salmonella and VTEC</td>
</tr>
<tr>
<td>WP Leader</td>
<td>Roberto LA RAGIONE</td>
</tr>
<tr>
<td>Name and Address</td>
<td>Veterinary Laboratories Agency (VLA) (Weybridge) Dept. Food and Environmental Safety Woodham Lane, Addlestone Surrey KT15 3NB UNITED KINGDOM</td>
</tr>
<tr>
<td>Project Start date</td>
<td>1 March 2006</td>
</tr>
<tr>
<td>Project End date</td>
<td>31 October 2009</td>
</tr>
</tbody>
</table>

**Progress summary**

During the final year of Med-Vet-Net, Workpackage 26 proceeded extremely well with excellent participation by all institutes. The development and implementation of diagnostic array platforms and PCR tests for virulotyping of *E. coli* and Salmonella across Europe have been major project outputs.

The studies during Year 5 have been wide-reaching and included *in vitro* and *in vivo* characterization of Salmonella virulotypes, together with a vast amount of molecular work. Detailed microarray analysis of Salmonella has revealed that the five most prevalent human serovars all possess distinct virulence gene repertoires. Identical or similar virulo- and resistance-types were identified in all of the participating countries. VTEC microarray studies revealed that the majority of VTEC strains harbour a typical profile that is comparable to that of the type pathogen *E. coli O157:H7*. Furthermore, many serogroups, including O157, have distinct virulence profiles more similar to those normally associated with enteropathogenic *E. coli* (ETEC) or enterotoxigenic *E. coli* (ETEC). The workpackage outputs are currently being drafted into seven peer-reviewed papers.

Following the success of the previous Workpackage 26 meetings held at the VLA, BfR and ISS, a final meeting was held at the VLA. This final gathering not only enabled detailed scientific discussion of the data collected within Workpackage 26, but also initiated multiple, novel collaborations. Through Med-Vet-Net contacts, and presentations of Workpackage 26 results, a number of Short Term Missions have been facilitated, including exchange visits to the VLA from Italy, Ireland, Sweden, Poland, Hungary and Holland.

**Objectives**

The overall aim of Workpackage 26 was to gather genetic data relating to specific virulence determinants from participating institutes in order to determine the distribution of such virulence determinants in Salmonella and *Escherichia coli* within Europe, and harmonize data sets. The long-term goal was to exploit the data by devising appropriate platforms for rapid analysis in routine diagnosis, for example microarray type. This was undertaken through the following sub-objectives:

- establishing a core group of institutes (Istituto Superiore di Sanita (ISS), Federal Institute for Risk Assessment (BfR) & Veterinary Laboratories Agency (VLA)) with expertise in the polymerase chain reaction (PCR) and microarray area
- linking with Workpackage 21’s *Salmonella* Genome Island 1 (SGI1) project (with isolates from SGI1 included)
- evaluating array platforms currently being developed for future applications in rapid molecular detection and diagnosis of enteric pathogens in the food chain
- identifying suitable methods for data analysis and collating the generated data into a database
- identifying gaps within the virulotype database that could be exploited in future studies within the Network
- performing targeted and functional virulotyping of verocytotoxin-producing *E. coli* (VTEC) and atypical *E. coli O157:H7*
- studying the effect of specific mutations on the *Salmonella* virulence plasmid and pathogenicity islands using a day-old chick model
- identifying differences at the genomic level to define the virulence genes for VTEC pathogenic to humans
- developing PCR tools for Stx2-converting phage typing
- identifying possible virulence predictors for the early detection of VTEC of public health significance
- performing specific virulence arrays on an extended subset of *Salmonella* isolates in order to study pathogenicity and epidemiology
- performing analyses of data generated and undertaking *ad hoc* analysis of the micro and nano array analysis on selected VTEC and *Salmonella* isolates.

**Key achievements to date**

- Successful workpackage workshop on virulotyping of *E. coli* and *Salmonella* held at the VLA.
- Development of virulotyping platforms for *Salmonella* and *E. coli* for routine diagnostic use across partner institutes in Europe.
- Identification of possible virulence predictors for VTEC of significant public health impact.
- Comparison of O1 genes present in the top non-O157 VTEC serotypes compared to those found in VTEC O157.
- Microarray comparison of VTEC seropathotype B, C, and D, with seropathotype A (O157).
- Multiple training and visiting scientist exchanges.

**Background**

*Salmonella* and VTEC are important food-borne pathogens, and identifying new and emerging pathotypes is essential if intervention strategies are to be developed. There is a wealth of expertise on *Salmonella* and VTEC within Med-Vet-Net and one of the principal aims of this project was to exploit this expertise.

Virulotyping is a newly emerging genomic-association approach to bacterial molecular epidemiology involving the use of DNA arrays designed to determine the presence (*conservation*) or absence (*divergence*) of potential virulence-related genes. Institutes within the Network have established different genotypic and phenotypic typing methods that are being used to characterize virulence determinants in *Salmonella* and VTEC. This project aimed to bring together all interested parties and exploit molecular expertise within Med-Vet-Net by selecting appropriate isolates by PCR analysis and then using a subset of these isolates to assess the available virulence arrays for *Salmonella* and *E. coli*, developed by BfR, ISS and VLA.

**Key outcomes of scientific approaches**

**Selection of *Salmonella* strains for microarray analysis.**

DNA microarray experiments were performed as previously described in Deliverable D26.1. Normalized presence/absence data of each strain were imported in BioNumerics as character values. A cluster calculation analysis was performed with the simple matching binary coefficient using the un-weighted-pair group method with arithmetic averages (UPGMA dendrogram type). The maximum parsimony clade analysis was performed with 1,000 bootstrap cycles, and the exported rendered tree was performed with hidden branches and distance labels shorter or equal to one and rooted tree type.

Forty-two strains belonging to serovars Enteritidis, Typhimurium, Infantis and Virchow were included, together with 56 strains of other serovars for a total of 98 strains. These were investigated using a microarray covering 104 *Salmonella* virulence determinants, such as type 1 or type 3 secretion systems, outer membrane proteins, secreted proteins, Vf antigen encoding genes, virulence markers located in prophages, and genes belonging to various fimbrial clusters. Based on the presence/absence of 104 virulence gene markers, the relationship between the serovars was represented by a maximum parsimony tree, as shown in Figure 26.1.

The tested serovars harboured different sets of virulence genes. Strains belonging to the same serovar mostly clustered together with regard to the virulence repertoire they harboured, with one
Many isolates resembled EDL933 (Figure 26.2) and these were all O157 strains. Most of these strains harboured typical VTEC characteristics such as locus of enterocyte effacement (LEE)-encoded genes (eae, espA, espF, tir), stx2A and stx2B, pO157 plasmid encoded genes (hlyA, espP, espD, ktpA, tctxE, espI), and tccP. Nevertheless, variation was observed in the presence of stx1A, stx2AB, (with one O157 isolate lacking all stx genes), the non-LEE encoded effecter protein genes nleA, nleB, nleC and cdB. This analysis identified some core genes commonly harboured by VTEC strains that can be used as marker genes for this pathogen.

The remaining strains either contained the stx genes (defining them as VTEC) or missed the toxin genes. The VTEC isolates contained the majority of O26 strains as well as other serotypes (O157, O91, O113, O77), Serotypes O91, O113 and O77, which are not commonly associated with human disease, nevertheless harbour several VTEC characteristics similar to O26 VTEC strains that are the second most prevalent serotype, after O157, to be associated with human infection.

Genes typically present in the EDL933-like strains, such as the LEE encoding genes eae, tir, espF, and espP, were absent from these strains although the pO157 plasmid-encoding genes hlyA and espP were present in several isolates. The majority of strains that were stx positive harboured the iss gene for increased serum resistance. Several O157 isolates that were not EDL933-like were identified (indicated by an asterisk). Two of these O157 strains harboured typical EPEC genes bfp and perA as well as the LEE encoded genes eae, espA, espF, and tir. Several other atypical O157 strains were identified that missed stx1 and 2 but contained some LEE (eae, espA, espF) and non-LEE (ktpA, espI, nleB) genes. Several O157 strains did not harbour any virulence genes present on the array. One O157 strain, which was haemolytic, had ETEC-like characteristics and harboured the heat-stable toxin genes stx1 and stb, and the F18 fimbrial genes fedA and fedF, whereas it also harboured the EHEC fimbrial gene fimA, which has been

exception: Salmonella Hadar is divided into two groups, one bearing similarity to S. Virchow and the other similar to Salmonella Newport. Both groups differ mainly in the absence/presence of giiA (encoded by Gifsy-2) and sopE1 (encoded by phage SopEphi). No correlation was found between the country of isolation or source (for example, human or poultry) and the absence/presence of these genes.

Specific correlations with the source of the isolate (human, animal) or with the country of origin of the isolates could not be identified. Consequently, variations within a serotype are observed independently from the country where the strain was isolated. There were some indications that strains belonging to rarely isolated serovars harboured their own virulence and fimbrial gene repertoires. For instance, IpfD was absent in these but cdB was found exclusively present.

Genes from Salmonella Pathogenic Islands (SPIs) were highly conserved throughout the serotypes. Exceptions were avrK and avsB (SPI1) and the left region (rhuM and sugI) of SPI3, which were deleted in some strains. The highest variability between and within a serotype was recognised for prophage associated virulence markers. A proportion of the S. Typhimurium strains harboured the Salmonella Genomic Island 1 (SGI1), especially in definitive phage type (DT) 104 and phage type U302. Other serotypes harbouring the SGI1 were distributed in distinct branches across the parsimony tree, supporting the hypothesis that each serovar encodes a distinct virulence gene repertoire.

Selection of VTEC strains for microarray analysis
As part of the Workpackage 26 activities, an E. coli virulence gene chip for pathotyping E. coli was developed based on 60 genes that included 46 virulence genes plus controls. Fifty-three selected Salmonella isolates were virulotyped using this chip. Additional virulence genes were subsequently included; especially those associated with VTEC, so there were 80 genes present on the chip. Prior to its introduction to the routine reference laboratory, the chip was validated using a control sequenced VTEC strain, EDL933 (O157:H7).

Fifty-eight strains were selected for virulotyping using this extended chip to determine which strains harbour typical VTEC characteristics of EDL933 as a reference, and to distinguish these from more atypical isolates. The microarray hybridization data were analysed in GeneSpring V5 and clustered using Pearson Correlation Coefficient. The resulting virulence profiles from serotypes O157, O26, O113, O91, O77, O158, O159, O160 and O161 were analysed using the miniaturized virulence microarray EC03. The results indicated that the strains clustered in groups as shown in Figure 26.2.

Figure 26.2. Clustering of E. coli strains according to presence or absence of virulence genes, as determined by microarray. To the left are 24 O157 strains resembling VTEC EDL933. An asterisk identifies other O157 isolates that were not EDL933-like.
We were able to identify a single marker for non-O157 VTEC serogroups (described in detail in Molecular tools were employed to characterise the transducable VT-coding genes, respectively. The genes present in the top non-O157 VTEC serotypes were compared to those found in VTEC O157. The studies demonstrated that some genes were conserved within all non-O157 VTEC strains tested, but diagnostic tools to accurately distinguish between the different serotypes could not be designed based on the outcome of this study. Nevertheless, the data generated have provided additional information on the genetic makeup of VTEC, including the finding that, of all serotypes studied, VTEC O26 is most closely related to an O128 strain (E15711) from the United Kingdom (UK) while another (ECA95) was more similar to an O113 strain (ECA97) from France. The two UK O128 strains tested were not similar to each other.

This study defined a set of core genes for VTEC. The genes present in the top non-O157 VTEC serotypes were compared to those found in VTEC O157. The studies demonstrated that some genes were conserved within all non-O157 VTEC strains tested, but diagnostic tools to accurately distinguish between the different serotypes could not be designed based on the outcome of this study. Nevertheless, the data generated have provided additional information on the genetic makeup of VTEC, including the finding that, of all serotypes studied, VTEC O26 is most closely related to an O128 strain (E15711) from the United Kingdom (UK) while another (ECA95) was more similar to an O113 strain (ECA97) from France. The two UK O128 strains tested were not similar to each other.

We concluded that the chip was able to distinguish typical VTEC strains that showed virulotyping profiles similar to EDL933, and these were all of serotype O157. Conversely, however, not all O157 isolates were VTEC or EDL933-like. A number of atypical VTECs of various serotypes were also identified.

The chip was also able to distinguish O157 strains that lacked the stx genes (non-VTEC) but harboured a variety of genes typically associated with EPEC, ETEC and EAEC. The use of this chip in routine reference laboratories in future will provide a cheap and rapid method to spot the emergence of new pathotype combinations, as well as new serotypes normally not associated with human and/or animal disease. Indeed much of the technology described here is now offered in a commercially available chip.

Identification of possible virulence predictors for VTEC of significant public health impact

Two regions within the VT2-converting phage of human VTEC O157 strains belonging to Phage Type 8 (PT8) were identified that were polymorphic with respect to the published sequence of the reference VT2 phage BP933W. These regions span the genes gam/cil and ica/S and encode the genetic determinants governing the balance between lytic and lysogenic cycle and the transducible VT-coding genes, respectively. Molecular tools were employed to characterise these DNA traits (described in Deliverable D26.8) and to evaluate the virulence prediction value of this polymorphic phage for both O157 and non-O157 VTEC serogroups (described in detail in Deliverable D26.13).

We were able to identify a single marker for particular VTEC O157 strains linked to an increased potential of causing disease in humans.
## Workpackage 27: Annual Research Report

<table>
<thead>
<tr>
<th>WP number</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>Harmonization of Trichinella infection control methods, quantitative risk assessment in pigs and an early diagnosis in humans to increase treatment efficacy (TRICHIMED)</td>
</tr>
<tr>
<td>WP Leader</td>
<td>Pascal BOIREAU</td>
</tr>
<tr>
<td>Name and Address</td>
<td>French Food Safety Agency (AFSSA) — LERPAZ 23 avenue du Général de Gaulle 94700 Maisons-Alfort FRANCE</td>
</tr>
<tr>
<td>Project Start date</td>
<td>1 March 2006</td>
</tr>
<tr>
<td>Project End date</td>
<td>31 October 2009</td>
</tr>
</tbody>
</table>

### Progress summary

**Trichinella** is one of the most widespread parasites infecting animals and humans. Control and diagnostic methods are essential tools to investigate the epidemiology and the infectivity of the parasite. As such, the harmonization and improvement of control and diagnostic methods are of primary importance for the control of the infection. To that end a novel indirect ELISA was developed using an antigen specific to the early stage of *Trichinella* infection. This ELISA shows important advancements over the ELISA test currently on the market. A gold standard for the molecular typing of *Trichinella* has been selected following ring trials between various workpackage laboratories. The tool can be applied to both diagnosis and routine laboratory work.

Various microsatellite sequences have been identified which could be used to study gene flow in *Trichinella*, and identify double infections or to trace back the origin of infection. To obtain a better understanding of the host changes induced by *Trichinella* infection, clinical and pathological studies were performed using a rat model and the effect of infections (at low and heavy larvae load) was analysed. The role of rats as a reservoir of *Trichinella spiralis* was also studied in natural conditions.

The harmonization of the research between the partner laboratories depends on the use of well-characterized material; the production and characterization of reference samples achieved by the workpackage have been of outstanding importance, as they provide essential material for various scientists working in the field.

To round off the Workpackage 27 collaborations, a number of integrated projects (fundamental research and applied research) have been produced with the goal of extending the collaboration between the various institutes beyond the end of Med-Vet-Net.

### Background

**Harmonization and improvement of official meat inspection methods to detect Trichinella larvae in domestic pigs**

The goal of this task was to compare the accuracy of artificial digestion methods that are used for routine *Trichinella* inspections in pigs according to current European Union (EU) legislation, and to improve laboratory interpretation. The magnetic stirrer method is the current reference method for detecting larvae in meat.

The double funnel method is a variation of the magnetic stirrer method — repeating the step of settling the digestion liquid down in a separation funnel is the important difference. We compared the classic magnetic stirrer approach (single settling) with the double funnel variant using proficiency samples. The results showed no significant difference between the two approaches so the simpler method can be used to save resources.

An optimization of the direct detection test on unusual meat samples was also performed. Some human infections have been related to the consumption of salted or smoked pork meat. Salted samples from porcine material obtained from a parallel project were prepared and analysed using the magnetic stirrer method. The results showed good performance for both fresh and dry salted material although particular attention should be paid to remove fat from the dry samples, which can be difficult when meat and fat are closely entangled.

**Comparison of the sensitivity and specificity of existing and newly developed tests for the diagnosis of trichinellosis at the early stage of infection**

The objective of this task was the comparison, in terms of sensitivity and specificity, of various methods for the detection of *Trichinella* infection in pigs at the early stage of infection with the gold standard method prepared by the European Community Reference Laboratory (Istituto Superiore di Sanita, ISS). An early antigen (NBL1) was chosen, purified and characterized for the development of a new indirect ELISA. The ELISA was tested with negative sera from specific pathogen free (SPF) and conventional pigs and sera from experimentally infected pigs (*T. spiralis*, *Trichinella britovi*, *Trichinella pseudospiralis*, *Trichinella nativa*), with larvae burdens of 20,000 larvae/pig, 1,000 and 200 larvae/pig. The high level infections showed seroconversion as early as the third week using the new iELISA, which is an improvement of one to two weeks compared to conventional ELISA. Although the sensitivity of the test was lower for low level of infections it was still acceptable (Figure 27.1). The next step will be the coupling of NBL1 antigen with a late antigen, which should be able to keep the signal above detection during prolonged infections. The test has also been used on human samples.

### Objectives

The overall goal of Workpackage 27 was to harmonize and improve official meat inspection methods for detecting *Trichinella* larvae. To that end we formulated the following objectives:

- evaluate the double funnel methods on calibrated samples and optimise the official inspection method for unusual meat samples
- develop novel detection methods particularly suitable for the detection of *Trichinella* DNA from faeces in the early stages of *Trichinella* infection
- produce a map correlating animal and human infections that can be updated with novel data from the analysis of new samples to better understand the epidemiology of *Trichinella*, and investigate the use of microsatellite sequences for tracing back *Trichinella* infections
- prepare reference material (antigens, sera) and distribute throughout the workpackage
- extend collaboration between the various groups beyond the end of the workpackage.

### Key achievements to date

- Development of a new indirect enzyme-linked immunosorbent assay (iELISA) for the detection of early *Trichinella* infections in pigs.
- Identification of microsatellite sequences.
- Clinical and pathological studies on rats that shed light on host response after *Trichinella* infections.
- Development of a gold standard for molecular *Trichinella* typing.
- Production and characterization of well-characterized reference material for Med-Vet-Net laboratories and scientists working in the field.
- Maintenance and extension of the strain repository at the International *Trichinella* Reference Centre at ISS.

---

Scientists aimed to improve official meat inspection methods to detect *Trichinella* larvae in domestic pigs.
To standardize a molecular typing method and prepare reference DNA material

This task was dedicated to the definition of a gold standard for molecular typing, and the preparation of DNA for ring trials according to the chosen method.

To start with, various DNA extraction methods were reviewed and two were selected on the basis of concentration, purity, simplicity and suitability for multiplex-polymerase chain reaction (PCR), microsatellites testing and variable number of tandem repeats (VNTR). Of these three tests, multiplex-PCR was selected as the best method for typing, and seven workpackage laboratories undertook ring trials using reference material. Transportation methods were also compared, in particular freezing, lyophilization and absorption on Whatman paper (Figure 27.2). Freezing turned out to be optimal for shipping the material during the ring trials. The typing results from the seven laboratories showed that multiplex-PCR is easy to perform and is reliable with high sensitivity, which would recommended it for use in diagnostic assays as well routine laboratory work.

Analysis of the reservoir(s) of Trichinella parasites

The objective of this task was to assess the risk of Trichinella in humans from wildlife in geographically defined areas. A map showing the spatial distribution of infections in humans
Figure 27.3. Immune response of T. spiralis infected rats. Average normalized OD-Blank values are given per infection dose group. OD values of the groups that received 12,000 or 14,000 muscle larvae per animal declined (results not shown). Animals of those groups also displayed the lowest average body weight throughout the experiment. Standard deviations are not displayed for the sake of clarity.

Figure 27.4. ELISA results of serum and meat juice samples from pigs experimentally infected with T. spiralis and T. britovi after slaughter (larvae per gram, lpg, is given for the diaphragm muscle).

Trace back of Trichinella infection

Genetic polymorphisms can be used to trace the origin of food infections or contaminations. Various marker sequences were selected and analysed during the previous year in search of genetic variability in T. spiralis. The screening of 1,000 sequences allowed the identification of eight polymorphic loci. This is probably an underestimation of the true polymorphism in microsatellites.

The availability of the T. spiralis genome sequence will enable identification of a higher number of polymorphic loci and studies of the population structure of the nematode. The use of microsatellites to study single larvae has detected genetic variability inside each isolate as well as between isolates.

In the past, the identification of single larvae allowed detection of mixed infections only when different species were present, but now we can detect double infections due to the same species in order to study gene flow in Trichinella. This opens a window of opportunity to study the genetic population of these zoonotic parasites and to trace back the origin of infection in humans and animals. The same approach could be applied to at least one other European species, namely Trichinella britovi.

Maintenance of the repository

The previously established Trichinella strains and isolates repository represented a source of reference materials for the workpackage partners. Trichinella isolates and strains from infections in humans and animals are maintained at the International Trichinella Reference Centre, ISS. This central repository has been the official reference laboratory for the World Organisation for Animal Health since 1988, and for the International Commission on Trichinellosis since 1992. Moreover, ISS is now the Community Reference Laboratory for Parasites (see www.iss.it/crlp).

Twenty-two reference strains are maintained in CD-1 outbred mice by serial passage. An additional 44 Trichinella isolates are maintained in vivo as a genetic bank for further identification.

Production, storage and characterization of reference sera

The aim of this task was to produce reference pig sera for all partner institutes. For this purpose, pig experiments using SPF and conventional pigs, were conducted with two Trichinella species: T. spiralis and T. britovi.

Sera and meat juices from SPF infected pigs were collected, and a molecular characterization was undertaken (Figure 27.4). Workpackage 27 partners used this material as reference material.

The analysis using the classical ELISA demonstrated seroconversion at four weeks after infection with T. spiralis, and five weeks after infection with T. britovi. The results obtained with the IELISA were confirmed by western blot experiments (same time of early seroconversion and same frequency of antibody detection). Long-lasting infection experiments (nine months) were also performed with conventional pigs infected with T. spiralis and T. britovi, and sera and meat juice samples were characterized.

Sera from the various pig experiments were selected, aliquoted, lyophilized and stored for use by workpackage partners in various tests — and it proved to be a very useful resource.

Participation in a European network and preparation of a new integrated project on Trichinella

Two projects have been prepared for future collaboration between Workpackage 27 partners.

The main objective of the first, fundamental project is to discover the key players of host cell and parasite interaction during the Trichinella cycle with a main focus on the nurse cell. The second project focuses on applied research.
Workpackage 28: Annual Research Report

WP number 28
Title Methods of attributing human Salmonella and Campylobacter infections with different animals, food and environmental sources
WP Leader Tine HALD
Name and Address Danish Technical University (DTU)
Danish Zoonosis Centre
Danish Institute for Food and Veterinary Research
Mørphej Bygade 19, DK-2860 Søborg, DENMARK
Project Start date 1 March 2006
Project End date 31 October 2009

Progress summary
To identify and prioritize effective food safety interventions, it is critical to attribute human zoonotic infections to the responsible sources. A variety of approaches exist for source attribution differing in characteristics, data requirements, advantages and limitations.

Workpackage 28 aimed at applying source attribution approaches, and comparing, discussing and recommending the appropriate use of four of these approaches to address different public health questions. Four source attribution approaches were applied to Salmonella and two approaches to Campylobacter. Methods were applied to different populations and time periods. All projects were completed during this final year of the workpackage.

Two microbial subtyping approaches were applied to attribute human salmonellosis. In the first, human salmonella infections reported in Denmark were attributed to the responsible sources using a three-dimensional (3D) approach that constitutes a methodological improvement of the model developed by Hald et al. (published in 2004). In the second approach, the original model described by Hald and colleagues was adapted to Salmonella and two approaches to Campylobacter. Methods were applied to different populations and time periods. All projects were completed during this final year of the workpackage.

A systematic review was undertaken of case-control studies, including studies on sporadic campylobacteriosis and salmonellosis published in the past two decades (not restricted to EU Member States). The objective of the work was to identify the most important risk factors for the two diseases. Sub-analyses of risk factors by geographic region, age group, and study period were also conducted. The approach proved useful for the attribution of these and other food-borne pathogens.

An analysis of data from outbreak investigations for source attribution used data, provided by EFSA, from Salmonella and Campylobacter outbreaks reported in European countries over two years. Since reporting of the causative vehicles of outbreaks was not harmonized between and within countries, we organized the implicated foods in mutually exclusive food categories based on the implicated simple and complex foods. The developed method can be applied to a variety of food-borne hazards and is appropriate for agents that are frequent causes of outbreaks.

All methods, including a previously described comparative exposure assessment model for Salmonella source attribution, were discussed and compared in light of data requirements, method characteristics and utility of the results. A comparison of all obtained results revealed that the estimated relative importance of the most important sources was in agreement for all methods, confirming that all of the methods provide useful tools to attribute human food-borne illness. In conclusion, the different approaches produced useful answers to various public health questions, whereby the integration of results from more than one method can potentially add insight into the contribution of different sources for the human burden of disease, and can strengthen confidence in the results. We present recommendations on how to choose one or more source attribution methods to address different risk management problems. For this purpose, a tool-box (source attribution toolbox) was published in the thesis of the PhD student based on Workpackage 28 experiences was developed. A PhD project was also conducted under the Workpackage 28 umbrella. The resulting thesis describes in detail all of the materials, methods and findings summarized in this report.

Objectives

- To estimate the number and/or proportion of Salmonella and Campylobacter human infections attributable to various food sources through four different approaches: microbial subtyping, risk (exposure) assessment, analytical epidemiology, and outbreak investigations.
- To compare and discuss the results obtained from the four different approaches.
- To make recommendations on the approach that may best answer different questions.
- To make recommendations on how the results can be used for prioritizing sources for control.

Key achievements to date

- Review paper on the harmonized concepts, definitions and methods used for the attribution of human illness to specific sources (Food-borne Pathogens and Disease, 2009, 6(4): 417-424).
- Development of a three-dimension Bayesian model for the attribution of human salmonellosis to specific sources, and the estimation of the public health impact of different Salmonella subtypes (Food-borne Pathogens and Disease, 2009, in press).
- Paper describing an analysis of data from outbreak investigations for source attribution of human salmonellosis and campylobacteriosis in European countries, submitted to the European Food Safety Authority (EFSA) and European Union (EU) Member States, and ready for submission to a scientific journal.
- PhD thesis, entitled “Attributing human salmonellosis and campylobacteriosis to food, animal and environmental sources”, printed and available for interested parties.
- Systematic review of case control studies of sporadic salmonellosis and campylobacteriosis completed.

Background

Many countries have implemented intervention programmes to prevent and control food-borne zoonoses during the past decades. However, data on the public health impact of such measures is still limited since information on the number or proportion of cases associated with a particular source is often lacking. In order to identify and prioritize appropriate food safety interventions, it is crucial to relate the zoonotic pathogen to the responsible source(s) of infection.

Several approaches to attribute human illness to the responsible source are available, including microbial subtyping, exposure assessment, analytical epidemiological studies, analysis of outbreak data, population studies and expert elicitations. The methodologies present different data requirements, advantages and limitations, and their usefulness is therefore dependent on the public health questions requiring answers. Workpackage 28 aimed to apply the different methodologies and explore their usefulness in different situations. Four methods were applied to Salmonella spp. and two methods to Campylobacter spp. The approaches were compared and discussed, and recommendations on choosing the most appropriate method were subsequently formulated.

Approaches

Microbial subtyping

Two microbial subtyping approaches were applied to attribute human salmonellosis; firstly to data originating from Denmark, and secondly to other European countries. In the first, human Salmonella infections reported in Denmark over a three-year period were attributed to the responsible sources using a 3D microbial subtyping approach that constitutes a methodological improvement of the model developed by Hald et al. (2004).

Table eggs, imported chicken, and pork were assessed as the most important sources of salmonellosis in Denmark, with results showing that travel was the single most important cause of salmonellosis in Denmark (illustrated in Figure 28.1).

Salmonella Newport, S. Virchow, S. Thompson and S. Enteritidis were found to be the serotypes with the highest impact on the number of reported cases. The 3D model achieved more robust estimates of the proportion of subtype-related factors that describe the ability of different Salmonella subtypes to cause disease and, consequently, of the proportion of disease
attributed to each source. In addition, the developed model can be applied to data with a lower discriminatory power, when, for example, only serotyping information is available as is the case in many countries, making the model suitable for worldwide use.

In the second approach, the original model, as described by Hald and colleagues, was adapted to Salmonella surveillance data from Sweden, the United Kingdom, The Netherlands, France, Germany and Poland. The required adaptations of the model were successful for the first four of these countries. The relative importance of sources varied between countries reflecting differences in data availability, the coverage of surveillance systems, the subtypes causing reported disease, and the epidemiology of salmonellosis in each country. Source attribution results for Sweden, the United Kingdom and The Netherlands were further compared with results of the Salmonella source attribution from Denmark.

Systematic review of case-control studies

Workpackage 28 carried out a systematic review of collected case-control studies, and an analysis of the literature on sporadic campylobacteriosis and salmonellosis published throughout the world in the past two decades. The aim of the study was to identify the most important risk factors for the two diseases. Data were extracted from 71 studies — 34 of which investigated risk factors for human salmonellosis, and 37 that focused on campylobacteriosis.

We assessed that eating undercooked chicken, raw eggs, and travelling abroad were the most important risk factors for salmonellosis, whereas travelling abroad, daily contact with a pet, and eating undercooked chicken were estimated to be the most important risk factors for campylobacteriosis. Sub-analyses of risk factors by geographic region, age group, and study period suggested that the major sources and risk factors of Salmonella and Campylobacter are similar in different populations and time periods, although they may vary to some extent in order of importance.

An analysis of data from outbreak investigations was the fourth approach applied for source attribution of human food-borne disease. The method used data, provided by EFSA, from investigations of Salmonella and Campylobacter outbreaks reported in European countries in 2005 and 2006. Because the reporting of simple foods (that is, foods belonging to one single food category) and complex foods (belonging to multiple food categories). We estimated that the most important food sources for human salmonellosis were eggs, meat, and poultry-meat, and the majority of the cases of campylobacteriosis were attributed to chicken. For both pathogens, a large proportion of cases could not be linked to any source. Results also revealed regional differences in the relative importance of specific sources (Figure 28.2).

All methods were discussed and compared in light of data requirements, method characteristics and utility of the results among Workpackage 28 members. The methods presented here were further compared with a previously developed comparative exposure assessment approach for the attribution of human salmonellosis in Denmark. A comparison of all obtained results revealed that all methods (four methods for salmonellosis and two for campylobacteriosis) agreed on the estimated relative importance of the most important sources. This suggests that all methods constitute useful tools to attribute human food-borne illness.

Figure 28.1. Proportion of human sporadic cases attributed to specific sources, travel and unknown (%), in Denmark 2005–2007.

Eating raw eggs is one of the most important risk factors for salmonellosis.
Table 28.1 presents a comparison of obtained results for the attribution of \textit{Salmonella} infections. Furthermore, some methods investigated the role of non-food routes of transmission (for example, direct contact with animals and environmental transmission), and results showed that routes other than food-borne should be considered when identifying and prioritizing control measures to prevent and reduce the human incidence.

We concluded that the different approaches proved useful to answer different public health questions, and the integration of results from more than one method can add insight into the contribution of different sources for the human burden of disease, and strengthen confidence in the results. We presented recommendations on choosing one or more source attribution methods to address different risk management problems. To that end, a “source attribution tool-box” was developed based on the workpackage experiences and is published in full in the PhD thesis funded by this Workpackage.

\textbf{Workshop}

As all methods and results had been fully discussed with the Workpackage participants during the course of the project, a final workshop was not considered necessary. Deliverable D28.12 was therefore withdrawn.

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
\textbf{Point of attribution} & \textbf{Point of reservoir} & \textbf{Point of reservoir/ exposure} & \textbf{Point of exposure} \\
\hline
\textbf{Methods} & MSA (DK, 2005)$^\text{1,4}$ & CEA (DK, 2005)$^\text{1}$ & SR-CC$^\text{3}$ & OUTBAN$^\text{4}$ \\
\hline
Eggs & 10.0 & 29.7 & 19 (3.4; 2.4; 2.3) & 20.8 \\
Pork & 11.4 & 16.1 & 5.6 & \\
Beef & 1.9 & 1.2 & 5.2 (2.0) & 2.9$^\text{2}$ \\
Chicken & 2.8 & 5.8 & 22 (7.3; 2.5) & 25.5$^\text{1}$ \\
Turkey & – & – & 0 & \\
Ducks & 2.0 & 0.4 & – & \\
Poultry & 4.8 & 6.2 & – & 25.5$^\text{2}$ \\
Imported pork & 0.8 & 5.3 & – & \\
Imported beef & 3.1 & 2.7 & – & \\
Imported chicken & 6.9 & 6.8 & – & \\
Imported turkey & 0.7 & 6.2 & – & \\
Imported ducks & 0.9 & – & – & \\
Imported poultry & 8.5 & 13.9 & – & \\
Vegetables & – & 0.3 & 8.0 & \\
Fruits & – & 12.3 & 0 & \\
Dairy products & – & – & 0.7 & \\
Travel & 27.2 & – & 10 (5.4) & 1.1 \\
Unknown & 27.9 & – & – & 22.3 \\
\hline
\end{tabular}
\caption{Results of source attribution methods applied to \textit{Salmonella}.}
\end{table}

MSA. Microbial subtyping; CEA: comparative exposure assessment; SR-SCC: systematic review of case-control studies on sporadic cases; OUTBAN: outbreak investigation data

1 Results are presented in percentage (%).
2 Results as estimated in the 3D model. These estimates are only used in this comparison, and not in the comparison between microbiological approaches.
3 Results are presented in % (relative weights), and odds ratio for risk factors within main reservoirs in brackets.
4 Results of the sub-analysis for the north of Europe.
* Sum of all poultry categories.
† Group domestic and imported products.
# odds ratio <1.

Data from outbreak investigations were analysed.
Workpackage 29: Annual Research Report

WP number 29
Title Surveillance of emerging antimicrobial resistance critical for humans in food, environment, animals and man
WP Leader Bruno GONZÁLEZ-ZORN
Name and Address Universidad Complutense de Madrid (UCM)
Departamento de Sanidad Animal
Facultad de Veterinaria
Avda. Puerta de Hierro s/n
28040-Madrid, SPAIN
Project Start date 1 March 2006
Project End date 31 October 2009

Objectives
The overall objective of Workpackage 29 is to study the spread and dissemination of antimicrobial resistance determinants between humans and animals. It will focus on novel and emerging antimicrobial resistance determinants such as 16S rRNA methylases and fluoroquinolone resistance genes of the qnr family.

As the emergence of resistance is a dynamic process, a third and ongoing objective was to monitor emerging AMR within the European Union (EU), and respond to, and focus on, antimicrobial resistance determinants important to human and animal health.

Key achievements to date
- Characterization of human isolates in Poland as a major reservoir of 16S rRNA methyltransferases.
- First identification of the rmtC methyltransferase in the EU.
- First identification of a 16S rRNA methyltransferase in a food isolate.
- First identification of armA in a food isolate.
- First identification of a qnr gene in Salmonella enterica.
- Review, nomenclature and detection of aminoglycoside resistance determinants in bacteria.
- Aminoglycoside resistance and virulotyping in Escherichia coli.
- Diversity of tet(A) in Salmonella in Europe.

Antimicrobials (CIAs) in human medicine.

We detected rmtC in Salmonella enterica Virchow in clinical and food isolates in the United Kingdom (UK). This is the first time rmtC has been detected in Europe, and the first time rmtC has been identified from food isolates. All isolates were clonally related and bore the rmtC gene on the bacterial chromosome. These results confirm spread of rmtC and imply a novel route of transmission for these emerging resistance determinants.

Five types of 16S rRNA methyltransferase genes (armA, rmtA, rmtB, rmtC and rmtD) conferring resistance to aminoglycosides have been identified — armA and rmtB have spread in enterobacteria worldwide and are the only methyltransferases identified in Europe so far. With the exceptions of armA and rmtB in porcine E. coli from Spain and China respectively, all methyltransferase genes described to date have been identified in human clinical samples, for which any role of food in the transmission of these determinants remains largely unknown.

In this study, 81,632 salmonellae and 10,700 E. coli, from the Health Protection Agency’s (HPA) Centre for Infections’ culture collection, isolated from January 2004 through to October 2008 were screened for the presence of 16S rRNA methyltransferases. Salmonella enterica (56 isolates) and E. coli (24 isolates) were selected based on their resistance to amikacin (breakpoint concentration = 4µg/mL).

Since 16S rRNA methyltransferases confer high-level resistance to amikacin, 13 S. enterica isolates were selected based on their ability to grow on Lissosistent agar containing 500mg/L amikacin — none of the E. coli grew under these conditions. Further antimicrobial susceptibility testing by microdilution confirmed high-level resistance to 4,6-disubstituted 2-deoxystreptamines.

Polymerase chain reaction (PCR) screening of the 13 isolates for armA, rmnA, rmnB, rmnC and rmnD, identified the rmtC gene. Nucleotide sequencing of the amplicons confirmed an rmtC gene with 100% identity with those originally identified in the Proteus mirabilis isolated from a patient in Japan, and a P. mirabilis strain (JIE273) from Australia. To our knowledge this is the third report of rmtC-bearing bacteria.

PCR of the emerging transferable fluoroquinolone-resistance determinants qnr-like, qepA and aac(6’)-Ib-cr, was performed on all isolates. Sequencing of the resulting amplicons revealed that all isolates bore the qnrB2 gene. Class one integrons were amplified and sequenced. Isolates resistant to neomycin bore the aac(6’)-Ib-cr cassette whereas the dfrA1 gene was responsible for resistance to trimethoprim.

Twelve S. enterica strains were originally isolated over a four-year period from cases of clinical infection, and one strain from frozen produce. Seven of the 12 strains were from patients with a history of foreign travel with four of those seven reporting recent travel to India. Interestingly, JIE273 was also isolated from a patient who had recently returned from India. Investigations into

rmrC 16SrRNA methyltransferase from Salmonella Virchow in the United Kingdom

Aminoglycosides are used for the treatment of a wide range of infections caused by both gram-negative and gram-positive bacteria, and have been classified by the World Health Organization as Critically Important

Figure 29.1: Dendrogram of 16S rRNA methyltransferases from pathogenic bacteria and environmental actinomycetes.
Attempts to isolate rmtC by conjugal transfer to rifampicin-resistant *E. coli* were unsuccessful, as was electroporation into *E. coli* and ElectroMAX DH10B cells using plasmid preparations. An approximately 100kb rmtC-bearing plasmid was previously transferred from *P. mirabilis* AR568 by electroporation but could not be mobilized by conjugation, while attempts to transfer the rmtC plasmid from *P. mirabilis* JIE273 by electroporation and conjugation failed. This is in contrast to other methyltransferases, such as armA and mttB, which are mostly located on conjugative plasmids.

Location of the rmtC gene was determined with PFGE using *S. Braenderup* H9812 size standard. Hybridization of plasmid extractions with the rmtC probe was negative.

We describe, for the first time, the occurrence of 16S rRNA methyltransferase rmtC in *Salmonella*, and the rmtC gene in Europe. This is also the first report of a 16S rRNA methyltransferase-producer being isolated from food. The overall isolation frequency of 16S rRNA methyltransferase-producing *S. enterica* is low (13 out of 81,632 strains) in the UK, and these genes were absent in *E. coli*. The spread of multi-resistant isolates expressing 16S rRNA methyltransferase, amplified by the association of these genes with the ISεc7 element, raises clinical concern that further spread is likely.

**First identification of armA in a food isolate**

The 16S rRNA methylase armA is a worldwide emerging gene confering high-level resistance to most clinically relevant aminoglycosides. We identified a multi-drug resistant *Salmonella* isolate bearing the armA methylase in association with *bla*f1, *bla*TEM, and *bla*CMY from a food product. The isolate was recovered from chicken meat sampled in February 2009 in La Réunion, a French island in the Indian Ocean. The isolate was identified as *Salmonella* 4,12:i:-. Antimicrobial testing showed resistance to ampicillin, amoxicillin/clavulanic acid, cefotaxime, cefuroxime, streptomycin, kanamycin, gentamicin, sulfonamides, trimethoprim, tetracycline and chloramphenicol, and susceptibility to quinolones and imipenem.

PCR analysis revealed the presence of *Salmonella* genomic island 1, usually responsible for ACCsUT phenotype, three beta-lactamase genes (*bla*TEM, *bla*SHV, *bla*CMY), and the *armA* gene. Resistance to beta-lactams, sulphonamides and aminoglycosides were transferred through conjugation to an *E. coli* recipient cell.

This is the first report of the *armA* methylase gene in food.

**qnrB in Salmonella from human origin**

Resistance to fluoroquinolones through plasmid-mediated *qnr* genes, especially when associated with resistance to cephalosporins by extended-spectrum beta-lactamases (ESBLs), is an emerging phenomenon in enterobacteria. This is worrisome in the case of *Salmonella*, as these are two of the CIAs, as defined by the World Health Organization, for treatment of invasive salmonellosis.

We have now identified a *qnrB2* gene in a *Salmonella* Bredeney clinical isolate from Spain. The gene was borne by a self-transferable IncH2 plasmid, pB1004, associated with *bla*TEM-1 and *bla*CMY-2. The genetic environment of *qnrB2* revealed a common evolutionary origin with plasmid pEC-IMPQ, recently described in Enterobacter cloacae clinical isolates from Taiwan.

Complete antimicrobial resistance profiling revealed that the bacterium possessed an unusual multi-drug resistance profile. Resistance to beta-lactams, aminoglycosides, chloramphenicol, tetracycline and fluoroquinolones could be transferred en masse into *E. coli* at a frequency of $3 \times 10^{-5}$ per donor colony forming unit.

The size of the plasmid was estimated at approximately 315 kilo base pairs. Hybridization with 8-lactamase probes (OXA, CMY, CTX, cefazidime, cefotaxime, streptomycin, kanamycin, gentamicin, sulfonamides, trimethoprim, tetracycline and chloramphenicol, and susceptibility to quinolones and imipenem.**
The resulting transformant showed reduced terminator regions, and cloned into pCR2.1, together with the putative promoter and terminator regions, and cloned into pCR2.1. Sequence analysis of the 7,613bp fragment revealed the presence of the qnrB2 gene described to date. Grey shading denotes ≥99% nucleotide identity. Black lines beneath S. Bredeney indicate the regions that have been amplified by overlapping PCR and sequenced using the nucleotide sequence of pEC-IMPQ for the design of appropriate primers.

Figure 29.3. Schematic representation of the genetic environments of the qnrB2 gene described to date. Grey shading denotes ≥99% nucleotide identity. Black lines beneath S. Bredeney indicate the regions that have been amplified by overlapping PCR and sequenced using the nucleotide sequence of pEC-IMPQ for the design of appropriate primers.

PCR assays were initially performed and amplicons of approximately 600, 3,000, 2,500 and 5,700bp were partially sequenced. A class 1 integron carrying the trimethoprim resistance gene dfrA25 as the unique gene cassette was identified, and the class 1 integron was located adjacent to the 3’CS of the integron, within an ISCR1 element. Two PstI fragments of approximately 2,200bp and 2,900bp containing the qnrB2 gene and the region flanking the ISCR1 were cloned from plasmid p137.25 into the PstI-pZeo-2 kanamycin-resistant vector and fully sequenced. DNA sequence comparison showed that ISCR1 carried the sapA-like, orf2, qnrB2 and ospA open reading frames, followed by a second copy of the qacEdel/tol13’CS.

The same structure was previously described for other five plasmids: the pSE936/05 from S. Enteritidis of human origin from Taiwan, the plasmid of S. Keurmassar from France, the IncI/M pJBB401 of Klebsiella pneumoniae from Australia, the IncI/pEC-IMPQ of Enterobacter cloacae from Taiwan and the IncHI2 p81004 of S. Bredeney from Spain.

The genetic environment of qnrB2 has been described in Salmonella Enteritidis and Salmonella Keurmassar, as well as in Klebsiella strains and partially in Citrobacter koseri, being highly similar in all bacteria described to date (Figure 29.3).

Further analysis by PCR and sequencing of the genetic environment of qnrB2 revealed the existence of an 8,939bp deletion including an ISCR1 element and a class I integron with the blaIMP-4 metallo-beta-lactamase gene.

Previously, qnrB2 has been identified in Enterobacter spp., qnrA has been detected in E. coli, Enterobacter cloacae and K. pneumoniae, whereas the qnrS gene has been shown to be present in a K. pneumoniae clinical isolate. In countries such as France, the United Kingdom, Germany, Israel, Australia, the United States, Taiwan, The Netherlands and Senegal, the qnrS gene is largely present. In the latter four countries, Salmonella spp. were the host bacterium of the qnrS gene. In Taiwan, the gene was present in the S. Enteritidis, and possessed a similar genetic environment to that described previously for S. Keurmassar.

The single qnrB2-bearing Salmonella, originating from a Dutch broiler chicken, also belongs to S. Bredeney and was suspected to be potentially linked to the S. Bredeney isolate from Spain. The genetic environment flanking the qnrB2 gene and the plasmid incompatibility groups were completely different for the two S. Bredeney isolates from The Netherlands and Spain (IncN-p137.25 and IncHI2-p81004 respectively), suggesting independent acquisition events of qnrB2-carrying plasmids in these isolates.

The genetic environment of this qnrB2, as well as the partial sequence of the plasmid backbone, has revealed a striking common evolutionary origin between p81004 and pEC-IMPQ. This is further supported by the plasmid size of Taiwanese plasmid, which has been shown to be 324kbp as compared to the 315kbp of p81004. These results confirm the emergence in Spain of an IncHI2 plasmid related to pEC-IMPQ, that associates the qnrB2 gene with SHV-12 and TEM-1.

**qnrB2 in Salmonella from chicken**

Little information is available on the strains of animal origin carrying qnr genes. Thus, we investigated the genetic environment surrounding the qnrB2 gene in the IncN p137.25 plasmid. This S. Bredeney strain was a unique qnr-positive strain from animal sources, identified among the collection of the Dutch Central Veterinary Institute, including 15,011 Salmonella isolates from humans, poultry, pigs, cattle and other sources in The Netherlands in the period 1999–2006. This isolate originated from a Dutch broiler chicken and showed low-level resistant to ciprofloxacin (MIC 0.25mg/L) but it was still susceptible to nalidixic acid (MIC 16mg/L).

TEM and SHV) and sequencing revealed that the genes encoding TEM-1 and SHV-12 were plasmata-located.

The plasmid was extracted, digested with HindIII, ligated into pUC19, transformed into E. coli, and plated on agar plates containing ampicillin (50mg/L). Two different clones were obtained, bearing a 7,613 base pair (bp) and a 4,489bp fragment that were completely sequenced in both strands by genome walking. The 4,489bp fragment encoded the trhA and trhL genes, as well as most of the putative replication origin of the plasmid. It was 100% identical to the IncHI2 plasmids pK29 from Klebsiella pneumoniae, R478 from Serratia marcescens, and the recently described plasmids pEC-IMP and pEC-IMPQ in Enterobacter cloacae from Taiwan.

When compared with the rest of the sequences present in the databases, the fragment differed by 26 nucleotides with other IncHI2 plasmids. Thus, this plasmid can be assigned to the R478 subgroup of the IncHI2 plasmids described to date.

Sequence analysis of the 7,613bp fragment revealed the presence of the qnrB2 gene, the structural gene of the qnrB2 protein. To assess whether qnrB2 was responsible for quinolone resistance in this strain, the gene was amplified together with the putative promoter and terminator regions, and cloned into pCR2.1. The resulting transformant showed reduced susceptibility to quinolones and fluoroquinolones demonstrating that qnrB2 contributes to the quinolone resistance phenotype of the organism.

The genetic environment of qnrB2 has been described in Salmonella Enteritidis and Salmonella Keurmassar, as well as in Klebsiella strains and partially in Citrobacter koseri, being highly similar in all bacteria described to date (Figure 29.3).

**Five types of 16S rRNA methyltransferase genes (armA, rmtA, rmtB, rmtC and rmtD) conferring resistance to aminoglycosides have been identified.**
Plasmid p137.25 shows a new genetic environment, but it is closely related to that one of the Salmonella pSE936/05 from Taiwan, since both contained the same class 1 integron carrying the blaA25 gene. Two open reading frames encoding hypothetical proteins (ORF5 and ORF6) and the insertion element IS6100 were located after the second 3'CS of the ISCR1 element (the sequence of the pSE936/05 plasmid in this region was not determined). The ORF5, ORF6 and IS6100 region has been previously described at the 3'-end of several ISCR1 elements, including that one located on another IncN plasmid named pKp96, identified in K. pneumoniae isolated in China in 2002.

Plasmid pKP96 differed from p137.25 as it was positive for qnrA1 instead of qnrB2, and also carried the aac(6')-Ib-cr gene as the integron-borne gene cassette (NC_011617). The other qnrB2-genetic environments previously described are different: pFJBE401 showed the insertion of IS4321-orf97-orf98 and the deletion of orf6 while a second ISCR1 was identified in the plasmid from S. ke尿massara, pB1004 and in pEC-IMPQ. In the latter, the acquisition of the ISCR1-qnrB2 element was generated by integration into the related pEC-IMP plasmid by homologous recombination of the ISCR1 rolling circular intermediate of replication, carrying the qnrB2-aac(6')-Ib-cr-sul1 resistance genes.

The qnrB2 gene was also identified on an IncFI plasmid that was carrying the blaOXA-48 extended spectrum ß-lactamase gene in E. coli from a dog in Portugal. Plasmids carrying the blaOXA-48 gene and belonging to the IncFI group have been reported worldwide in E. coli of human origin but none carried the qnrB2 gene, suggesting a recent acquisition of this resistance determinant on IncFI carrying CTX-M-5.

The presence of related elements carrying the qnrB2 gene on a variety of unrelated plasmids from Salmonella or E. coli of animal origin opens the possibility that genetic exchange and plasmid acquisition of the qnrB2 gene could occur more frequently in the faecal flora of animals, particularly poultry.

qnrS1 in an animal E. coli isolate

Three plasmid-mediated quinolone resistance mechanisms have been described so far: Qnr peptides capable of protecting DNA gyrase and topoisomerase IV from quinolones; Aac(6')-Ib-cr aminoglycoside acetyltransferase modifying the quinolones with a piperazinyl substituent (for example, ciprofloxacin); and the quinolone efflux pump QepA. Plasmid-mediated quinolone resistance is being increasingly recognized in Enterobacteriaceae from human infections but seems very rare in strains of animal origin.

In a recent study from China, 17% of isolates from food-producing animals contained one or more plasmid-mediated quinolone resistance determinants. In Europe, qnr-carrying Escherichia coli strains have not yet been described to occur in animals, and qnr peptides have been reported to occur only in Salmonella enterica. Infantis isolates from chicken carcasses in Germany, and in S. Bredeney isolates from chicken meat in The Netherlands.

In this study, the occurrences of qnr, aac(6')-Ib-cr, and qepA genes in 73 E. coli strains of avian origin were investigated. The 73 strains all showed ciprofloxacin minimal inhibitory concentrations (MICs) of 0.125 μg/ml among 113 isolates recovered between April 2003 and December 2006 (18, 25, 27, and 43 isolates collected in 2003, 2004, 2005, and 2006, respectively) during the surveillance activities of the Istituto Zooprofilattico delle Venezie, Italy.

The 113 isolates (74 from poultry with cocolibacillosis and 39 from poultry at slaughter) represented over 10% of all the E. coli isolates from poultry collected during the study period in the Italian region that hosts the greatest number of poultry farms. Of the 73 isolates analysed, 65 were fully resistant to ciprofloxacin (MIC range, 4 to 32 μg/ml) and eight showed reduced susceptibility (MIC range, 0.125 to 0.5 μg/ml). The screening for the qnrA, qnrB, qnrS, aac(6')-Ib-cr, and qepA genes was performed by multiplex and simplex PCR amplifications, and amplicons were sequenced to determine the gene variants. One qnrS7-positive isolate (strain 3963) was detected among the eight isolates showing reduced susceptibility to ciprofloxacin (12.5%); all other isolates were negative for the qnr, aac(6')-Ib-cr, and qepA genes. Strain 3963 was isolated from a regularly slaughtered chicken in 2006. This strain belonged to phylogenetic group D and to multilocus sequence type 398 (see http://mlst.ucc.ie/mlst/dbs/Ecoli/).

Strain 3963 showed resistance to enrofl oxacin and reduced susceptibility to nalidixic acid, ciprofloxacin, and levofloxacin. This strain was also resistant to ampicillin but susceptible to broad-spectrum cephalosporins. No mutations were identified in the quinolone resistance-determining regions of the gyrA, parC, or parE genes. Strain 3963 also carried the blaTEM-1 gene, as demonstrated by PCR and sequencing.

Plasmid DNA from strain 3963 was used to transform competent E. coli TOP10 cells. Transformants were selected on LB agar plates containing 0.06 μg/ml of ciprofloxacin. TOP10-3963 transformants contained both qnrS1 and blaTEM-1 genes, and showed resistance to ampicillin and increased MICs for fluoroquinolones. Strain 3963 failed to produce transconjugants when rifampin (rifampicin)-resistant E. coli CSH26 was used as the recipient strain.

The transferred qnrS1 plasmid, of approximately 45 kb, was further analysed by restriction analysis, Southern blot hybridization experiments, cloning, and DNA sequencing of the regions flanking the qnrS1 gene. In particular, the 3,592- and 2,851-bp PstI fragments containing the qnrS1 and blaTEM-1 genes, respectively, were both cloned into the PstI-gZero-2.1 kanamycin-resistant vector. The DNA sequences of the cloned PstI fragments perfectly matched the sequence of the resistance region from plasmid pINF5, a qnrS1-positive plasmid previously identified in S. Infantis isolates from chicken carcasses in Germany. In particular, the 3,592-bp PstI fragment contained the 3' end of the trpA gene of transposon Tn3, the relict of the insertion sequence IS2, and the entire qnrS1 gene. The 2,851-bp PstI fragment contained the blaTEM-1 gene, the resolvase gene of Tn3, and part of the 5' end of the Tn3 trpA gene.

Since no information is available on the pINF5 plasmid scaffold and the 3963 transformant strain was found untypeable for the 18 incompatibility groups tested by PCR-based replicon typing, a further characterization of the 3963 plasmid was performed. Several recombinant clones were randomly selected and fully sequenced. Three clones contained 359, 739, and 1,467 bp inserts matching at 95–99% with the DNA sequence of the IncX1 virulence plasmid pOU1114, a 35 kb plasmid previously identified in Salmonella Dublin strain OU7025, isolated in Taiwan.

The sequenced inserts from plasmid 3963 targeted three regions scattered along a large portion of the pOU1114 scaffold, including the pilT, pilX2, and pilX4 genes. These sequence data suggest that the 3963 plasmid scaffold is similar to that described to occur in the pOU1114 plasmid of S. Dublin, although the latter did not contain the qnrS1 gene.

Aminoglycosides are used for the treatment of a wide range of infections caused by both gram-negative and gram-positive bacteria.
Our findings indicate that qnr determinants are present in E. coli from poultry in Europe and cannot be associated with the quinolone resistance-determining region mutations as previously described for other qnrS1-positive Enterobacteriaceae.

These data suggest genetic exchanges among Salmonella and E. coli strains of animal origin, and open up new perspectives on the potential animal reservoirs of qnr genes.

**Review, nomenclature and detection of aminoglycoside resistance determinants**

The aminoglycosides can be classified into groups that include different classes: Streptomycin (streptomycin); Spectinomycin (spectinomycin); Apramycin (apramycin); Destomycin (destomycin, hygromycin); Neomycins (neomycin, paromomycin, lividomycin, ribostamycin, butirosin); Kanamycins (kanamycin, bekamycin, tobramycin, dibekacin, amikacin, arbekacin); Gentamicin (gentamicin C, gentamicin B, sisomicin, gentamicin); and Fortimicin (astromycin).

Several mechanisms conferring aminoglycoside resistance have been described: These include active efflux of the antibiotics or reduced intake into the bacterial cell, the newly described methylation of the action target (16S rRNA), and enzymatic inactivation of the aminoglycosides. This modification, made by aminoglycoside modifying enzymes (AMEs), can be phosphorylation, acetylation, or adenylylation.

Within Workpackage 29, a sub-project focusing on the AMEs and methylases was initiated to:

- resolve the confusion related to the nomenclature of the AMEs encoding genes
- to make a collection of primers (already used or new designed), and collect information on phenotypic tests (related to the detection methods)
- collect information on control strains (that is, which institution has what)
- collect and distribute general information about aminoglycosides and resistance mechanisms.

**Resolve the chaos related to the nomenclature of the AMEs encoding genes**

This was achieved by making revisions of the literature and Genbank research, contributions of some Workpackage partners and external collaborators, and collecting information in large tables (actualized up to January 2009) that were distributed to Workpackage 29 members and external collaborators. Some of the information gained is reported below:

**a) Phosphorylases: “APH”**

Phosphorylases have different regiospecificity for phosphate transfer. Seven classes have been described to date. They add PO3 to the following radicals: (3'), (6'), (2''), (3''), (7''), (9), (4). Some of them are bi-functional (phosphotransferase+acetyltransferase).

All the data related to their nomenclature were collected in a comprehensive table, distributed to participants. Examples of the names given to these enzymes are:

- **aph(3')-** = aphA
  - a = aphA1, aphA-1, aph, rplD, aphI
  - b = aphA2, aphA-2, kn, rplD, kan, neo
  - c = aphA3
  - d = aphA4
  - a - b = (including variants): aphA4-aphA8

**b) Acetyltransferases: “ACC”**

This is the largest AME group, containing more than 50 enzymes, and have different regiospecificity for acetyl transfer. Four classes have been described so far: ACC: (1), (3), (2'), (6'). Some of them are bi-functional (phosphotransferase+acetyltransferase: APH(2'')-AAC(6')).

All the data related to their nomenclature has been collected in a comprehensive table, distributed to participants. Examples of the names given to these enzymes include:

- **aac(3')-** = aacA
  - a = aacA1-aacA1, aacC1
  - b = aacA2
  - c = aacA3
  - d = aacA4
  - e = aac-C10

- **aac(3'')** = aacC2

- **aac(3')-** to X: aacC10

**c) Adenylyltransferases: AAD = ANT**

Adenylyltransferases have different regiospecificity for nucleotidyl (adenylyl) transfer. There are four classes that transfer the adenylyl group to the (6), (4'), (2''), (3'') radicals. All the data related to their nomenclature has been collected in a comprehensive table, distributed to participants. Some examples of the names given to these enzymes are:

- **ant(3'')-** = aadA
  - a = aadA1, aadA, aadA1a, aadA1b
  - b = aadA2
  - aadA3 - aadA4

**d) Methylases**

Methylases are not AMEs. They just modify the target where the aminoglycoside bind, but not the aminoglycosides itself. The first methylases were discovered in 2003. They produce the methylation of the 16S rRNA A site. The most wide spread methylation encoding genes are armA (Enterobacteriaceae, Acinetobacter spp.) and armB (Enterobacteriaceae). NpmA methylates the A1408 position of the A site. All others methylate the G1405 position.

All the data related to methylase nomenclature were collected in a comprehensive table and distributed to participants.

**Collection of primers, phenotypic tests and control strains**

Several participants and external partners contributed to this part of the work providing their own protocols and so on. A large table was generated comprising all the information. This table was distributed to all Workpackage 29 participants and external collaborators for revision, with a definitive table to be provided.

**Collect and distribute general information about aminoglycosides and resistance mechanisms.**

Part of the information is provided in this report. The collected information will also be used to write a review on the subject.
Workpackage 30: Annual Research Report

WP number 30
Title Towards a combined microbiological and epidemiological approach for investigating host-microbe interactions of Campylobacter jejuni – CampyNet III
WP Leader Thomas ALTER
Name and Address Federal Institute for Risk Assessment (BfR), Diersdorfer Weg 1, 12277 Berlin, GERMANY
Project Start date 1 March 2006
Project End date 31 October 2009

Progress summary
CampyNet III is a network of microbiologists and epidemiologists working on campylobacteriosis in humans, with emphasis on host-pathogen interactions. In the fifth and final year of CampyNet III, the following progress was made.

A database was compiled and extended with features of all strains from the CampyNet strain collection that had previously been established. In parallel, a virtual database of strains from partner institutes was created with a public part to be linked to the CampyNet website. The database is a Campylobacter reference collection of epidemiological and clinical interest.

Three workpackage meetings were held during the year. The fourth meeting was jointly held with Workpackage 34 at the French Food Safety Agency (AFSSA), Ploufragan, from 19–21 November 2008. The progress on the review paper and the MLST analysis were discussed along with the array comparative genomic hybridization (aCGH) of Campylobacter spp. on a Nimblegen array. Strains for aCGH analysis were selected, and tasks distributed.

The fifth workpackage meeting (Med-Vet-Net Workpackage 30 Microarray Workshop II) was held at Utrecht University in The Netherlands, in collaboration with CVI, from 6–8 May 2009. The workshop provided the opportunity to discuss the microarray activities (exchange of strains, practical details of microarray analysis, handling of software for data analysis), the upcoming deliverables and milestones, and the sustainability of the CNET strain set such as deposition in the German Collection of Microorganisms and Cell Cultures (DSM).

The workshop also held a satellite meeting on 5 June 2009, during the Med-Vet-Net annual conference in El Escorial, Spain, to discuss final tasks and the details about DSM deposition.

Work to complete MLST results for the CNET strain set was progressed. DNA for 38 strains was prepared at CVI and sent for analysis to the Health Protection Agency (HPA) in the United Kingdom. Results were obtained for 23 isolates and the database has been updated. A subset of 16 isolates failed and cultures were provided by CVI for repeat analysis, which has been finalized.

Due to initial problems with preparation and labelling of samples, the aCGH analysis of selected CNET strains and additional strains fell behind schedule. The problems were overcome and a total of 92 CNET strains have been tested by the Federal Institute for Risk Assessment (BfR) and the data sent to CBS for analysis. Due to the experimental delay, analysis of the results is still ongoing. A manuscript is in preparation.

Background
Campylobacter is the most commonly reported gastrointestinal pathogen in humans in the European Union (EU) with approximately 200,000 laboratory-confirmed cases recorded in the EU Member States in 2007 (European Food Safety Authority, 2009). Strains are highly diverse by phenotypic and genotypic typing methods, but little is known about diversity in virulence. The host-pathogen interaction of campylobacters, and diversity thereof between subtypes is, likewise, poorly understood, largely resulting from the absence of appropriate in vivo disease models. This knowledge gap hinders accurate risk assessment for campylobacters in the food chain and the development of relevant intervention strategies.

Objectives
The overall objectives of Workpackage 30 were to complete the outstanding tasks previously described, and to extend our knowledge about virulence-related properties of selected CampyNet (CNET) strains by applying pan-genome microarray analysis. This will:
- enable transfer of the appropriate technologies to laboratories of workpackage members (by hosting a second workshop on microarray applications and analysis)
- enable further characterization of CNET strains by array comparative genomic hybridization (aCGH) in conjunction with maintenance of the database
- ensure sustainability of CNET strain sets by providing general access to strains and to the associated information on strain typing markers/virulence assays/host diseases association/epidemiology.

Key achievements to date
- Genotyping of CNET strains by multilocus sequence typing (MLST) completed.
- Comparative genomic hybridization (CGH) by microarray analysis on the CNET strains performed.
- Expansion of CampyNet database with inclusion of epidemiological data relevant to investigation of host immunity
- Joint workpackage meeting with Workpackage 34 at the French Food Safety Agency (AFSSA).
- Workpackage 30 microarray workshop II held at University of Utrecht, The Netherlands, in collaboration with the Central Veterinary Institute (CVI).

This workpackage built on CampyNet II by working with epidemiologists to match epidemiological requirements for investigating human campylobacteriosis with microbiological and molecular tools for investigating host-microbe interactions.

Since Workpackage 30 was initiated, there have been major advances in the number of available Campylobacter genome sequences. Consequently, a clearer understanding of C. jejuni population structure has been developed, notably by multilocus sequence analysis (MLST) in elucidation of allelic markers for host-attribute based on core housekeeping genes, and by pan-genomic analysis using DNA microarrays (genomotyping).
Pan-genome microarray analysis offers a suitable alternative to complete genome sequencing for extracting the necessary gene content to construct a realistic phylogenetic tree based on conserved gene content, to identify gene networks present in subsets of strains, and predict additional virulence properties. Pan-genomic microarray analysis can, moreover, identify predictor genes of relevance to known virulence properties that may be part of the variable section of the pan-genome. Where these approaches reveal genetic markers predictive of host specificity they may also provide valuable clues for further elucidation of host-pathogen interactions.

A high density Campylobacter pan-genome microarray (on a Nimblegen platform) was created, based on all genes found present in currently available genome sequences. That pan-genome array includes a core set of genes present in all strains as well as many genes found in particular strains only. Additionally, an MLST scheme has been added to the panarray, consisting of tiling probes.

To facilitate the transfer of technologies to several participating Workpackage 30 laboratories, a standardized operating procedure for the pan-genome microarray assay was established.

**Approaches**

*Array comparative genomic hybridization (aCGH)*

Three laboratories participated in comparative genomic hybridization of Campylobacter strains by microarray analysis: CBS, BFR and the Veterinary Laboratories Agency (VLA). After establishing inter-centre congruence by testing 10 core strains of the CNET strain set, each participating partner tested an individually unique set of well-characterized strains of *C. jejuni*, selected to extend the range of hosts and diversity of disease pathologies, by aCGH.

During the fourth workpackage workshop, strains for aCGH analysis were selected, and the protocol for pan-genome microarray analysis was discussed. During the fifth workshop, the first data of the microarray analysis were presented by workpackage members and the analysis procedure of array data was discussed.

The standard operating procedure for pan-genome microarray analysis (based on the Nimblegen protocol for aCGH); created during Year 4, was applied successfully in three participating laboratories. Using one harmonized protocol guaranteed maximum performance harmonization among the workpackage laboratories involved in aCGH analysis of CNET strains.

**Genotyping**

MLST analysis was completed for the CNET strain set. DNA for 38 strains was prepared at CVI and sent to HPA for analysis. Results were obtained for 22 isolates and the database has been updated. A subset of 16 isolates failed and cultures were reprocessed. Of these 22 isolates, 16 strains were from other *C. jejuni* from the CNET strain set generated. The clonal complex (CC) distribution among the analysed strains is shown in Figure 31.4. The most common clonal complexes represented in the set were CC21 (n= 25 strains) and CC45 (n=20 strains). This is in agreement with data from the literature on the most common CCs in the UK. Six strains could not be assigned to a clonal complex, and three strains were found to be a mix of types.

A diverse range of 41 different sequence types (STs) were represented in the set. The most common were ST 45 (n=15), ST 21 (n=6) and ST 53 (n=8). The other STs were represented by three or fewer strains. STs for three additional genetic loci, which are not part of the MLST target genes, were available for 32 isolates: flaA, flaB, and porA. These data will be included in the online CNET dataset. Apart from CNET 16, 17 and 18, which they might contain genes not hitherto found in *C. jejuni*, but that might potentially occur in novel *C. jejuni* isolates.

After scanning, tiff-files were transferred via WS FTP (file transfer protocol) to CBS. Data analysis was undertaken centrally at CBS, and data processing was performed using the oligo package in R. Data were processed like expression data and normalized with Robust Multi-chip Average, which also does probe summary calculations using median polish. From the distribution of the processed array signals (distribution plot) it can be derived which genes are present and which are not. A test of the signal quality of the arrays was also performed using the National Collection of Type Cultures (NCTC) 11168 strain: since its genome was completely represented on the chip, its hybridization signals could be cross-checked in detail. This is shown in Figure 31.2.

**Results and conclusions**

*aCGH*

An example of the outcome of microarray analysis for array comparative hybridization of thermophilic *Campylobacter* spp. is given in Figure 30.1.

The arrays used in this project were based on the NimbleGen 12-plex platform, officially released on 19 November 2008. The custom probe set for the arrays was built around a set of 18 core genomes representing all publicly available *Campylobacter* genomes in GenBank at the time. Of these 18 genomes, 11 were from *C. jejuni* while the remaining genomes were from other species within the *Campylobacter* genus. Although the arrays were primarily designed for use with *C. jejuni*, the theory behind the inclusion of the non-*C. jejuni* genomes was that

**Figure 30.1. Hybridized Nimblegen Campylobacter Panarray (reference strains and CNET strains were used for hybridisation).**

virulence genes) in human strains with different types of well-described clinical cases.

The addition of more contemporaneous strains with associated epidemiological data from disease cases, also enables studies to identify possible shifts in geno- and virulo-types due to, for example, new environmental or other selection pressures. Members of a workpackage subgroup recognised the difficulty of obtaining access to matched isolate/serum collections. To ensure strains from the panels could be made available for general research use by the campylobacter community it was agreed to exclude any potential strain sets that may be impossible to obtain, or are no longer sourced. *Campylobacter jejuni* is a species within the campylobacter community it was agreed to subgroup recognised the difficulty of obtaining access to matched isolate/serum collections.

**Figure 30.2. Validation of design, distribution of processed signal**

The raw data of these experiments are currently stored at CBS and will be made publicly available after publication of the results. The current aim is to rely on an internationally established database such as the Gene Expression Omnibus (see www.ncbi.nlm.nih.gov/geo/) that stores aCGH data.

**Distribution of Processed Signal**

Based on the gene content detected by the analysis a dendrogram was created of the tested Campylobacter strains, as shown in Figure 31.3. The raw data of these experiments are currently stored at CBS and will be made publicly available after publication of the results. The current aim is to rely on an internationally established database such as the Gene Expression Omnibus (see www.ncbi.nlm.nih.gov/geo/) that stores aCGH data.

**Distribution of Processed Signal**

The raw data of these experiments are currently stored at CBS and will be made publicly available after publication of the results. The current aim is to rely on an internationally established database such as the Gene Expression Omnibus (see www.ncbi.nlm.nih.gov/geo/) that stores aCGH data.

**Figure 30.2. Validation of design, distribution of processed signal**

(NCTC 11168, RefSeq id NC_002163. Signal from probes derived from the specific genome sequence of this strain are shown by the yellow curve and signal from the remaining genomes are shown in blue.)

Intestinal infections, including the attack-rate of reactive joint pain following with comprehensive epidemiological information. Campylobacter isolates recovered from the patients. Available samples for the study cases with associated epidemiological information and serological the same research group. This dataset comprised to two-year study investigating antibody responses linked immunosorbent assay.

Patient and serum MBL determined by enzyme-reactant in infectious disease) genotyping was molecule which may behave as an acute phase

Binding lectin (MBL; a pathogen recognition of infection. HLA-B27 tissue typing and mannose joint pain, reported joint pain within four weeks accompanying inclusion: the study should have included publications had to fit specific criteria for host-pathogen interaction studies. The publications had to fit specific criteria for inclusion: the study should have included paired samples from both the patient and the accompanying Campylobacter strain together with comprehensive epidemiological information. Only three studies fitted the criteria including a case-case comparison study aimed at estimating the attack-rate of reactive joint pain following intestinal infections, including Campylobacter spp. Of 1,003 patients presenting with Campylobacter, 131 patients previously free of joint pain, reported joint pain within four weeks of infection. HLA-B27 tissue typing and mannose binding lectin (MBL; a pathogen recognition molecule which may behave as an acute phase reactant in infectious disease) genotyping was undertaken on the blood sample taken from the patient and serum MBL determined by enzyme-linked immunosorbent assay.

A second set was from an older (1996–97) two-year study investigating antibody responses to Campylobacter infections in 210 patients, by the same research group. This dataset comprised epidemiological information and serological samples for the study cases with associated isolates recovered from the patients. Available from workers who were asymptomatic at the time of collection. Accompanying material included sera from short-term, long-term and blood donor controls, with follow-up sera available for 51 subjects. Study five, a post-infection antibody study designed to determine whether systemic and mucosal antibodies induced by a confirmed infection remain at elevated levels for prolonged periods, did not have accompanying isolates. Serum, saliva and urine samples were available and subjects were followed up for up to one year post-infection.

Finally, isolates from a sporadic infection acquired following a visit to a poultry abattoir with accompanying abattoir environment strains, and sera from infected patient were available from the VLA. This set was included as the strain recovered from the infected individual had been genome-sequenced.

Workshops and meetings

Fourth workpackage meeting

The fourth Workpackage 30 meeting was held as joint meeting with Workpackage 34 at the French Food Safety Agency in Ploufragan from 19–21 November 2008. Gwennola Ermel from Université de Rennes I was invited to speak on “The usefulness of Microarray analysis to study interaction between Campylobacter and host”. The progress on the review paper and the MLST of Campylobacter spp. on a Nimblegen array was discussed; strains for aCGH analysis were selected and tasks distributed.

Microarray workshop II

The fifth workpackage meeting doubled as the second Med-Vet-Net Microarray workshop, and was held at Utrecht University in collaboration with CVI in The Netherlands from 6–8 May 2009. Fourteen workpackage members were present along with Med-Vet-Net Project Manager, John Threlfall. Ten members of the University of Utrecht also joined the afternoon session of the workshop, and three invited experts gave presentations:

Fimme Jan van der Wal (Animal Sciences Group, Wageningen University and Research Centre): “Nucleases encoded by phage-like integrated elements inhibit natural transformation of C. jejuni”.

Marc Wösten (University of Utrecht): “Regulons in Campylobacter jejuni”.

Dave Ussery (Technical University of Denmark): “Microbial pan-genomics”.

The workshop provided the opportunity for WP30 members to discuss the microarray activities (exchange of strains, practical details of microarray analysis, and handling of software for data analysis), workpackage deliverables and milestones, and the sustainability of the CNET strain set.

Satellite meeting

The workpackage also held a satellite meeting during the Med-Vet-Net annual conference in El Escorial, Spain, in June 2009. The future of the CNET strain set was discussed and it was agreed to submit the strain set to a strain collection, preferably DSM. Details about DSM deposition were considered. An update on the virtual CampyNet database was also provided, and the final tasks of the workpackage were discussed.

Figure 30.3. Dendrogram of the analysed CNET strains based on the complete hybridization data obtained in this study.

Figure 30.4. Number of C. jejuni strains by MLST clonal complex (mixed/no data: CNET 48 and CNET 112).
**Workpackage 31:**

**Annual Research Report**

**WP number:** 31

**Title:** Food producing animals as a potential source of emerging viral zoonoses (ZOOVIRNET)

**WP Leader:** Franco M. RUGGERI

**Name and Address:** Istituto Superiore di Sanità (ISS) Department of Food Safety and Veterinary Public Health Viale Regina Elena 299 00161 Rome ITALY

**Project Start date:** 1 March 2006

**Project End date:** 31 October 2009

**Objectives**

Workpackage 31 aimed to generate knowledge and methodologies on *Hepatitis E virus* (HEV), *Anellovirus* and *Encephalomyocarditis virus* (EMCV) strains circulating in pigs, and on *Tick-borne encephalitis virus* (TBEV) primarily related to goats’ milk transmission. The main objectives were to:

- harmonize molecular methods, including RealTime and quantitative polymerase chain reaction (Q-PCR) assays, in order to detect and characterize these viruses in animals and food
- better understand the epidemiology and zoonotic role of these pathogens
- implement a virus and genome database
- clone and express viral proteins and generate immune reagents for virus diagnosis and typing
- implement serological assays for TBEV
- develop immunohistochemistry (IHC) and in situ hybridization (ISH) methods for swine virus detection in cell cultures or tissues.

**Key achievements to date**

- Cell culture methods for swine HEV replication, monitored for viral genome and antigen synthesis.
- Control sera and monoclonal antibodies (MAbs) to HEV and EMCV for use between partners.
- Data on widespread HEV infections in swine and wild boars in Europe, including asymptomatic G3 HEV infection in slaughter-age pigs.
- Serological data on EMCV in swine, and TBEV in goats.
- Harmonisation of HEV diagnostic procedures within the workpackage.
- Options prepared for staining virus and HEV sequence databases.

**Progress summary**

Workpackage 31 further progressed the study of emerging zoonotic viruses through co-ordinated field investigations, particularly across Europe, refinement of laboratory methods, and production of control reagents for molecular, immunological and cultivation assays, and ring tests.

HEV studies involved most of the workpackage partners, who used the powerful diagnostic tests developed to investigate HEV spread from pig farms, in wild animals, and in the course of experimental in vivo and in vitro infection. Reference sera and newly developed MAbs, in addition to efficient real-time assays, proved critical in performing these activities, including development of cell culture protocols for HEV.

New HEV nucleotide sequences, including long genome stretches, were generated from the field studies, mostly in farmed pigs and wild boars, further confirming the wide presence of G3 HEV throughout Europe, and its interspecies, zoonotic and environmental transmission. Most of these data have already been uploaded into the ZOOVIRNET database at the Health Protection Agency (HPA) in the United Kingdom. The database will be maintained for future use by workpackage partners as part of a continuing collaboration plan.

Collaborative actions also involved EMCV animal serology in France and Germany, *Torque teno virus* (TTV) virological studies in France and Hungary, and TBEV sero-epidemiology in Poland and Hungary. The overall emerging picture is that all these agents are widely diffuse in Europe, and need further attention in relation to zoonotic and food-borne transmission, animal health and farm control.

Resulting data were disseminated in publications and meetings. The ZOOVIRNET PhD student obtained her doctorate, which examined three-dimensional (3D) cell systems for in vitro replication of HEV.

Animal ribonucleic acid (RNA) viruses capable of transmitting via zoonotic routes represent the largest emerging infectious disease threat to humans, as recently exemplified by the epidemic diffusion of influenza strains of avian and swine origin, several flavivirus and bunyavirus. Spread of RNA viruses is favoured by the high evolution rates of their RNA genome, through genetic drift and shift mechanisms, that allow the viruses to infect novel hosts and evade immune responses. The increased circulation of viral pathogens across different environments and geographic areas also plays a major role, creating new circuits between domestic and wild animals, and humans. The effects of global marketing and travelling, intensive farming practices, and the resistance of viruses in waste and environmental matrices, particularly surface water, act in synergy to make the exposure of animal and human populations to pathogens more intimate and difficult to control.

Workpackage 31 (ZOOVIRNET) focused on three viruses — *Hepatitis E virus* (HEV), *Anellovirus,* and *Encephalomyocarditis virus* (EMCV), which appear to be widely distributed in domestic swine worldwide, and *Tick-borne encephalitis virus* (TBEV) as a paradigm of re-emerging food-borne pathogen.

Animal ribonucleic acid (RNA) viruses capable of transmitting via zoonotic routes represent the largest emerging infectious disease threat to humans, as recently exemplified by the epidemic diffusion of influenza strains of avian and swine origin, several flavivirus and bunyavirus. Spread of RNA viruses is favoured by the high evolution rates of their RNA genome, through genetic drift and shift mechanisms, that allow the viruses to infect novel hosts and evade immune responses. The increased circulation of viral pathogens across different environments and geographic areas also plays a major role, creating new circuits between domestic and wild animals, and humans. The effects of global marketing and travelling, intensive farming practices, and the resistance of viruses in waste and environmental matrices, particularly surface water, act in synergy to make the exposure of animal and human populations to pathogens more intimate and difficult to control.

Workpackage 31 (ZOOVIRNET) focused on three viruses — *Hepatitis E virus* (HEV), *Anellovirus,* and *Encephalomyocarditis virus* (EMCV), which appear to be widely distributed in domestic swine worldwide, and *Tick-borne encephalitis virus* (TBEV) as a paradigm of re-emerging food-borne pathogen.

Animal ribonucleic acid (RNA) viruses capable of transmitting via zoonotic routes represent the largest emerging infectious disease threat to humans, as recently exemplified by the epidemic diffusion of influenza strains of avian and swine origin, several flavivirus and bunyavirus. Spread of RNA viruses is favoured by the high evolution rates of their RNA genome, through genetic drift and shift mechanisms, that allow the viruses to infect novel hosts and evade immune responses. The increased circulation of viral pathogens across different environments and geographic areas also plays a major role, creating new circuits between domestic and wild animals, and humans. The effects of global marketing and travelling, intensive farming practices, and the resistance of viruses in waste and environmental matrices, particularly surface water, act in synergy to make the exposure of animal and human populations to pathogens more intimate and difficult to control.

Workpackage 31 (ZOOVIRNET) focused on three viruses — *Hepatitis E virus* (HEV), *Anellovirus,* and *Encephalomyocarditis virus* (EMCV), which appear to be widely distributed in domestic swine worldwide, and *Tick-borne encephalitis virus* (TBEV) as a paradigm of re-emerging food-borne pathogen. The approach chosen for implementing knowledge, skills and expertise on these pathogens included developing and harmonizing methods for detection, typing and epidemiological surveillance of viruses, exchanging samples and reagents, creating databases and inventories, training human resources (in addition to several post-docs, a PhD student was involved in the project), and collaborating with other scientific networks in Europe.

**Hepatitis E virus (HEV)**

Recent evidence indicates that HEV has both a global distribution and a local evolution, with Genotype 3 (G3) strains being the ones mostly spread throughout Europe and Northern America. Although HEV of Genotype 1 (G1) is still responsible of human cases in European countries, mostly related to travelling to the G1 endemicity areas in Eastern Asia, G3 HEV is now being recognized as the prevalent genotype in both European humans and swine, with autochthonous cases more frequent than previously estimated.

According to Med-Vet-Net activity and similar studies by other groups, HEV is present in Europe as a disease of low endemicity in humans, whereas it is associated to an extremely high prevalence in farmed swine, virtually in a completely asymptomatic way. Because of this and because G3 HEV strains infecting pigs and wild boars appear to show a very high degree of genetic relatedness to the strains causing disease in man in the same geographic areas, the risk of zoonotic transmission, via either professional exposure (swine and pork chain) or food-borne transmission (consumers of raw pork products) requires accurate investigation.

Similar correlations have also been revealed with the other viral genotypes in different parts of the world, including Asiatic countries from where direct confirmation of animal (pork, wild boars, deer) food involvement in human *Hepatitis E* outbreak derives.
Workpackage 31 thus concentrated on establishing state-of-the-art molecular diagnostics and typing protocols, collecting epidemiological and virological data, and developing novel approaches to cell culture methods and immune reagents to enhance the capabilities of the partnership to study HEV antigenic characteristics and infectivity. Taking advantage of new harmonized methods and reagents (Table 31.1), several prevalence studies (from Sweden through Italy) on HEV in settings other than pig farms were also conducted, particularly in wild boars, looking both at the occurrence in samples of serum antibodies (Figure 31.1) and viral genomic RNA (Figure 31.2). Investigation of the possible influence between conditions of pig farming and extent of HEV infection spread within farms did not show any significant correlation, rather these studies further identified a general widespread diffusion of asymptomatic G3 HEV infection also in old slaughter-age pigs independent of the farming strategy adopted in the different European countries.

From the several field studies conducted, the overall picture describes a continuous flow of different G3 HEV strains throughout farms, in different countries of Europe, with still unknown pathway(ies) of transmission that also involve wild boars and other wild animals, and sometimes humans. If particular activities, such as game hunting and pig farming and slaughtering, were regarded a risk of acquiring HEV infection, it is still unclear if the viral genome traces detectable in pork and wild boar liver from slaughter and from the market may represent a risk for consumers.

The preliminary data obtained on these aspects within ZOOVIRNET point to the need of further specific investigations, also using an Risk Assessment approach, that can be undertaken by some of the partners in future collaborative activities within the Med-Vet-Net Association as well as other European Commission projects (for example, FP7 VITAL). The expertise acquired in the field is valuable, as are the methods implemented for detection. In addition, ZOOVIRNET has produced a basis for novel technological platforms that will hopefully be adapted in due course.

Table 31.1. Swine HEV ELISA Ring Test results: OD values in serum samples A-D, Labs: 1a–9a.

<table>
<thead>
<tr>
<th>Sample</th>
<th>1a</th>
<th>2a</th>
<th>3a</th>
<th>3b</th>
<th>4a</th>
<th>5a</th>
<th>6a</th>
<th>7a</th>
<th>8a</th>
<th>8b</th>
<th>8c</th>
<th>9a</th>
<th>9b</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.95</td>
<td>0.738</td>
<td>0.517</td>
<td>1.189</td>
<td>0.78</td>
<td>NA</td>
<td>0.575</td>
<td>0.206</td>
<td>0.661</td>
<td>1.087</td>
<td>NA</td>
<td>1.666</td>
<td>0.404</td>
</tr>
<tr>
<td>D</td>
<td>0.72</td>
<td>0.386</td>
<td>0.075</td>
<td>0.196</td>
<td>0.13</td>
<td>NA</td>
<td>0.754</td>
<td>0.279</td>
<td>0.233</td>
<td>0.509</td>
<td>NA</td>
<td>0.859</td>
<td>0.276</td>
</tr>
<tr>
<td>A</td>
<td>0.35</td>
<td>0.521</td>
<td>0.00</td>
<td>0.023</td>
<td>0.08</td>
<td>NA</td>
<td>0.736</td>
<td>0.233</td>
<td>0.119</td>
<td>0.241</td>
<td>NA</td>
<td>0.358</td>
<td>0.976</td>
</tr>
<tr>
<td>B</td>
<td>0.28</td>
<td>0.393</td>
<td>0.011</td>
<td>0.009</td>
<td>0.08</td>
<td>NA</td>
<td>0.766</td>
<td>0.233</td>
<td>0.24</td>
<td>0.226</td>
<td>NA</td>
<td>0.33</td>
<td>0.243</td>
</tr>
</tbody>
</table>

Figure 31.1. Serum anti-HEV antibody detection by an in-house ELISA test, built with swine G3 HEV antigen expressed in recombinant baculovirus (AFSSA). Assay was adapted to use on swine, wild boar and other animal sera. Left panel: example of a test microplate. Right panel: a comparison between in-house and a commercial ELISA kit.

Figure 31.2. Detection and phylogenetic analysis of HEV genomic RNA in Wild boar liver samples in different areas of Germany.

Figure 31.3. Pig liver L3 cells infected with swine G3 HEV, stained by immuno-peroxidase with anti-G3 HEV recORF2 MAb 2H1, and an irrelevant antibody to Pestivirus.
course for evaluation of infectivity of HEV found in tissues and food matrices. An inter-laboratory effort (mainly the Veterinary Laboratories Agency (VLA), The French Food Safety Agency, Istituto Superiore di Sanità, and the Central Veterinary Institute) has in fact led to the establishment of 3D hepatocyte and other cell cultures, where differentiation processes in the absence of gravity have allowed productive replication of a swine G3 HEV strain, monitoring viral growth by both decrease with viral passage of Ct in a HEV real time RT-PCR and immuno-cytochemical staining of infected cell cytoplasm with MAbs to HEV ORF2 capsid protein (Figure 31.3).

Among a large (61) panel of MAbs produced against the soluble form of the recombinant HEV capsid protein, more than 60% were able to stain the linear form of the protein by Western blotting after polyacrylamide gel electrophoresis, whereas only a minority could immuno-stain efficiently insect cells expressing the recombinant protein. Four MAbs also recognized the recombinant ORF2 protein, assembled in 3D-structured virus-like particles (VLP), by ELISA. These data indicate that the MAb panel developed recognizes at least three distinct antigenic determinants on the ORF2 capsid protein, two of which are “linear” and at least a third one that is “conformational”.

None of the MAbs developed has a high affinity to the VLP antigen being consequently unsuitable for setting up an “antigen-capture” method for immuno-concentration of diluted HEV in difficult matrices such as surface waters, sewage or other environmental samples. Deliverables D31.27 and D31.28 can therefore be regarded as failed. Further hybridoma cells and MAbs therefore need to be produced to implement a virus concentration immune system enabling proper sensitive testing of HEV contamination in water and sewage. Preliminary results in fact suggest that HEV can be present to a significant extent in these environmental matrices (up to 17% of real time RT-PCR positive river samples in The Netherlands).

Anellovirus
Anellovirus is a new genus of viruses (prototype Torque teno virus strain, TTV) with a circular DNA genome that is found to be very prevalent in humans, a wide range of farm and wild animals, and pets including pigs and wild boars. A role of Anellovirus as a direct cause or cofactor in some human liver pathologies and infections of the immune system is still debated. Besides the occurrence of several genotypes in humans, two genogroups have been described in swine. The possible impact of the continuous massive viral load excretion into the environment and entry into the food chain deserves attention.

Data obtained from several swine farms and animals at different ages in Hungary (National

**Figure 31.4. Serum antibody detection in German domestic pigs by sampling area, analysed by recombinant VP1 ELISA.**

Two genotypes of Anellovirus have been described in swine.
Between different TTV strains. Specificity of TTV strains and the occurrence of mixed infection in pigs as published earlier for viral strains), and confirmed the occurrence of (less than 80% nucleotide identity to database identified a proposed new swine TTV genotype different from the ones in liver samples. The study on humans, swine and wild boar from France reagents and tests), and virus prevalence studies recombinant antigens and MAbs, immune molecular and virological methods (cell culture, for possible zoonotic transmission of EMCV. Antigen-antibody interactions were also investigated transmission routes of viruses, and virus prevalence studies of recombinant viral proteins and monoclonal antibodies between ZOOVIRNET groups. Data were presented on the presence of HEV, EMCV and TTV in swine and wild animals, and on the characterization of strains from different countries and hosts. Reports were also presented and analysed on TBEV occurrence in goat milk and ticks in Poland and Hungary, highlighting the risks of new consumer habits in resort locations.

**Methodologies developed or used**

Starting from the initial inventory of reagents, expertise and approaches, state-of-the-art protocols for detection and characterization of viruses studied within ZOOVIRNET were implemented and made accessible to all partners. A complete list of assays and methods have been presented in the Workpackage 31 deliverables D31.2, D31.4, D31.7, D31.8, D31.16, D31.18, D31.19, which are available for consultation and shared use. The diagnostic methods are complemented by a wide range of specific laboratory techniques used in partner laboratories, including immunological, biochemical, cell culture and microscopic methods, and recombinant protein technologies in prokaryotes and eukaryotes. Ring test and shared common reagents were used for harmonizing methods, and joint actions were undertaken to investigate viruses throughout countries to produce comparable results.

**Workshops and meetings**

The third ZOOVIRNET meeting was held at the VLA Weybridge in the United Kingdom on 12 February 2009, to revise the status of Workpackage 31’s collaborative research on zoonotic viruses, and plan future activity. All partner laboratories were represented at the meeting, and the activity outcomes reported were in line with the tasks and timing of the workpackage milestones and deliverables. The use of the workpackage web page for exchange and elaboration of documents and file drafts between members was discussed, as well as the virus database developed with HEV sequence and strain information. The options for sustaining the database for use by partner institutes beyond Med-Vet-Net were also considered.

The recent developments in viral molecular and immunological diagnostics were presented, particularly concerning the results of the ring test on ELISA assays for swine HEV antibody detection in sera, and plans were made to favour exchange of recombinant viral proteins and monoclonal antibodies between ZOOVIRNET groups. Data were presented on the presence of HEV, EMCV and TTV in swine and wild animals, and on the characterization of strains from different countries and hosts. Reports were also presented and analysed on TBEV occurrence in goat milk and ticks in Poland and Hungary, highlighting the risks of new consumer habits in resort locations.

Workpackage partners expressed an overall interest in continuing contacts beyond Med-Vet-Net to both complete the present collaborative studies and establish new joint scientific projects at the European level.

---

### Table 31.2. Goat breed characteristics according to farmer attitude towards dairy consumption.

<table>
<thead>
<tr>
<th>Household Members Consume Dairy</th>
<th>only pasteurized (n=22)</th>
<th>unpasturized (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Farm size (number of goats)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>6</td>
<td>27.3%</td>
</tr>
<tr>
<td>50–100</td>
<td>10</td>
<td>45.5%</td>
</tr>
<tr>
<td>&gt;100</td>
<td>6</td>
<td>27.3%</td>
</tr>
<tr>
<td>Farm type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agrotourism/hobby</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Dairy production</td>
<td>22</td>
<td>100%</td>
</tr>
<tr>
<td>Goat handling (spend &gt;1 hour daily in tick habitats)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In woods</td>
<td>2</td>
<td>9.1%</td>
</tr>
<tr>
<td>In grasslands</td>
<td>5</td>
<td>22.7%</td>
</tr>
<tr>
<td>In wooden paths</td>
<td>2</td>
<td>9.1%</td>
</tr>
<tr>
<td>Ticks attached to goats</td>
<td>8</td>
<td>36.4%</td>
</tr>
<tr>
<td>Frequency of dairy consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>daily</td>
<td>5</td>
<td>22.7%</td>
</tr>
<tr>
<td>&gt; 1 per week</td>
<td>3</td>
<td>13.6%</td>
</tr>
<tr>
<td>less frequently</td>
<td>4</td>
<td>18.2%</td>
</tr>
</tbody>
</table>

Centre for Epidemiology, NCE/Veterinary Medical Research Institute, VMRI) confirmed the high prevalence of TTV infection in swine, showing more than 30% of adult swine sera and up to 72% of piglets to be positive for TTV genomic DNA, in the absence of clinical symptoms. The completion of viral characterization studies by molecular methods on swine TTV isolates from different organs (liver and gut) showed the occurrence of markedly different TTV sequences by site of replication. Phylogenetic studies confirmed a close correlation between piglet TTVs detected in serum and the gut, which were different from the ones in liver samples. The study identified a proposed new swine TTV genotype (less than 80% nucleotide identity to database viral strains), and confirmed the occurrence of mixed infection in pigs as published earlier for human cases, suggesting both the changing cell specificity of TTV strains and the occurrence of conditions for possible recombination events between different TTV strains.

**Encephalomyocarditis virus (EMCV)**

EMCV can infect many animal species including primates and humans, and is capable of cross-species infections. The virus has been associated with endemic infections in pig herds worldwide, resulting in acute fatal myocarditis and reproductive failure or asymptomatic infections. EMCV infection in humans is associated with fever, neck stiffness, lethargy, delirium, headaches, or vomiting, or aseptic meningitis, polymyelitis-like disease and Guillain-Barré syndrome, but a causal relationship in the few studies of cases remains unconfirmed. Pigs might represent a reservoir for possible zoonotic transmission of EMCV. Following the implementation and validation of molecular and virological methods (cell culture, recombinant antigens and MAbs, immune reagents and tests), and virus prevalence studies on humans, swine and wild boar from France and Spain, the circulation of EMCV was further investigated in Germany by looking at serum EMCV antibody in farm swine from different parts of the country (Figure 31.4).

Testing by an in-house ELISA based on recombinant EMCV capsid protein VP1 revealed serum antibody prevalence to EMCV ranging from 4.0–27.0%, that had no apparent link to specific animal handling procedures, and might imply risks for professional workers in the pig industry in the regions with peak virus diffusion.

**Tick-borne encephalitis virus (TBEV)**

TBEV represents a re-emerging zoonosis expanding mainly in Central-Northern Europe, with fatality rates close to 1.0%. Although human infections mainly result from bites of infected ticks, recent outbreaks in Central Europe confirm an increase of food-borne TBEV transmission via unpasteurized milk or cheese from infected cows, goats, or sheep, especially among tourists.

Early workpackage activity on TBEV was focused on developing diagnostic methods for animal samples that were used in pilot epidemiological investigations in humans and goats in Poland. Further studies identified areas with high-risk for food-borne TBEV transmission in Poland and Hungary, by serological analysis of more than 1,000 goats, and comparative analysis of human and animal risk maps throughout the countries. TBEV seroprevalence in goats ranged between 8.0% and 12% in endemic and non-endemic areas, respectively, showing an obvious correlation with human risks maps (4.3% vs. 0.8%). NCE/VMRI (Hungary) & Poland’s National Institute of Hygiene collaborated to study a major outbreak of TBEV in Hungary in 2007, associated with drinking raw milk from an infected goat farm. These results together with the official TBEV surveillance data have proven useful to implement a model for the risk assessment of milk-borne transmission of TBEV, also based on the geographical density and type distribution of farming milk activities (Table 31.2).

In summary, ZOOVIRNET has developed state-of-the-art methodologies for the study of selected emerging zoonotic viruses, and has confirmed the large spread of viral pathogens among farmed and wild animals. The project also investigated transmission routes of viruses, highlighting risks for zoonotic and/or food-borne virus passage to humans, generating useful knowledge for implementation of possible infection control measures.

---

**Table 31.2. Goat breed characteristics according to farmer attitude towards dairy consumption.**

<table>
<thead>
<tr>
<th>Household Members Consume Dairy</th>
<th>only pasteurized (n=22)</th>
<th>unpasturized (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Farm size (number of goats)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>6</td>
<td>27.3%</td>
</tr>
<tr>
<td>50–100</td>
<td>10</td>
<td>45.5%</td>
</tr>
<tr>
<td>&gt;100</td>
<td>6</td>
<td>27.3%</td>
</tr>
<tr>
<td>Farm type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agrotourism/hobby</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Dairy production</td>
<td>22</td>
<td>100%</td>
</tr>
<tr>
<td>Goat handling (spend &gt;1 hour daily in tick habitats)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In woods</td>
<td>2</td>
<td>9.1%</td>
</tr>
<tr>
<td>In grasslands</td>
<td>5</td>
<td>22.7%</td>
</tr>
<tr>
<td>In wooden paths</td>
<td>2</td>
<td>9.1%</td>
</tr>
<tr>
<td>Ticks attached to goats</td>
<td>8</td>
<td>36.4%</td>
</tr>
<tr>
<td>Frequency of dairy consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>daily</td>
<td>5</td>
<td>22.7%</td>
</tr>
<tr>
<td>&gt; 1 per week</td>
<td>3</td>
<td>13.6%</td>
</tr>
<tr>
<td>less frequently</td>
<td>4</td>
<td>18.2%</td>
</tr>
</tbody>
</table>
Workpackage 32: Annual Research Report

WP number 32

Title Public health surveillance for food-borne infections: Design of epidemiological studies and applying seroepidemiology to validate the surveillance pyramid

WP Leader Kåre MOŁBAK

Name and Address Statens Serum Institut (SSI) Artillerivej 5 2300 Copenhagen DÉNMARK

Project Start date 1 September 2006

Project End date 31 October 2009

Progress summary

During the final year of Workpackage 32, cross-sectional serological surveys of antibody levels against Salmonella and Campylobacter in eight EU member states (Denmark, Finland, Sweden, The Netherlands, France, Italy, Poland, Romania) were completed. In total, almost 7,500 sera were analysed. Sero-incidence of Salmonella infections ranged from 56 (95% confidence limits 8-151) infections per 1,000 person-years in Finland to 567 (943-813) in Poland. Sero-incidence was not correlated with incidence of cases reported through routine surveillance, and it exceeded incidence of reported cases by a factor of 124 to approximately 10,000, depending on country. Translations with other epidemiological data showed significant correlations with infection rates. In Swedish travellers returning from the respective countries, and with prevalence of Salmonella in holdings of layer hens and broilers (using data from EU baseline surveys).

Sero-incidence estimates for Campylobacter were consistently higher (64 to 913 per 1,000 person-years) than those of Salmonella and showed much less variation among participating countries. Comparison with infection rates in Swedish travellers to the respective countries showed no correlation. Veterinary data from the EU baseline survey of Campylobacter in Chickens were not yet available.

We collected serum samples from approximately 60 people affected by a waterborne Campylobacter outbreak in Denmark in June 2009. Preliminary data seemed to indicate that exposed, but asymptomatic individuals have considerably lower serum antibody levels than symptomatic cases. Serum collection and analyses will be completed after the end of Med-Vet-Net.

Key achievements to date

- Cross-sectional serological surveys of antibody levels against Salmonella and Campylobacter in eight EU member states completed.
- Data analysed, sero-incidence estimates for the eight countries generated, and correlation with other available data on Salmonella and Campylobacter epidemiology in Europe investigated.
- Assessed feasibility of using sera from screening programme in pregnant women in The Netherlands as more convenient substitute for population-based serum collections.
- Sera from exposed, but asymptomatic persons, collected from a Campylobacter outbreak.
- Two scientific papers published in peer-reviewed journals, and three manuscripts produced.

Background

National and European agencies working in the areas of food safety and public health need information on the incidence of zoonotic bacterial infections commonly transmitted through food. Data on human bacterial infections are usually derived from passive public health surveillance systems — physicians or clinical microbiology laboratories report cases diagnosed by positive stool cultures. Such surveillance systems capture only a fraction of all cases occurring in the population. Their sensitivity is influenced by many factors including patients’ perceived severity of illness, health seeking behaviour, clinical and laboratory practices, sensitivity of laboratory methods, and compliance with reporting requirements. These factors vary widely throughout the EU.

In all Member States, Salmonella and Campylobacter are by far the most frequently reported bacterial enteric pathogens (European Food Safety Authority 2007). Due to a lack of systematic validation of the surveillance systems, one reported case of, for example, a Salmonella infection in countries A and B is likely to represent different numbers of infections in the respective populations. Hence the reported incidence rates are inadequate as the basis for comparisons between countries and for the calculation of overall burden, cost of diseases, priority settings, validation of risk assessment models and monitoring of the impact of control measures.

Approaches

Earlier in Workpackage 32 we developed a novel approach of estimating the population incidence of infections with Salmonella and Campylobacter on the basis of cross-sectional serological population surveys.

Using ELISA, we measured concentrations of serum immunoglobulin (IgG, IgM, and IgA against Salmonella and Campylobacter), respectively, in population-representative serum collections from eight EU countries. Antibodies against Salmonella were measured with a validated in-house mix-ELISA at Statens Serum Institut, using lipopolysaccharide antigens from Salmonella Enteritidis (O-antigens 1,4,5,12) and Salmonella Typhimurium (O-antigens 1,4,5,12) in the solid phase of the microtiter plates. All analyses were done in duplicate. A negative control and a serial dilution of a reference serum were included on all plates. Antibodies against Campylobacter were measured at Stichting Samenwerkende Delftsche Ziekenhuizen in Delft, The Netherlands, with an in-house ELISA using whole-cell extract as antigen, which had been previously developed there.

Incidences of seroconversions (representing infections) was estimated with the help of a Bayesian back-calculation model, which has been described in detail elsewhere. The model is based on the kinetics of IgG, IgM, and IgA observed during a follow-up study of 302 adult Danish patients with stool culture-confirmed Salmonella infections and 210 patients with stool culture-confirmed Campylobacter infections. It incorporates inter-individual variation of peak antibody response and decay rates.

In a “reverse” application, the model generates a probability distribution of the likely time since last infection for a given set of IgA, IgG and IgM values measured in any single serum. Applied to a large number of antibody measurements from a cross-sectional sera survey in a population, the individual estimates of the individual times since last infection were finally converted to an estimate of the annual infection incidence (sero-incidence) in that population. By dividing
The usefulness of the methodology was demonstrated but also some barriers for wider application were identified. One of these barriers was the limited availability of sera that were sampled in a representative fashion from the general population. Hence we embarked on studying the feasibility of more easily accessible sera, for example surplus sera from pregnancy screening programmes. If valid sero-incidence estimates can be derived from such sera, this would greatly simplify the application of the methodology as a monitoring tool in many more countries.

**Results**

The European multinational study was completed by finishing tests for *Campylobacter* antibodies on previously collected sera and analysing additional sera obtained from the Netherlands and Sweden for both *Salmonella* and *Campylobacter* antibodies. Eventually, approximately 7,500 sera from eight countries (Denmark, Finland, Sweden, The Netherlands, France, Italy, Poland, Romania) were tested (Table 32.1). This included a historical comparison of sera from two different time periods in the Netherlands (1998–2002 and 2006–2007). In addition, we tested 530 surplus sera from the screening programme for pregnant women in The Netherlands collected between January and December 2006. Based on the antibody measurements, an incidence estimate of *Salmonella* and *Campylobacter* infections was generated for each serum collection with our back-calculation model.

Table 32.2 shows the sero-incidence estimates for *Salmonella*, the reported incidences, the incidence estimates based on a study of infection rates in Swedish travellers, and the ratios (“multipliers”) between these estimates.

Sero-incidence estimates ranged from 56 and 58 infections per 1,000 person-years in Finland and Sweden, respectively (corresponding to one infection per person approximately every 17 years), to 547 infections per 1,000 person-years in Poland (equivalent to approximately one infection per person every other year).

Sero-incidence and incidence of reported cases are inversely correlated, albeit not significantly (Figure 31.2a). The lowest sero-incidence estimates were found in the Scandinavian countries—Finland, Sweden and Denmark—which at the same time show rather high incidences of reported cases. The multipliers, defined as the ratio between sero-incidence and incidence of reported cases, were in the range from 124 for Finland to almost 10,000 for Romania. They tended to increase with increasing sero-incidence.

In a further study the back-calculation model was applied to estimate *Salmonella* sero-incidence in cohorts from three different European countries at varying time periods. For this purpose the model was extended such that it was able to estimate the ratio between the incidence ratios, with belongings confidence intervals (Deliverable D32.11).

A significant positive correlation was demonstrated between sero-incidence and incidence estimates derived from infection rates in Swedish citizens returning from travel to the respective country (Figure 32.1b). The country-specific multipliers between these estimates and corresponding sero-incidence estimates varied less widely (26 to 227), with a trend to decrease with increasing sero-incidence.

*Salmonella* sero-incidence showed a significant correlation with prevalence of *Salmonella* in holdings of layer hens (Figure 32.2a) and broilers (data not shown—very similar to layer hens), and a non-significant correlation with prevalence in slaughter pigs (Figure 32.2b).

The *Salmonella* incidence estimate based on analysis of sera from pregnant women in the Netherlands in 2006 was 86 (90% CI: 28-175) per 1,000 person years. This is not significantly different from the estimate of 149 per 1,000 person years based on population-representative sera from 2006–2007, but the point estimate is about 40% lower.

Analysis of sera from 16 individuals affected by a food-borne outbreak of *Salmonella* Heidelberg in Denmark showed that the mix-ELISA was able to detect antibodies in similar concentrations as antibodies after infections with *S. Enteritidis* or *S. Typhimurium*. This was to be expected, because *S. Heidelberg* has the same O-antigens (1,4,5,12) as *S. Typhimurium*.

Sero-incidence estimates for *Campylobacter* were higher for *Salmonella* and showed much less variation among participating countries with overlapping credibility intervals (Table 32.3). Comparison with incidence of reported cases was limited by the lack of operational surveillance systems for human *Campylobacter* infections in Italy, Romania and Poland. Comparison with

<table>
<thead>
<tr>
<th>Country</th>
<th>Serum Incidence* (95% CI)</th>
<th>Reported cases*</th>
<th>Multiplier</th>
<th>Incidence estimate based on (de Jong 2006)*</th>
<th>Multiplier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>56 (8,151)</td>
<td>0.45</td>
<td>124</td>
<td>0.08 (700)</td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>58 (8,155)</td>
<td>0.43</td>
<td>134</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Denmark</td>
<td>84 (41,141)</td>
<td>0.29</td>
<td>289</td>
<td>0.81 (104)</td>
<td></td>
</tr>
<tr>
<td>NL 2006–2007</td>
<td>149 (78,245)</td>
<td>0.14</td>
<td>1,064</td>
<td>0.98 (152)</td>
<td></td>
</tr>
<tr>
<td>NL 1998–2002</td>
<td>169 (91,271)</td>
<td>0.20</td>
<td>939</td>
<td>0.98 (172)</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>239 (115,411)</td>
<td>0.12</td>
<td>1992</td>
<td>2.71 (88)</td>
<td></td>
</tr>
<tr>
<td>Romania</td>
<td>385 (217,613)</td>
<td>0.04</td>
<td>9625</td>
<td>14.57 (26)</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>404 (272,573)</td>
<td>0.20</td>
<td>2010</td>
<td>1.78 (227)</td>
<td></td>
</tr>
<tr>
<td>Poland</td>
<td>547 (343,813)</td>
<td>0.42</td>
<td>1302</td>
<td>16.26 (33)</td>
<td></td>
</tr>
</tbody>
</table>

* = per 1,000 person-years.

95% CI = 95% credibility limits, i.e. the 2.5th and 97.5th percentile of the probability distribution of the estimate.

n/a = not applicable.
incidence estimates derived from infection rates in Swedish citizens returning from travel to the respective country (Ekdahl, 2004) showed no correlation. Veterinary data from the EU baseline survey in chickens were not yet available.

**Conclusions**

We were able to demonstrate that our novel approach to estimate population incidence of infections works well for *Salmonella*. Triangulation with other data that are independent of the sensitivity of routine public health surveillance systems, such as infection rates in returning Swedish travellers and prevalence in major food animals, confirms the plausibility of the sero-incidence estimates.

*Salmonella* sero-incidence did not correlate with incidence of reported cases. This is no surprise and is very likely a reflection of the fact that in countries that prioritize *Salmonella* control, such as the Scandinavian countries, two effects occur simultaneously: the number of reported cases increases due to intensified surveillance, while the true incidence of human infections goes down as a result of mitigation efforts. As such, reported incidence rates do not reflect the infection risk in the human population.

For *Campylobacter* the situation is less clear. Sero-incidence estimates showed little variation among countries, in contrast to infection rates in Swedish travellers as shown by others. The uniformity of our estimates may reflect the fact that much less is done to control *Campylobacter* compared to *Salmonella*. In addition, ubiquitous environmental transmission may be more important for *Campylobacter*, defying control efforts. However, our estimates may also be biased due to particular characteristics of the ELISA or of the longitudinal data set from Danish patients, on which the parameters describing antibody kinetics in the back-calculation model were based. Further validation of the methodology is needed for *Campylobacter*.

An important lesson learnt during Workpackage 32 is that truly population-representative serum collections are not available in all countries, and, where they do exist, they may be considered a precious commodity and access may be denied. As a first step to explore if other more readily accessible serum collections could be a suitable substitute, we analysed sera collected for screening purposes from pregnant women in The Netherlands. The results of this pilot study were ambiguous and more work needs to be done to explore the suitability of easily accessible, pre-existing serum collections. These could include, among others, pregnant women, blood donors, individuals consulting for health checks, and orthopaedic patients.

Following an outbreak of *Campylobacter jejuni* caused by a temporary drinking water contamination in a small town in Denmark in mid-June 2009, we started collecting serum and faecal samples from approximately 60 people, including sick people and asymptomatic people believed to be exposed. This provided a long-awaited opportunity to study the antibody response in asymptomatically infected individuals. While only some of these samples could be analysed within the lifetime of the workpackage, the preliminary data seem to indicate that exposed, but asymptomatic, people may have lower serum antibody levels than symptomatic cases. Further analyses will be continued after the end of Med-Vet-Net.

**Table 32.3. Sero-incidence estimates for Campylobacter.**

<table>
<thead>
<tr>
<th>Country</th>
<th>Sero incidence* (90% CL)</th>
<th>Reported cases*</th>
<th>Multiplier</th>
<th>Infections per 1,000 Swedish travellers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finland</td>
<td>668 (454, 929) 0.71</td>
<td>945</td>
<td>C/A/B</td>
<td>0.009</td>
</tr>
<tr>
<td>Denmark</td>
<td>720 (568, 892) 0.66</td>
<td>1,095</td>
<td>ND</td>
<td>0.04</td>
</tr>
<tr>
<td>NL 2006–2007</td>
<td>827 (635, 1,051) 0.40</td>
<td>2,068</td>
<td>ND</td>
<td>0.09</td>
</tr>
<tr>
<td>NL 1998–2002</td>
<td>674 (514,862) 0.42</td>
<td>1,605</td>
<td>3,970*</td>
<td>0.08</td>
</tr>
<tr>
<td>Italy</td>
<td>913 (658, 1,227) 0.23</td>
<td>3,080</td>
<td>ND</td>
<td>0.009</td>
</tr>
<tr>
<td>Romania</td>
<td>775 (545, 1,054) 0.40</td>
<td>2,356</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>644 (481, 840) 0.27</td>
<td>816 (584, 1,100) 0.50</td>
<td>ND</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*a = per 1000 person-years.  
b = based on surveillance data from a limited geographical area.  
90% CL = 90% Credibility Limits.  
ND = no comprehensive surveillance data available (for year of serum collection).
Workpackage 33: Annual Research Report

WP number 33

Title Early host responses to Salmonella and Campylobacter

WP Leader Riny JANSSEN

Name and Address Laboratory for Health Protection Research National Institute for Public Health and the Environment (RIVM) Bilthoven NETHERLANDS

Project Start date 1 March 2007

Project End date 31 October 2009

Progress summary

The aim of Workpackage 33 was to identify host-factors that are regulated in the intestine at early time points after infection with Salmonella or Campylobacter. To that end end microarray analysis, confocal microscopy and conventional immunological techniques were used.

During the previous reporting period a workshop was held at the Veterinary Laboratories Agency (VLA) during which data obtained by workpackage partners were discussed. It appeared that Salmonella and Campylobacter induced only weak responses in the intestine of infected mice. Based on the findings, recommendations for further research were made and addressed during the third and final year of the workpackage. As a result, immune responses were measured:

- in vivo, using a colitis model of infection
- in vitro, using cell lines infected with Salmonella or Campylobacter
- in chicken as a natural host of the bacteria
- in cell lines using well-defined bacterial mutants
- in cell lines bearing well-defined genetic deficiencies.

Attempts were also made to extend the findings in the murine model to humans.

The major conclusion of the work was that the in vivo response to Salmonella and Campylobacter in the intestine is very weak, whereas the response of cell lines is much stronger. In addition, there is very little overlap between the in vivo and in vitro response. Strains that replicate better in the host induce stronger responses.

Taken together, these data indicate that responses to Salmonella and Campylobacter in the intestine are tightly controlled by the host, which would protect the host from potentially harmful inflammatory intestinal responses to pathogens that cause relatively mild disease. This may have implications for the induction of long-term protective immunity to infection. The obtained data suggest that when exposure to Salmonella and Campylobacter is very low, long-term protection is not efficiently induced whereas frequent low dose exposure may result in the induction of some protection. This fits quite well with epidemiological observations, particularly for campylobacteriosis.

Objectives

The overall objective of Workpackage 33 was to identify host-factors that are regulated in the intestine at early time points after infection with Salmonella or Campylobacter using microarray analysis, confocal microscopy and conventional immunological techniques.

Key achievements to date

- Host responses were found to be weak in a colitis model of disease where mice were pre-treated with streptomycin and then infected with Salmonella or Campylobacter.
- In vitro experiments identified much stronger responses in murine cell lines exposed to Salmonella and Campylobacter.
- Host responses in a natural host, using a chicken model, were also weak following colonisation but were much stronger in vitro using chicken cell lines.
- It seems the host down-regulates immune responses to Salmonella or Campylobacter during colonisation or infection, which could be a means of damage control by the host during infection.

Background

The overall aim of this project was to identify host-factors that are regulated in the intestine in response to infection with Salmonella or Campylobacter, making use of a mouse model of colonization. It was hoped that this would facilitate a better understanding of the interaction of these pathogens with the host immune system, that the findings would increase our knowledge on specific host-pathogen interactions, and that they would be valuable to researchers working on these pathogens in the fields of risk-research, epidemiology, and detection and control.

During the first two years of Workpackage 33, efforts concentrated on a comparison of early host-responses to Salmonella in highly susceptible, as well as relatively resistant mice. In addition, a comparison was made of early host-responses to Salmonella wild-type and knockout mutants with different replication profiles, and an attempt to analyse the early host-responses to Campylobacter colonization was undertaken. These data demonstrated that responses to infection, as measured by microarray analysis of the intestine to infection, were rather weak, although three regulated murine gene clusters could be identified that responded in presence of the pathogens: a stress gene cluster, a cluster of genes involved in metabolism, and an immune gene cluster. The latter was mainly induced following Salmonella infection but not during Campylobacter infection. These results lead to four scientific questions that were addressed in Workpackage 33’s final year:

1. How strong are the immune responses in a model of intestinal disease, as opposed to colonization only? For this purpose responses were analysed in a colitis model where mice were pre-treated with streptomycin and then infected with Salmonella or Campylobacter.

2. What responses are seen in a natural host? To address this, responses were analysed in vivo in chickens using a Salmonella colonisation model, as well as in vitro using chicken cell lines.

3. How do the observed responses relate to data generated by in vitro models? For this, experiments were performed in murine cell lines exposed to Salmonella or Campylobacter.

4. Is it feasible to measure markers of Th1 immunity in humans suffering from Salmonella or Campylobacter infection?

Results

Responses in a model of intestinal disease

Mice were pre-treated with streptomycin to exclude intestinal flora and were subsequently infected with Salmonella. Responses in the ileum or colon were investigated and even though the mice displayed symptoms and were visibly sick, responses at the transcription level were similar to those observed in all previous colonization experiments where symptoms had been absent. Figure 33.1 illustrates some of the findings.

Host responses in chicken as a natural host were measured.

Figure 33.1 illustrates some of the findings.
Analysis of responses in a natural host of Salmonella

To investigate responses in a natural host, chicken cell lines, as well as chicks, were infected with Salmonella (serotypes Typhimurium, Hadar and Enteritidis), and with various isogenic knockout mutants. Host responses were evaluated in parallel with the phenotypic pathogenicity indicators of cell invasion and intestinal colonization/organ invasion of the Salmonella strains used.

The first series of experiments was performed using strains S. Enteritidis SE-147, S. Typhimurium ST-F9B, and S. Hadar strain SH-18. The latter is a representative of group "C" strains, isolated from a broiler chick in Hungary that had previously been shown to have some cross-inhibitory activity against other Salmonella serovars.

Wild-type SH-18 lacks the Salmonella virulence plasmid (spv), but contains the Salmonella Pathogenicity Island 1 (SPI1) for which, within Workpackage 26, the question of its role in pathogenicity had been raised. Therefore, specific (targeted) knockout (KO) mutants were produced to delete SPI1 genes hiIA, spvP, and sipB in SH-18. Subsequent in vitro infection of Vero and chicken embryo fibroblast (CEF) cells revealed that all three strains of Salmonella were equally invasive to both cell types and induced similar IL-8 responses in both cells. Comparing wild-type SH-18 with its isogenic single SPI1 gene deletions, invasiveness and cytokine stimulation had both increased for the sipB mutant (in both Vero and CEF cells) but, in contrast, hiIA and sipB mutants were less invasive and induced fewer IL-6 production, although those decreases were not always significant. These results indicate that invasion and cytokine stimulation, as measured in vitro, are under complex regulation and that SPI1 genes may be involved in this, but multiple gene deletions would be necessary to obtain a conclusive answer to their role in invasion and cytokine induction.

In vivo studies on S. Hadar and its mutants were performed using a day-old chick oral infection model, and organ invasion in liver and spleen was assessed. In accordance with the in vitro data, SH-18 and its sipB mutant behaved similarly, but organ invasion was significantly decreased for sipB and dhIA. Cacal IL-8 responses were also determined and the same differences were observed: the mutants, sipB and hiIA, produced a reduced IL-8 response but the sipB mutant behaved like the wild-type strain.

A similar series of experiments was performed using S. Enteritidis strain 11, which is a representative of group "D" strains isolated from a broiler chick in Hungary and had lower colonizing and organ invasion abilities compared to the reference wild-type S. Enteritidis 147. For these experiments the immune response was assessed using a custom-made (Agilent) microarray bearing chicken-specific 15,206 gene probes (out of the 44,000 Galuss genes). A one-colour labelling system was used for hybridization. Results (normalized for GAPDH and 28S rRNA) were analysed by one-way analysis of variance using GeneSpring V9, with significant (p<0.05), at least two-fold, up- or down-regulated genes identified and grouped using Database for Annotation, Visualization and Integrated Discovery (DAVID). The in vitro analysis of SE-147 infected CEF cells identified 907 up-regulated genes, of which 447 could be interpreted. These were clustered into six groups, and included genes related to immune response. Far fewer genes (59 genes) were down-regulated. Results for strain SE-11 were less pronounced. For the SE-11 fliD spv double mutant these numbers were 388 and 15, respectively, but the findings did not result in a clear pattern that could be attributed to invasiveness or cytokine production.

The cellular expression results were compared with in vitro invasiveness and the elicited in vitro IL-8 response. The results, shown in Figure 33.2, indicate a parallel decrease in invasion and IL-8 stimulation when fliD or spv genes are mutated in SE-11, suggesting that both motility and the spv plasmid could play a role in these phenotypes.

The microarray was also used to analyse the in vivo situation where chicks were colonized with SE-147, SE-11 and the SE-11 double mutant. Epithelial cell RNA was analysed at 16 hours and 40 hours post-infection. In this analysis only few genes were observed up- or down-regulated.

Comparing the chicken RNA microarray responses to the same Salmonella strains under in vitro (CEF) and in vivo (caecum) conditions (using the same bioinformatic analytic criteria), we can conclude that there is a much stronger transcriptional response to infection in vivo than there is in vivo. Contra-intuitively, the strongest in vivo response detectable was in the negative control group after 16 hours post-hatch (without Salmonella challenge), most likely due to the development of the normal intestinal flora. As a result of a Salmonella challenge, it seems that the caecal early pro-inflammatory response is actively down-regulated (maybe except the non-pathogenic non-motile, plasmid-less mutant). Thus, the ‘under-responding’ in vivo chicken host, and the ‘over-responding’ in vitro chicken cells somewhat parallels the in vivo and in vitro findings obtained with the mouse models.

In vitro experiments performed in murine cell lines exposed to Salmonella and Campylobacter All obtained data showed that responses to infection, as measured by microarray analysis of the intestine to infection, are rather weak even when a colitis model for Salmonella infection...
was used, which is in sharp contrast to infection data in cell lines where strong responses were observed, and that had also been described in the literature.

In a next step, microarray analysis was performed on cells infected with wild-type (WT) *Salmonella*, and *Salmonella* PCP, a mutant with a greater capacity to replicate in vivo. Wild-type Campylobacter was compared to a non-motile (NM) Campylobacter mutant. Four hours after *in vitro* infection, cellular RNA was extracted. The responses of up-regulated genes with fold-ratios (FR) greater than 1.5 and 2.0 are summarized below:

<table>
<thead>
<tr>
<th></th>
<th>FR &gt; 1.5</th>
<th>&gt; 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> WT</td>
<td>460</td>
<td>81</td>
</tr>
<tr>
<td><em>Salmonella</em> PCP</td>
<td>685</td>
<td>110</td>
</tr>
<tr>
<td><em>Campylobacter</em> WT</td>
<td>390</td>
<td>24</td>
</tr>
<tr>
<td><em>Campylobacter</em> NM</td>
<td>675</td>
<td>110</td>
</tr>
</tbody>
</table>

For *Salmonella*, gene ontology analysis of the up-regulated genes showed enrichment of the Gene Ontology (GO) terms "chemokines", "cytokines" and "inflammation" but no clear pathway enrichment was observed among the down-regulated genes. The *Salmonella* PCP mutant resulted in more genes being up- and down-regulated, suggesting that these more rapidly growing mutants also induced the strongest cellular response.

For Campylobacter infection, no clear enrichment of GO terms was seen in the corresponding genes, though the Campylobacter NM mutant indicated up-regulation of genes involved in "apoptosis", "transcription" and "development" and down-regulation for "translation" and "metabolism" genes.

![Figure 33.3. Response of a murine rectal cell-line to wild type and mutant *Salmonella* or Campylobacter.](image)

Such data indicate that wild-type *Salmonella* and, in particular its mutant PCP, induce a strong response whereas the response to wild-type *Campylobacter* is weaker. The response to the Campylobacter mutant, however, is much stronger. In total, 259 genes were regulated more than two-fold in any of the samples. Expression of these genes is shown in Figure 33.3. The highly expressed gene cluster at the top contains chemokines, cytokines and other immune/inflammation-related genes. Interestingly, these genes are not up-regulated in response to Campylobacter.

**Feasibility of measuring markers of Th1 immunity**

Human sera from patients suffering from *Salmonella* or Campylobacter infection are available albeit scarce. Since our murine studies showed that, in response to *Salmonella* (but not Campylobacter) infection, immune genes are activated, especially those involved in Th1 immunity, we investigated if determining Th1 protein expression in serum of groups of patients would be feasible. However, searching the literature for auto-immune models revealed that stable Th1 markers that can be readily measured in serum are not yet available. Therefore, we decided that it was not feasible to use the scarce human sera for these analyses.

If in the future stable, easy-to-measure Th1 markers become available, this would be a possible approach for further studies.

**Conclusions**

- Responses to Campylobacter and Salmonella in a colitis model of infection were very weak compared to *in vitro*-determined responses.
- The far stronger *in vitro* responses to *Salmonella* and Campylobacter are almost completely distinct from responses measured in a murine cell line. In the case of Salmonella, the strength of the response correlates to the replication capacity of the bacteria.
- In a natural host (chicken), *in vivo* responses were very low and clearly distinct from the much stronger *in vitro* response observed in cell lines.
- The early host-response in the ileum is surprisingly weak and dependent on the infecting pathogen.

**The workpackage aimed to identify host-factors that are regulated in the intestine.**
Workpackage 34: Annual Research Report

WP number 34
Title Sources, control and prevention of Campylobacter in poultry
WP Leader Diane G. NEWELL
Name and Address Veterinary Laboratories Agency (VLA) (Weybridge) Dept. Food and Environmental Safety Woodham Lane, Addlestone Surrey KT15 3NB UNITED KINGDOM
Project Start date 1 September 2008
Project End date 31 October 2009

Progress summary
Campylobacteriosis is the most common food-borne zoonotic disease reported in the European Union (EU), and the handling and consumption of poultry meat is a major attributable source of the disease. The prevalence of Campylobacter colonization in EU broiler flocks is currently under investigation but is assumed to be high in Member States. The control and prevention of the organism in poultry meat is therefore a strategic public health target. In this workpackage, Med-Vet-Net scientists successfully collaborated to extend our understanding of the development of interventions to control Campylobacter in poultry pre-harvest. This work included the delivery of an up-to-date database of the published literature on such interventions, and the development of a draft survey-based tool for investigating stakeholder attitudes to the role of biosecurity in prevention of flock colonization. In addition, an international scientific workshop on the potential for vaccine development was organized with outputs delivered to the 15th International Campylobacter Workshop in Japan in September 2009 and presented to the poultry industry.

Fundamental work was carried out, using post-genomic and bioinformatics approaches, to develop understanding of the mechanisms of colonization by Campylobacter in chickens, which resulted in an evidence-based database of Campylobacter genes associated with colonization. A systematic review on the molecular mechanisms of Campylobacter survival in the poultry environment was undertaken with the information contributing to an analysis of non-food-borne routes of human infection. Finally, we completed an analysis of published in vivo models of chicken colonization with the aim of making recommendations for standardization and harmonization.

Background
Campylobacter jejuni and Campylobacter coli are the most common causes of human acute enteritis in Europe with 200,507 cases reported in 2007. This is a considerable under-estimation of the prevalence of disease because many EU Member States do not undertake effective surveillance and, even in those countries with adequate surveillance, only approximately one in eight cases is reported. At the time of initiating this project, epidemiological studies and quantitative risk assessment models indicated that 20–40% of campylobacteriosis cases were attributable to the handling or consumption of contaminated poultry meat. More recently, population studies based on multilocus sequence typing (MLST) indicate that up to 85% of cases may be attributed to Campylobacter derived from poultry. The disparity between these prevalences may reflect a general contamination of the environment with poultry Campylobacters.

Campylobacters are a common asymptomatic colonizer of broilers and in many EU countries up to 100% of tested broiler flocks are colonized at least during the summer months. During processing, faecal contamination occurs and carcasses become contaminated. Although organisms do not grow in the environment, they survive for long periods and cross-contamination with contaminated chicken juices during cooking is a well recognised route of human infection. Modelling studies clearly indicate that although interventions post-harvesting could be cost effective, the most effective public health measure would be prevention of colonization on the farm.

In 2008, a baseline survey of Campylobacter colonization in broiler flocks in EU Member States was undertaken to provide a basis for determining effective interventions against Campylobacter in poultry flocks. However, currently it would be difficult, if not impossible, to develop recommendations for practical intervention strategies at the farm level. The epidemiology of colonization indicates that Campylobacters are horizontally transmitted to the flock from sources within the farm environment. Many potential on-farm sources and routes of transmission have been reported, nevertheless the control and prevention of Campylobacter colonization in poultry by biosecurity measures has proven fraught with difficulties. This failure reflects the ubiquitous nature of the organisms within the environment and their ability to survive in many hostile environments. Such survival mechanisms could be targets for the development of novel interventions but little is known of the molecular basis of the mechanisms involved.

Experimental evidence from avian models of colonization suggests that birds are highly susceptible to colonization, indicating that the avian gut is a preferential niche for these organisms. Despite considerable studies, the molecular mechanisms of colonization and the roles of Campylobacter colonization factors in birds are largely unknown. In part, this is due to the variety of models of colonization used.

Objectives
Workpackage 34’s overall objective was to develop and expand knowledge of Campylobacter in poultry by:
• collating, managing and archiving Europe’s information base
• developing and collating knowledge of the molecular basis of colonization of the avian gut, and disseminating knowledge on the epidemiology of poultry colonization and of the survival of Campylobacters in the poultry environment
• critically evaluating the indirect poultry-derived routes of Campylobacter infections
• investigating and developing understanding of effective methods of control and prevention.

Key achievements to date
• An evidence-based database of Campylobacter genes associated with colonization in chickens and their functions and the publications on interventions for Campylobacter in poultry.
• An international workshop on vaccination of poultry against Campylobacter.
• A systematic review of the molecular mechanisms for survival of Campylobacter in the poultry environment.
• A review of in vivo models of Campylobacter colonization in poultry, and recommendations for standardization and harmonization.
• A critical review of potential indirect (non-poultry, meat-borne) routes of transmission of “poultry Campylobacters” to humans.
• A draft survey-based tool for determining stakeholder attitudes to biosecurity, to prevent Campylobacter colonization at the farm level.

Approaches and results
Workpackage 34 was a multidisciplinary project utilizing the skills of epidemiologists, geneticists, bioinformaticists and microbiologists to further understand the interaction between the organism and its environment and host, and to exploit this information to develop intervention strategies. The project was undertaken in four major tasks:
1. Managing current knowledge.
2. Understanding chicken host colonization.
3. Understanding the basis for, and consequences of, Campylobacter survival in the poultry flocks and other environments.
The prevalence of Campylobacter colonization in EU broiler flocks is currently under investigation.

1. Managing current knowledge
While there is extensive worldwide knowledge on Campylobacters, because the organism is diverse in the host species that can be colonized, the outcomes of infection and genome complement, this information base is highly fragmented. Most information is focused on the disease aspects in humans, especially epidemiology and pathogenesis, but little is understood of the non-human host aspects or on aspects of the farm-to-fork transmission of this agent. In particular, because colonization in domestic animals and livestock is generally asymptomatic, there are few groups in the world interested in colonization in these hosts, and most of the interested groups are based in Europe.

Workpackage 34 has provided an integrated research network of experts from within the Med-Vet-Net partnership as well as external collaborators invited to contribute specific information, such as at the vaccination workshop. Historical published information was collected using a literature search (based on Endnote X1) from primarily white literature using the search term "Campylobacter AND poultry OR chicken OR broiler OR turkey OR duck". This information database, updated in February 2009, was distributed to the partnership and also provided to the European Food Safety Authority working group on Campylobacter in poultry.

A joint workshop aiming to develop a combined microbiological and epidemiological approach for investigating host-microbe interactions of Campylobacter jejuni was held with Workpackage 30 in November 2008. The workshop facilitated an information exchange, particularly regarding the use of molecular epidemiology to investigate the attribution of sources of Campylobacter for humans and chickens.

Ongoing discussions with the poultry industry are working towards the delivery of a regular web-based newsletter on Campylobacter in poultry to provide knowledge and education about current scientific approaches and research outcomes on prevention and control.

2. Understanding chicken host colonization
Experimental evidence suggests that both the host and the bacterium have roles in the establishment, persistence and extent of colonization. Over the past 20 years considerable effort has gone into the development of chicken models of colonization, and the use of these models for the comparison of strain colonization potentials and ad hoc identification of bacterial genes involved in colonization using defined mutants. Several models have been developed and are routinely used by members of Workpackage 34. These models can differ in the chicken age, type, microbiological status, methods of maintenance, as well as the number of organisms; organism growth conditions, challenge conditions, and so on. All of these factors may have impacts on the outcome of challenge. Little of this information, however, is recorded and much is anecdotal. Therefore, a review was undertaken to collate and utilize existing knowledge to develop recommendations for the chicken model of colonization.

The review comprised two phases. In the first phase, models of chicken colonization were identified from the literature and relevant parameters were extracted and collated into a spreadsheet. The information was then interpreted to analyse model variables in terms of the bacterium and bacterial factors and experimental procedures. Finally, recommendations to enable some model standardization and harmonization were delivered. These recommendations focused on the need for detailed information disclosure in publications, availability of a panel of reference strains for model testing, and the requirements for comparing parent strains with isogenic mutants.

With the recent publications of a number of Campylobacter genomes, and with more sequences becoming available in the near future, a rapidly growing number of isogenic mutants have been generated and tested for colonization potential in vivo. In addition, microarray data from colonization studies are also now becoming available, including from work within Workpackage 33.

Studies using structured post-genomic approaches to the identification of colonization factors are now feasible. It is anticipated that the number of genes associated with colonization of the chicken gut and the available information about these genes will grow exponentially over the next few years. In order to proactively support such studies, a list of described genes affecting colonization (positively or negatively) and their properties, based on a search of the current literature and supplemented by data provided by Workpackage 34 partners, has been developed.

A total of 52 such genes were identified from the available information sources. Working on the basis that genes involved in colonization of chickens would need to be highly conserved in all strains that could colonize chickens, bioinformatic analysis of the genomes of C. jejuni to compare these genes with the core genome (the fraction of genes present in every Campylobacter genome) as opposed to the variable genome fraction. A "guilty by association" strategy followed to identify further putative colonization genes using two complementary approaches.

The first approach investigated those genes that consistently flanked proven colonization genes in all, or nearly all, sequenced genomes. The rationale of was that genes consistently co-localized in a genome are more likely to be involved in the same process. The second approach identified genes that were predicted to be involved in the same biological processes as proven colonization genes, using two methods—firstly the annotation of genes was tested using Gene Ontology terms, and secondly the genes were investigated in maps representing their roles in metabolic and physiological processes using the Kyoto Encyclopaedia of Genes and Genomes. Both approaches led to the identification of novel putative colonization genes through their association with published colonization-associated genes. The conservation of all putative colonization genes was assessed, and the potential strength of their association with colonization, as indicated by gene relatedness, was recorded. Such information enabled a score to be developed for the likely strength of the affect on colonization expected when the putative genes were mutated. These "predicted-scored" putative colonization genes provide suitable targets for mutagenesis studies that might generate evidence of their role in colonization.

3. Understanding the basis for, and consequences of, Campylobacter survival in the poultry farm and other environments
One important factor in colonization from horizontal sources around the poultry environment would be the ability of Campylobacters to survive in that environment. A multitude of stresses exist in and around the poultry farm to which the bacteria are exposed, such as temperature changes, dessication, osmotic shock due to water exposure, disinfectants, bacteriophages and (ultraviolet) sunlight. Mechanisms of
survival in Campylobacters have been poorly investigated and there are few obvious survival mechanisms indicated in the genome sequence of the organism. The consequences of effector(s), like exposure to water, may result in loss of colonization capacity without an effector on culturability. Some genetic mechanisms potentially associated with survival have been identified, including hypervariable sequences and/or genetic rearrangements and instability, but the frequency and role of such mechanisms in the poultry farm environment is largely unexplored. Environmental contamination may act as source for the contamination of poultry flocks but also as an indirect source of Campylobacter to humans (for example, surface water contamination or contamination of vegetables).

This task comprised two sub-tasks: (i) a systematic review of Campylobacter genes and mechanisms associated with environmental survival as defined using in vitro models, and (ii) a critical review of the role of poultry in environmental contamination and its contribution to indirect routes of transmission to humans.

Although Campylobacter does not grow outside the host, it can survive for extended periods in a variety of hostile environments. Such survival requires the organism to detect and respond to environmental stresses. Understanding such responses contributes to the development of intervention approaches targeted at controlling the organism in the farm environment.

A systematic review of the published literature demonstrated that a variety of approaches and technologies have been used to investigate Campylobacter responses to environmental stresses. Campylobacter mechanisms associated with survival of such stresses have been identified using a variety of models involving exposure of the organism to a defined stress, and the measurement of the effect of that stress exposure on phenotypic properties such as viability, colonization potential or gene expression. The types of exposure investigated in these studies included aerobic/oxidative stresses, heat stress/thermoregulation, nutrient deprivation/starvation, osmotic stress, nitrosative stress, acid pH, and UV. Most recently, post-genomic investigations have been used, including genome sequence comparison, global expression microarrays and proteomics, in conjunction with a range of isogenic mutants, to identify putative survival-associated genes.

From the literature, a list of 63 genes associated with Campylobacter survival (positively or negatively), and their properties, was generated in a spreadsheet format, which was then used as the basis for a review of models for measurement of stress survival, and the molecular bases for the survival of specified environmental stresses.

The review highlighted differences in assay conditions between studies of similar stress exposures. To some degree these differences reflected the stated objectives of the studies. For example, studies were designed to investigate the types of stresses encountered in the host gut, such as temperature up-regulation and acid pH, while other studies focused on external conditions potentially found in the farm environment, such as nutrient deprivation or atmospheric oxygen.

In agreement with the early analysis of the Campylobacter genome sequence, the diversity of genes shown to be associated with survival by Campylobacter of environmental stresses are limited, indicating that novel genes or mechanisms of survival, have yet to be identified in this species. Of the genes identified as stress-associated, several were involved in the response to multiple stresses. This is unsurprising given that the environments to which this organism is exposed are highly complex and variable.

A critical review of the literature concluded that environmental contamination with Campylobacters from poultry was potentially widespread based on knowledge of the numbers of organisms associated with poultry, the survival potential of these organisms, and the ease of transmission from major reservoirs. However, there is little evidence of the degree of such contamination throughout the environment.

Published evidence suggested that these environmental “poultry Campylobacters” could infect humans, contributing to the non-food-borne disease burden. Interestingly, recent observations from Workpackage 32 and Workpackage 23 indicate that human exposure to Campylobacter from the consumption or handling of poultry meat is far more frequent than previously assumed. One hypothesis would be that indirect non-poultry meat routes could be important to such exposure. Such an explanation could account for the reported differences in observations of source attribution as indicated by epidemiological studies as against models using MLST.

This review will be submitted for publication.

4. Biosecurity-based and non-biosecurity-based control and prevention

Expert opinion and advice on strategies to prevent or control Campylobacter in poultry is actively sought by industry and public health authorities. Currently the only strategy available at the farm level involves improved biosecurity to exclude Campylobacter from entering the flock. Unfortunately the sources and routes of transmission for a poultry farm are manifold, highly variable and may differ between farm, season and region. A critical review of these issues was recently undertaken for the Food Standards Agency in the United Kingdom by an international research team that included members of Workpackage 34 (see www.foodbase.org.uk/results.php?ff_category_id=&f_report_id=251).

Current approaches to biosecurity include general measures, such as educating catching crews in the United Kingdom, or specific targets such as the use of fly screens over poultry houses in Denmark. Many such strategies, though suitable for conventionally-reared flocks in some countries, may be inappropriate for all geographical locations, and for flocks reared under different management systems. Importantly, expert opinion is uncertain as to the most effective or practical approaches to adopt, which provides stakeholders and policymakers with confused advice. In order to understand the diversity of expert opinion, a survey-based tool that could be widely distributed, has been drafted. The survey is in English but has been reviewed and edited by participants from several countries for clarity and global relevance. Feedback by way of the survey could be incorporated into future studies, and indicate expert bias in policyholder advice. Moreover, such information could be used to target education of industry representatives.

There are no currently available non-biosecurity-based strategies for on-farm intervention. However, vaccines, bacteriophages and bactericins are being actively investigated. Vaccination has made a highly successful contribution to the control of Salmonella in chickens but, until recently, vaccination against Campylobacter in poultry has been poorly investigated. As such Workpackage 34 hosted a workshop entitled “Vaccination of poultry against Campylobacter” in April 2009 involving 40 participants from 11 countries including the United States. The workshop also included contributions from all the leading research groups in this field.

A CD-ROM of the meeting presentations was produced and distributed to all participants, and is available on request. The conclusions of the workshop were incorporated into a keynote lecture given at the 15th International Campylobacter Workshop in Japan in September 2009, and a report appeared in the November 2009 edition of Poultry World, a monthly magazine for the poultry industry.
The aim of the Med-Vet-Net Network of Excellence was to integrate the research activities of over 300 scientists from 14 public health and veterinary institutes and a learned society. In the EC Referees’ Report for Year 4, the Network was rated as ‘good to excellent’ having “fully achieved its objectives and technical goals for the period and even exceeded expectations”.

**Management issues and resolution**

In Year 5 the main issues addressed were:

- Governing Board leadership
- Administration Bureau
- Financial management
- Durable integration and sustainability
- Delivery of high quality research programme
- Workpackage interactions
- Training
- Communications Unit
- Expansion of international collaborations.

**Governing Board leadership**

From 1 September 2008 to 18 May 2009, the Chair and Deputy Chair of the Med-Vet-Net Governing Board continued under the leadership of Dr. Valérie Baduel of the French Food Safety Agency, and Dr. Carmen Audera of the Institute of Public Health Carlos III (ISCIII), Spain, respectively. In May 2009, Dr. Audera tendered her resignation as Deputy Chair following her appointment to a position in the World Health Organization, based in Switzerland. Professor Carlos Segovia, also of ISCIII, replaced Dr. Audera as Deputy Chair.

Both the Chair and Deputy Chair provided invaluable input and support to all Network management, sustainability and scientific activities throughout their tenures. We are also particularly grateful to Dr. Audera for her input into the planning of Med-Vet-Net’s 5th Annual Scientific Meeting held in El Escorial, Spain, in June 2009.

**Administration Bureau**

In December 2008, Sandra Daignot resigned from the Administration Bureau to take up a post elsewhere, and was replaced in January 2009 by Alice Créty.

The Administration Bureau continued to work closely with the Project Manager, the Communications Unit, the Co-ordinating Forum, the Governing Board and the EC representative to ensure delivery of the annual activity report and the JPAS plans in an acceptable time frame. Additionally, the Project Management Team continued to participate in the European Community Project Managers’ Association (ECPMA).

**Financial management**

It is difficult to forecast a year in advance what have to be exact expenditures from 15 partners in order to optimize total expenditure by the project.

The main financial challenge for Year 5 therefore was establishing a consistent JPAS budget and achieving planned expenditures that would comply with the following parameters:

- To set up a JPAS budget not too low and not too high to optimize the total expenditures claimed from the European Commission (EC) such that the €14.4 million total EC grant allocated to the project is fully spent, and self-financing from partners is controlled in acceptable proportions.
- To take into account the impact of receipts declared by partner institutes over the full duration of the project on financial statements.
- To decide how to share the self-financing generated by the project among the partnership.
- To anticipate the final payment from the EC, and final settlement from the Co-ordinator to the partners.

**Durable integration and sustainability**

As reported for Year 4, durable integration and sustainability for the Network for the future continued to be addressed by two subcommittees in Year 5 — a Sustainability subcommittee formed with Governing Board and Co-ordinating Forum members and including the Project Manager and Co-ordinators Representative, and a Legal Officers subcommittee comprising the Legal Officers from several of the participating institutes. These two subcommittees continued to work closely together in developing the concept of a Med-Vet-Net association to ensure the Network’s sustainability after the cessation of EU funding on 31 August 2009.

Those efforts to ensure long-term stability have borne fruit with the formation of the self-funded Med-Vet-Net Association, which was officially launched in Brussels on 6 October 2009. It is noteworthy that all current scientific institutes elected to join the Association as full members for an initial period of three years.

The durable integration of scientific activities was also addressed at the scientific level, and is clearly demonstrable in the Key Performance Indicators. Med-Vet-Net scientists at all levels continued to pursue opportunities for funding within FP7 and other areas, with the primary objective of maintaining and expanding multi-disciplinary research teams and incorporating research groups and institutes outside the present network into the proposals. In that respect, several collaborative projects that will continue beyond the EU-funded period of Med-Vet-Net, have ensued. These are listed elsewhere in the report.

**Delivery of high quality research programme**

All of Med-Vet-Net’s partner institutes continued to be actively involved in the research programme under the leadership of the Health Protection Agency. During Year 5, fifteen scientific workpackages were ongoing — Workpackage 6 and Workpackages 21–34. In all, 22 research-associated meetings were held throughout the Network, involving over 300 scientists, of which at least 100 were external. Additionally, results from Med-Vet-Net scientific workpackages have been presented to at least 70 external meetings with an overall audience of more than 1,500 people from Europe and elsewhere.

Summaries of the major workpackage achievements are provided elsewhere in this report and more detailed information is given in the deliverables. The outputs of the research are also summarized in the Key Performance Indicators.

**Workpackage interactions**

Collaborations between scientific workpackages continued to develop and become more evident. Six workpackages with common interests held joint meetings, and other workpackages held joint meetings with Special Interest Groups. These meetings ensured a high level of interaction between participants and were of significant benefit to the Network in terms of exchange of ideas, technologies and the exploitation of methods and analyses.

Efforts to ensure long-term stability have borne fruit with the formation of the self-funded Med-Vet-Net Association, which was officially launched in Brussels on 6 October 2009.
In several instances such collaborations resulted in either the submission of, or publication of, peer-reviewed papers with joint authorships from several different workpackages. An additional outcome of the inter-workpackage collaborations has been the informal linkage of scientists to develop and submit applications for funding, which will continue after the cessation of EU funding for the Med-Vet-Net Network of Excellence.

**Training**

Training activities continued to be afforded high priority and remained an essential element of the Med-Vet-Net during the final year. Three well-attended training courses were funded, and short-term missions (STMs) and exchanges continued to be well subscribed, with 15 STMs funded. The Network also continued to provide partial funding for five PhD students within four workpackages. Two of those PhDs were awarded. A further seven interns were also trained in the modular science communication programme, which was established in Year 2.

**Communications Unit**

In March 2009, Jennie Drew Greaney resigned from the Communications Unit to take up a post elsewhere, and was replaced in the same month by Tania Cutting. Charlene Mills also joined the team in March 2009 to manage the graphic design. The Communications Unit worked closely with the Project Manager and the Administration Bureau in ensuring knowledge dissemination and spreading excellence were undertaken to the highest standards.

**Expansion of international collaborations**

Raising awareness and developing collaborations in the international context of food safety and zoonoses continued to be an important component of the Network. In Year 5 efforts were made to increase Med-Vet-Net’s level of collaboration with other institutes and networks. Subsequently, 12 new international collaborations were initiated. Strong contacts with other Networks of Excellence working in associated areas were also strengthened. In addition, Med-Vet-Net became officially represented in the International Federation for Animal Health Europe through membership of the EC-funded Disease Control Tools project. Other new international collaborations were developed through the Special Interest Groups, and Med-Vet-Net scientists continued to support the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) through membership of ad-hoc and other fora.

**Project timetable status**

The project is now 100% through the scheduled workplan. Table B1 shows progress against plans, as a Gantt chart, for the overarching Workpackages 1–3 and the scientific Workpackages 6 and 21–3. Table B2 provides a list of meetings held within the Network.

Workpackage 6 continued from JPA1 through to JPA5. All other Workpackages continued as expected with new milestones and deliverables. Following receipt of financial information, the possibility of extending workpackages for a three-month period after the first quarter of 2009 was investigated. Workpackage leaders were invited to submit proposals for extensions, which were peer-reviewed by members of Med-Vet-Net’s Co-ordinating Forum. All workpackages were provided with additional funds under the proviso that all work was to be completed before 31 August 2009.

Of the 117 expected milestones in Year 5, 111 (95.2%) were fully achieved, one (0.8%) was partially achieved, one (0.8%) was withdrawn and only four (3.2%) failed. Of the expected 142 deliverables, 132 (93%) were fully achieved, one (0.7%) was partially achieved, three (2.1%) were achieved with modifications, two (1.4%) were withdrawn and four (2.8%) failed.

None of the modifications was considered to require management intervention. The appropriate reasons are provided in the relevant workpackage reports (see Deliverables).

**Communication between partners, project meetings, possible co-operation with other projects/programmes**

The partnership continued to make full use of all forms of communication. Of particular note was the use of web-based conferencing. In Year 5, 31 web-based meetings involving 136 participants were held. The web-based conferencing facility was particularly useful in the planning of the 5th Annual Scientific Meeting, during which 7 such conferences took place over a period of five months.

**Co-operation with other projects/programmes**

<table>
<thead>
<tr>
<th>Task Name</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Med-Vet-Net project</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP1 – Virtual Institute</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP2 – Strategic Scientific Integration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP3 – Spreading Excellence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP6 – Geographical Information Systems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP21 – Epidemiology of Salmonella Genomic Island 1(SG11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP22 – Zoonotic Protozoon Network (ZOOPNET)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP23 – Prioritizing food-borne and zoonotic hazards</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP24 – Campylobacter risk assessment models</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP25 – Improved diagnostics for Q-fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP26 – Virulotyping Salmonella and VTEC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP27 – Trichinella control, QMIRA &amp; early diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP28 – Attributing human zoonotic infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP29 – Emerging antimicrobial resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP30 – Host-microbe interactions of C. jejuni (CampyNet II)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP31 – Potential sources of emerging viral zoonoses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP32 – Epidemiological studies for surveillance pyramid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP33 – Early host-pathogen responses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP34 – Campylobacter in Poultry</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- The Co-ordinator’s Representative is a member of the European Technology Platform for Global Animal Health Steering Committee.
- The Project Manager represents Med-Vet-Net on the EU-funded Disease Control Tools (DISCONTOOLS) project and has been appointed to the DISCONTOOLS Management Board.
- The Communications Unit is a member of CommNet.
- The Project Management Team are active members of EUPMAN, which currently has over 60 project managers from EU-funded projects within FP6 thematic areas one and five.

All Med-Vet-Net partners are involved in many other EU-funded projects, and the sharing of knowledge between these projects occurs regularly within all the partner institutes. All of the zoonoses-related Community Reference Laboratories (CRL) are also partners in Med-Vet-Net, and the contacts within the Network are used to support CRL activities.

**Non-EC collaborations**

- Club 5, a European communication and collaboration network consisting of five national reference veterinary laboratories.
- Food Safety Rapid Response and Research Network, a research network of the United States Department of Agriculture. The collaboration has involved joint meetings and short-term missions through the associated EUUS-SAFEFood project.
- The Workpackage 23 leader is a member of the steering group of the Food Safety Research Consortium, an American research network.
• Workpackage 24 has collaborated with the New Zealand Food Safety Group.
• Exchanging newsletters and information with the Australian Biosecurity Cooperative Research Centre.

**FP7 and other proposals prepared during Year 5** with Med-Vet-Net participants actively involved

- FoodCom, HealthCom (Workpackage 3).
- SAFEOOD-ERA. Project started in April 2009 and involves four Workpackage 21 partners and one other partner.
- Collaborative project between three Med-Vet-Net partners on method development for plasmid characterization by micro-array (Workpackage 21).
- Workpackage 24’s CRAF 2.0 extension with the American-based organization, FoodRisk.org.
- Ongoing EFSA-funded project, ‘Development of harmonized schemes for monitoring and reporting of rabies and Q-fever in animals in the European Union’ (Workpackage 25).
- Club 5-funded project, ‘Implementation and harmonization of diagnostic tools for Coxiella burnetii (Q-fever) in ruminants or cattle, goats and sheep’ (Workpackage 25).
- Submission by three partners, including two from Workpackage 25, to an EMIDA ERA-NET proposal regarding the study of the resistant forms of Coxiella burnetii.
- FP7 proposal with several Med-Vet-Net partners and other collaborators in parallel with a participation of the consortium in the recent EFSA call on zoonotic parasites (Workpackage 27).
- ERA-NET proposal in November 2009 on Hepatitis E virus (Workpackage 31).
- Four Med-Vet-Net partners are involved in the FP7 project VITAL, integrated monitoring and control of food-borne viruses in European food supply chains (Workpackage 31).
- ECDC call for tender ‘Sero-epidemiology as a tool to assess incidence of Salmonella and Campylobacter infections.’ Four Med-Vet-Net Workpackage 32 partners are collaborating to submit an offer.
- FP7 application to respond to new food call in September 2008 (Workpackage 33).
- Part of the work performed in Workpackage 33 is being submitted into a Wellcome Trust proposal.
- KBBE-2009-1-3-05: Improving Campylobacter control measures in primary production of poultry, involves Workpackage 34 partners.

### Table B2. Meetings held within network in Year 5.

<table>
<thead>
<tr>
<th>WP no.</th>
<th>Date</th>
<th>Meeting type</th>
<th>Location</th>
<th>Number of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>WP1</td>
<td>October 2008</td>
<td>Co-ordinating Forum</td>
<td>Rome, Italy</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>November 2008</td>
<td>Governing Board</td>
<td>Weybridge, UK</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>December 2008</td>
<td>EC Reviewers</td>
<td>Brussels, Belgium</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>April 2009</td>
<td>Co-ordinating Forum</td>
<td>Milton Keynes, UK</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>October 2009</td>
<td>Final Joint Co-ordinating Forum and Governing Board</td>
<td>Brussels, Belgium</td>
<td>41</td>
</tr>
<tr>
<td>WP2</td>
<td>September 2008</td>
<td>Meeting with ECDC officials</td>
<td>Stockholm, Sweden</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>December 2008</td>
<td>EC reviewers</td>
<td>Brussels, Belgium</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Meeting with DG Sanco</td>
<td>Brussels, Belgium</td>
<td>12</td>
</tr>
<tr>
<td>WP2B (SIG WIREDZ)</td>
<td>December 2008</td>
<td>Discussion meeting — wildlife diseases</td>
<td>Budapest, Hungary</td>
<td>22</td>
</tr>
<tr>
<td>WP3</td>
<td>October 2008</td>
<td>Pre-conference planning meeting</td>
<td>El Escorial, Spain</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>July 2009</td>
<td>Communications Unit Meeting</td>
<td>Milton Keynes, UK</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>October 2009</td>
<td>Med-Vet-Net Association kick-off meeting</td>
<td>Brussels, Belgium</td>
<td>37</td>
</tr>
<tr>
<td>WP6</td>
<td>March 2008</td>
<td>Workpackage meeting</td>
<td>Bilthoven, The Netherlands</td>
<td>22</td>
</tr>
<tr>
<td>WP21</td>
<td>April 2009</td>
<td>Workpackage meeting (joint with WP29)</td>
<td>Paris, France</td>
<td>22</td>
</tr>
<tr>
<td>WP22</td>
<td>November 2008</td>
<td>Workpackage meeting</td>
<td>Uppsala, Sweden</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>ZoopNet meeting (informal)</td>
<td>Madrid, Spain</td>
<td>20</td>
</tr>
<tr>
<td>WP23</td>
<td>June 2009</td>
<td>Workpackage meeting (joint with WP32)</td>
<td>Warsaw, Poland</td>
<td>22</td>
</tr>
<tr>
<td>WP24</td>
<td>February 2009</td>
<td>Workshop meeting</td>
<td>Berlin, Germany</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>CRAF training course</td>
<td>Madrid, Spain</td>
<td>30</td>
</tr>
<tr>
<td>WP25</td>
<td>June 2009</td>
<td>Workshop meeting</td>
<td>Warsaw, Poland</td>
<td>20</td>
</tr>
<tr>
<td>WP26</td>
<td>January 2009</td>
<td>Workshop meeting</td>
<td>Weybridge, UK</td>
<td>30</td>
</tr>
<tr>
<td>WP29</td>
<td>April 2009</td>
<td>Workshop meeting (joint with WP21)</td>
<td>Paris, France</td>
<td>22</td>
</tr>
<tr>
<td>WP30</td>
<td>November 2008</td>
<td>Workpackage meeting (joint with WP30)</td>
<td>Ploufragan, France</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>May 2009</td>
<td>Workshop meeting</td>
<td>Utrecht, The Netherlands</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Workshop satellite meeting</td>
<td>Madrid, Spain</td>
<td>20</td>
</tr>
<tr>
<td>WP31</td>
<td>February 2009</td>
<td>Workshop meeting/Third ZOOVIRNET meeting</td>
<td>Weybridge, UK</td>
<td>30</td>
</tr>
<tr>
<td>WP32</td>
<td>June 2009</td>
<td>Workshop meeting (joint with WP23)</td>
<td>Warsaw, Poland</td>
<td>22</td>
</tr>
<tr>
<td>WP34</td>
<td>November 2008</td>
<td>Workpackage meeting (joint with WP30)</td>
<td>Ploufragan, France</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>April 2009</td>
<td>Workshop meeting</td>
<td>Weybridge, UK</td>
<td>39</td>
</tr>
</tbody>
</table>

* Meetings to prepare/facilitate applications for future FP7 and other proposals were not funded by the Network.
Section 4: Other issues

Ethical
Ethical issues were specifically considered in the workpackages below in relation to work with experimental animals, exchanges of biological reagents, genetic engineering, genetically modified organism release, or release of patient data:

Workpackage 26: Animal studies conducted at the Veterinary Medical Research Institute, Hungary, were in line with the local regulations and approved by the appropriate ethics committee.

Workpackage 27: The participants involved in the project guaranteed to respect the Council Directive 93/88/EEC of 12 October 1993 amending Directive 90/679/EEC on the protection of workers from risks related to exposure to biological agents at work. The participants complied with EU and local regulations for all exchanges of biological reagents. *Trichinella* infected material was packed and forwarded as hazardous material in accordance with EU regulations. All requested import permits accompanied the shipments. All animal experiments were performed according to national regulations and were approved by the ethical committees of the institutes in charge.

Workpackage 32: All participating countries arranged permission with the appropriate authorities to use sera for the purpose of the workpackage. Available sera were shipped anonymised with only background information on age, gender and date of blood collection. No other issues were applicable.

Workpackage 33: All animal studies complied with all national legislation.

There were no ethical issues involved in the work undertaken in any other workpackage.

Gender
The male-female distribution of personnel within Med-Vet-Net was maintained at approximately 50:50, although some workpackages may have fluctuated by 10–20%. The primary consideration for staff recruitment was the ability of the person appointed to perform the tasks required in relation to the Job Description, irrespective of gender.

Wherever possible, meetings were held within the working week, avoiding school and public holidays. An exception to this was the annual scientific meeting, which for the past four years concluded at noon on a Saturday.
## Deliverables:
(1 September 2008 – 31 October 2009)

<table>
<thead>
<tr>
<th>WP no.</th>
<th>Deliverable no.</th>
<th>Deliverable title</th>
<th>Due date</th>
<th>Status</th>
<th>Lead contractor</th>
</tr>
</thead>
<tbody>
<tr>
<td>WP1</td>
<td>D1.08</td>
<td>Final report on animal experimental facilities</td>
<td>31/03/09</td>
<td>Withdrawn</td>
<td>AFSSA</td>
</tr>
<tr>
<td></td>
<td>D1.11</td>
<td>Statutes and internal regulations for the Med-Vet-Net Association</td>
<td>30/11/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D1.13</td>
<td>Report on EC reviewer’s meeting 2008</td>
<td>31/01/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D1.15</td>
<td>Fourth annual financial report and JPA5 plans</td>
<td>15/10/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D1.16</td>
<td>Fifth and final financial report</td>
<td>15/10/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP2</td>
<td>D2.16</td>
<td>Delivery of scientific annual report for Year 4</td>
<td>15/10/08</td>
<td>Achieved</td>
<td>HPA</td>
</tr>
<tr>
<td></td>
<td>D2.17</td>
<td>Delivery of JPA5 plans</td>
<td>15/10/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D2.24</td>
<td>Plans for workpackage extensions from March to June 2009</td>
<td>31/10/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D2.25</td>
<td>Reports on three additional workshops/training courses</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D2.26</td>
<td>Report on EC reviewer’s meeting 2008</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D2.27</td>
<td>Report on 5th Annual Scientific Meeting</td>
<td>31/07/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D2.28</td>
<td>Three Project Management Newsletters in JPA5</td>
<td>31/08/09</td>
<td>Modified</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D2.29</td>
<td>Final report of Med-Vet-Net activities</td>
<td>30/09/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D2.30</td>
<td>Submission of paper to peer-reviewed journal on Med-Vet-Net’s scientific achievements</td>
<td>30/09/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP3</td>
<td>D3.19</td>
<td>Public engagement activity</td>
<td>28/02/09</td>
<td>Achieved</td>
<td>SFAM</td>
</tr>
<tr>
<td></td>
<td>D3.22</td>
<td>Modules 3 &amp; 4 of the Science Communications Internship run for the second time with up to three participants</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D3.24</td>
<td>Up to eight participants complete Module 1 of the Science Communications Internship</td>
<td>30/04/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D3.25</td>
<td>Delegate packs for 5th Annual Scientific Meeting</td>
<td>31/05/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D3.26</td>
<td>Production of four newsletters in JPA5 period</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D3.27</td>
<td>Sustainable website designed for Med-Vet-Net beyond 31 August 2009</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP6</td>
<td>D6.08</td>
<td>Final product — Dynamic Salmonella Atlas</td>
<td>31/03/09</td>
<td>Modified</td>
<td>SSI</td>
</tr>
<tr>
<td></td>
<td>D6.09</td>
<td>Outcome of Salmonella geo-trend project (report)</td>
<td>30/04/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D6.10</td>
<td>Outcome of VTEC project (report)</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D6.12</td>
<td>Outcome of C. burnetii project (report)</td>
<td>31/05/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D6.13</td>
<td>Spatio-temporal Salmonella project: final analysis of 10 subtypes</td>
<td>15/12/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D6.14</td>
<td>Report on workshop in The Netherlands</td>
<td>15/04/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D6.16</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP21</td>
<td>D21.13</td>
<td>Second paper on the occurrence and distribution of SGI1 submitted for publication</td>
<td>31/03/09</td>
<td>Achieved</td>
<td>CVI</td>
</tr>
<tr>
<td></td>
<td>D21.14</td>
<td>Paper on analysis of multi-drug resistant salmonellas in France submitted for publication</td>
<td>31/03/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D21.15</td>
<td>Final WP21/WP29 meeting in Paris</td>
<td>30/04/09</td>
<td>Modified</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D21.16</td>
<td>Presentations at the 5th Annual Scientific Meeting</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D21.17</td>
<td>Report on the occurrence and characteristics of the SGI1 in S. Kentucky and S. Newport isolates</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D21.18</td>
<td>Submission of peer-reviewed publication on the mobilization of SGI1</td>
<td>31/08/09</td>
<td>Failed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D21.19</td>
<td>Submission of peer-reviewed publication on association between SGI1 and virulence</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D21.20</td>
<td>Manuscript on the frequency of the ASSuT island</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D21.21</td>
<td>Submission of manuscript on the plasmids harbouring qnrS1</td>
<td>31/08/09</td>
<td>Modified</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D21.22</td>
<td>Submission of manuscript on fingerprinting assay based on SYBR-green real-time-PCR</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D21.23</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP no.</td>
<td>Deliverable no.</td>
<td>Deliverable title</td>
<td>Due date</td>
<td>Status</td>
<td>Lead contractor</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------</td>
<td>-------------------</td>
<td>----------</td>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td>WP22</td>
<td>D22.02</td>
<td>Online database of relevant methodologies for the detection and control of Giardia</td>
<td>30/11/08</td>
<td>Achieved</td>
<td>ISS</td>
</tr>
<tr>
<td></td>
<td>D22.11</td>
<td>Online dynamic database for the analysis of sequence data from both Cryptosporidium and Giardia</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D22.12</td>
<td>Repositories of Giardia and Cryptosporidium reference material</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D22.15</td>
<td>Sequence data from Cryptosporidium isolates collected in different European countries and from different hosts</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D22.16</td>
<td>Sequence data from Giardia isolates collected in different European countries and from different hosts</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D22.17</td>
<td>Phylogenetic and spatial analysis of Cryptosporidium and Giardia isolates from different regions in Europe</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D22.19</td>
<td>Joint publication on the database and genotyping data: use and lessons learned</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D22.20</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP23</td>
<td>D23.06</td>
<td>Summary of available data</td>
<td>31/12/08</td>
<td>Achieved</td>
<td>RIVM</td>
</tr>
<tr>
<td></td>
<td>D23.07</td>
<td>Scientific paper on generated results</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D23.09</td>
<td>Interim report on telephone survey</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D23.10</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP24</td>
<td>D24.09</td>
<td>Report on consensus framework with directions for risk assessment</td>
<td>30/06/09</td>
<td>Achieved</td>
<td>RIVM</td>
</tr>
<tr>
<td></td>
<td>D24.10</td>
<td>CRAF 2.0 software tool</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D24.11</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP25</td>
<td>D25.12</td>
<td>Recommendation for best practice in PCR</td>
<td>31/12/08</td>
<td>Achieved</td>
<td>AFSSA</td>
</tr>
<tr>
<td></td>
<td>D25.13</td>
<td>Submission of second paper on molecular epidemiology of C. burnetii infections</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D25.14</td>
<td>Analysis and recommendations for serological tests (human)</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D25.15</td>
<td>Analysis of typing method (MST/VNTRs) comparisons</td>
<td>31/05/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D25.16</td>
<td>Analysis of a microarray genotyping for C. burnetii</td>
<td>31/05/09</td>
<td>Failed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D25.17</td>
<td>Presentation at the 5th Annual Scientific Meeting</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D25.18</td>
<td>Recommendation for best practice in typing</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D25.19</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP26</td>
<td>D26.02</td>
<td>Review paper submitted for publication by VLA</td>
<td>31/08/09</td>
<td>Achieved</td>
<td>VLA</td>
</tr>
<tr>
<td></td>
<td>D26.10</td>
<td>In vitro (primary cell work) characterization of selected Salmonella wild-type and knockout mutants</td>
<td>31/10/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D26.11</td>
<td>Completion of in vivo (poultry model) characterization of Salmonella knockout mutants</td>
<td>31/12/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D26.12</td>
<td>Gene sets for development into virulotyping platforms (for example, microarray) for routine diagnostic use</td>
<td>31/12/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D26.13</td>
<td>Identification of possible virulence predictors for VTEC of significant public health impact</td>
<td>31/01/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D26.14</td>
<td>Report of workpackage meeting held at the VLA</td>
<td>31/01/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D26.15</td>
<td>Selection of a panel of well-characterized strains from each of the VTEC seropathotypes B, C, and D, and microarray comparison with strains from seropathotype A (O157)</td>
<td>30/04/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D26.16</td>
<td>Define OI genes using a molecular approach</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D26.17</td>
<td>Paper submitted to peer-reviewed journal on virulotyping</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D26.18</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP no.</td>
<td>Deliverable no.</td>
<td>Deliverable title</td>
<td>Due date</td>
<td>Status</td>
<td>Lead contractor</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------</td>
<td>-------------------</td>
<td>----------</td>
<td>--------</td>
<td>-----------------</td>
</tr>
<tr>
<td>WP27</td>
<td>D27.12</td>
<td>Report on the double funnel method for the direct test</td>
<td>28/02/09</td>
<td>Achieved</td>
<td>AFSSA</td>
</tr>
<tr>
<td></td>
<td>D27.13</td>
<td>Draft report concerning the optimization of the magnetic stirrer method</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D27.15</td>
<td>Draft report to describe how to improve the direct method for wild animal muscle</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D27.16</td>
<td>Draft report to describe how to improve the direct method for salted muscle</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D27.18</td>
<td>Report on sensitivity and specificity of recombinant iELISA and recombinant western blot for pigs</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D27.19</td>
<td>Draft report concerning a typing method and DNA extraction (without validation)</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D27.20</td>
<td>A model to design plausible scenarios for the persistence and detection of the parasites in wildlife</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D27.21</td>
<td>Protocol for <em>Trichinella</em> typing</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D27.22</td>
<td>Additional VNTR markers for <em>Trichinella</em> isolate typing</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D27.23</td>
<td>Report on iELISA and western blot analysis of reference samples</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D27.24</td>
<td>Genetic variability of <em>Trichinella spiralis</em> in Europe</td>
<td>30/06/09</td>
<td>Partially Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D27.25</td>
<td>Report on clinical and pathological findings following a dose dependent <em>T. spiralis</em> infection in rodents</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D27.26</td>
<td>Report on comparison of different DNA transport methods and ring trial on <em>Trichinella</em> typing</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D27.27</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP28</td>
<td>D28.11</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td>DTU</td>
</tr>
<tr>
<td></td>
<td>D28.12</td>
<td>Report on workpackage workshop</td>
<td>31/01/09</td>
<td>Withdrawn</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D28.13</td>
<td>Paper on attribution of human <em>Salmonella</em> and <em>Campylobacter</em> cases using outbreak data</td>
<td>28/02/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D28.14</td>
<td>Paper on development of comparative exposure assessments for human <em>Salmonellosis</em></td>
<td>30/11/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D28.15</td>
<td>Paper on attribution of human <em>Salmonella</em> cases using microbial subtyping — a multi-country study</td>
<td>31/01/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP29</td>
<td>D29.05</td>
<td>Submission of paper on methylases in peer-reviewed journal</td>
<td>31/12/08</td>
<td>Achieved</td>
<td>UCM</td>
</tr>
<tr>
<td></td>
<td>D29.09</td>
<td>Evaluation of the similarity between human and avian extra-intestinal <em>E. coli</em> in relation to their ciprofloxacin resistance status</td>
<td>30/11/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D29.10</td>
<td>Evaluation of the dissemination of the qnr genes among human and avian extra-intestinal <em>E. coli</em></td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D29.11</td>
<td>Report on the presence of aminoglycoside resistance determinants other than armA in bacteria of human and animal origin</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D29.12</td>
<td>PCR screening from the collection of isolates with high-level aminoglycoside resistance for the genes armA and rmtA-D</td>
<td>31/03/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D29.13</td>
<td>Report on the emergence of aminoglycoside resistance methyltransferase genes in human and animal isolates in the participating countries during 2005–07</td>
<td>30/04/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D29.14</td>
<td>Minutes of joint meeting with Workpackage 21 on antimicrobial resistance in Paris, April 2009</td>
<td>31/05/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D29.15</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP30</td>
<td>D30.05</td>
<td>Electronic catalogue (virtual strain collection) of expanded CampyNet database</td>
<td>28/02/09</td>
<td>Achieved</td>
<td>BfR</td>
</tr>
<tr>
<td></td>
<td>D30.08</td>
<td>Manuscript ready for submission: CampyNet strain characterization</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D30.10</td>
<td>Virtual database of strains</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D30.12</td>
<td>Pan-genome microarray data on selected CampyNet strains</td>
<td>31/07/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D30.13</td>
<td>Manuscript ready for submission on pan-genomics</td>
<td>31/07/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D30.14</td>
<td>Report of second workshop at CVI</td>
<td>01/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D30.15</td>
<td>Microarray data on selected CampyNet strains publicly available</td>
<td>31/07/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D30.16</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP no.</td>
<td>Deliverable no.</td>
<td>Deliverable title</td>
<td>Due date</td>
<td>Status</td>
<td>Lead contractor</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------</td>
<td>-------------------</td>
<td>----------</td>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td>WP31</td>
<td>D31.16</td>
<td>Standard reference positive and negative porcine sera for Hepatitis E virus (HEV) antibody determination, and ring trial for harmonization of HEV antibody ELISA assays</td>
<td>30/09/08</td>
<td>Achieved</td>
<td>ISS</td>
</tr>
<tr>
<td></td>
<td>D31.18</td>
<td>Real-time nucleic acid sequence based amplification protocol for sensitive detection of HEV in pig samples</td>
<td>31/08/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D31.19</td>
<td><em>In vitro</em> system for efficient propagation of HEV to high titres, and <em>in vitro</em> model organoid system for studying pathogenesis of Hepatitis E</td>
<td>31/12/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D31.20</td>
<td>Data on HEV antigenic determinants</td>
<td>31/12/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D31.21</td>
<td>Data on the correlation between conditions of farming and spread of HEV in pigs</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D31.22</td>
<td>Data on the levels of HEV contamination in oysters, mussels, wild boar and deer meat in Europe</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D31.23</td>
<td>Data on the seroprevalence of HEV in wild boar and animal species other than pigs in European countries</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D31.24</td>
<td>Data on the prevalence of Encephalomyocarditis virus infection in humans and animals in Europe</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D31.25</td>
<td>Preliminary model assessing the risk of milk-borne transmission of Tick-borne encephalitis virus based on data collected in Poland and Hungary</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D31.26</td>
<td>A durable web-accessible database of HEV strains and sequences on bionumerics platform</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D31.27</td>
<td>Chemical and antibody-based methods for HEV concentration from water</td>
<td>30/06/09</td>
<td>Failed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D31.28</td>
<td>Data on the detection of genotype three HEV in water and sewage</td>
<td>30/06/09</td>
<td>Failed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D31.29</td>
<td>Publication of results in peer-reviewed journal</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D31.30</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP32</td>
<td>D32.06</td>
<td>Report on results of the sero-epidemiological pilot study</td>
<td>30/11/08</td>
<td>Achieved</td>
<td>SSI</td>
</tr>
<tr>
<td></td>
<td>D32.10</td>
<td>Scientific paper describing how to generate incidence estimates from measured antibody levels in different populations</td>
<td>31/12/08</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D32.11</td>
<td>Scientific paper describing possible alternative modelling techniques for back-calculation</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D32.12</td>
<td>Scientific paper on the sero-incidence of Salmonella infections in the studied European countries, compared to reported case numbers</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D32.13</td>
<td>Scientific paper comparing sero-incidence of Campylobacter infections between countries</td>
<td>31/07/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D32.14</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP33</td>
<td>D33.06</td>
<td>Workshop to discuss and evaluate approaches to study early responses to Campylobacter infection</td>
<td>28/02/09</td>
<td>Achieved</td>
<td>RIVM</td>
</tr>
<tr>
<td></td>
<td>D33.08</td>
<td>Report on pilot studies to evaluate gene expression in response to infection in <em>in vivo</em> and <em>in vitro</em> chicken models</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D33.09</td>
<td>Evaluation of gene expression in streptomycin pre-treated mice with subsequent colitis induced by Salmonella</td>
<td>30/06/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D33.10</td>
<td>Evaluation of gene expression in murine cell lines infected with Salmonella or Campylobacter</td>
<td>31/05/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D33.11</td>
<td>Scientific paper (submissible draft) on evaluation of gene expression studies</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D33.12</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>WP34</td>
<td>D34.01</td>
<td>Deliver an up-to-date database (endnote-based) of publications on interventions for Campylobacter in poultry</td>
<td>30/09/08</td>
<td>Achieved</td>
<td>VLA</td>
</tr>
<tr>
<td></td>
<td>D34.02</td>
<td>Deliver recommendations of the chicken model of colonization utilising existing knowledge</td>
<td>31/07/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D34.03</td>
<td>Collation and annotation of Campylobacter genes and mechanisms associated with environmental survival as defined using <em>in vitro</em> models</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D34.04</td>
<td>Workshop on vaccination of poultry against Campylobacter</td>
<td>31/04/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D34.05</td>
<td>Deliver a critical review of the indirect poultry derived routes of Campylobacter infections</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D34.06</td>
<td>Deliver a report on outcome of bioinformatics studies undertaken within Task 2</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D34.07</td>
<td>Inventory of Campylobacter genes affecting colonization properties as described in the literature</td>
<td>28/02/09</td>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D34.08</td>
<td>Final workpackage report</td>
<td>31/08/09</td>
<td>Achieved</td>
<td></td>
</tr>
</tbody>
</table>
## Milestones:
(1 September 2008 – 31 October 2009)

<table>
<thead>
<tr>
<th>WP no.</th>
<th>Milestone No.</th>
<th>Milestone name</th>
<th>Due date</th>
<th>Actual/forecast delivery date</th>
<th>Partner</th>
</tr>
</thead>
<tbody>
<tr>
<td>WP1</td>
<td>M1.20</td>
<td>Finalization of inventory of animal experimental facilities</td>
<td>28/02/08</td>
<td>Month 42</td>
<td>Withdrawn AFSSA</td>
</tr>
<tr>
<td></td>
<td>M1.22</td>
<td>Fourth annual financial report delivered to EC</td>
<td>15/10/08</td>
<td>Month 50</td>
<td>05/12/08</td>
</tr>
<tr>
<td></td>
<td>M1.23</td>
<td>JPAS financial plans delivered to EC</td>
<td>15/10/08</td>
<td>Month 50</td>
<td>05/12/08</td>
</tr>
<tr>
<td></td>
<td>M1.24</td>
<td>Second preliminary report on sustainability to Governing Board (GB)</td>
<td>30/11/07</td>
<td>Month 34</td>
<td>30/11/08</td>
</tr>
<tr>
<td></td>
<td>M1.25</td>
<td>Decision by GB on options for sustainability</td>
<td>28/02/09</td>
<td>Month 55</td>
<td>31/12/08</td>
</tr>
<tr>
<td></td>
<td>M1.26</td>
<td>Completion of financial organization of the 5th Annual Scientific Meeting</td>
<td>31/08/09</td>
<td>Month 60</td>
<td>31/08/09</td>
</tr>
<tr>
<td></td>
<td>M1.27</td>
<td>Contribution to annual and final scientific report</td>
<td>31/08/09</td>
<td>Month 60</td>
<td>15/09/09</td>
</tr>
<tr>
<td>WP2</td>
<td>M2.31</td>
<td>New workpackage on Campylobacter in poultry</td>
<td>30/09/08</td>
<td>Month 48</td>
<td>30/09/08 HPA</td>
</tr>
<tr>
<td></td>
<td>M2.32</td>
<td>Plans for extensions of scientific workpackage plans for period March–June 2009</td>
<td>31/10/08</td>
<td>Month 50</td>
<td>31/10/08</td>
</tr>
<tr>
<td></td>
<td>M2.33</td>
<td>Submission of plans for collaborative projects extending Network activities beyond 31/08/09</td>
<td>31/10/08</td>
<td>Month 50</td>
<td>01/04/09</td>
</tr>
<tr>
<td></td>
<td>M2.34</td>
<td>Wildlife-related Emerging Diseases and Zoonoses meeting on bat lyssaviruses</td>
<td>31/12/08</td>
<td>Month 52</td>
<td>31/12/08</td>
</tr>
<tr>
<td></td>
<td>M2.35</td>
<td>Further three workshop/training courses</td>
<td>28/02/09</td>
<td>Month 54</td>
<td>28/02/09</td>
</tr>
<tr>
<td></td>
<td>M2.36</td>
<td>80 short term scientific missions (accumulative)</td>
<td>30/06/09</td>
<td>Month 58</td>
<td>30/06/09</td>
</tr>
<tr>
<td></td>
<td>M2.37</td>
<td>Completion of 5th Annual Scientific Meeting</td>
<td>30/06/09</td>
<td>Month 58</td>
<td>30/06/09</td>
</tr>
<tr>
<td></td>
<td>M2.38</td>
<td>Workpackage leader meeting</td>
<td>30/06/09</td>
<td>Month 58</td>
<td>30/06/09</td>
</tr>
<tr>
<td></td>
<td>M2.39</td>
<td>Workpackage leader presentations at Annual Scientific Meeting</td>
<td>30/06/09</td>
<td>Month 58</td>
<td>30/06/09</td>
</tr>
<tr>
<td></td>
<td>M2.40</td>
<td>Contribution to final report</td>
<td>31/08/09</td>
<td>Month 60</td>
<td>31/08/09</td>
</tr>
<tr>
<td>WP3</td>
<td>M3.16</td>
<td>Three additional science communicators trained</td>
<td>28/02/09</td>
<td>Month 54</td>
<td>28/02/09 SFAM</td>
</tr>
<tr>
<td></td>
<td>M3.17</td>
<td>Applicants enrolled in Module 1</td>
<td>28/02/09</td>
<td>Month 54</td>
<td>28/02/09</td>
</tr>
<tr>
<td></td>
<td>M3.18</td>
<td>Complete registration of all MVN delegates and fee-paying delegates for 5th Annual Scientific Meeting</td>
<td>31/05/09</td>
<td>Month 57</td>
<td>31/05/09</td>
</tr>
<tr>
<td></td>
<td>M3.19</td>
<td>Complete abstract submission for delegates at the 5th Annual Scientific Meeting</td>
<td>31/05/09</td>
<td>Month 57</td>
<td>31/05/09</td>
</tr>
<tr>
<td></td>
<td>M3.20</td>
<td>Interns assist at the Annual Scientific Meeting in Madrid, June 2009</td>
<td>30/06/09</td>
<td>Month 58</td>
<td>30/06/09</td>
</tr>
<tr>
<td></td>
<td>M3.21</td>
<td>Contribution to final report</td>
<td>31/08/09</td>
<td>Month 60</td>
<td>31/08/09</td>
</tr>
<tr>
<td>WP6</td>
<td>M6.16</td>
<td>Dynamic atlas: deployment of system</td>
<td>31/05/08</td>
<td>Month 57</td>
<td>31/05/09 SSI</td>
</tr>
<tr>
<td></td>
<td>M6.17</td>
<td>VTEC project: Final analyses performed for majority of countries</td>
<td>31/12/08</td>
<td>Month 52</td>
<td>31/12/08</td>
</tr>
<tr>
<td></td>
<td>M6.19</td>
<td>EPEC analysis completed (Denmark only)</td>
<td>01/11/08</td>
<td>Month 51</td>
<td>01/11/08</td>
</tr>
<tr>
<td></td>
<td>M6.20</td>
<td>VTEC project: drafts of first manuscript</td>
<td>01/12/08</td>
<td>Month 52</td>
<td>31/05/09</td>
</tr>
<tr>
<td></td>
<td>M6.21</td>
<td>Q-fever data collected and analysed</td>
<td>28/02/09</td>
<td>Month 54</td>
<td>28/02/09</td>
</tr>
<tr>
<td></td>
<td>M6.22</td>
<td>Contribution to final report</td>
<td>31/08/09</td>
<td>Month 60</td>
<td>31/08/09</td>
</tr>
<tr>
<td>WP21</td>
<td>M21.06</td>
<td>Prevalence/occurrence study for SGI1 in other Enterobacteriaceae</td>
<td>31/12/08</td>
<td>Month 52</td>
<td>31/12/08 CVI</td>
</tr>
<tr>
<td></td>
<td>M21.07</td>
<td>Development of a database of SGI1 positive isolates</td>
<td>31/12/08</td>
<td>Month 52</td>
<td>30/03/09</td>
</tr>
<tr>
<td></td>
<td>M21.08</td>
<td>Meeting at AFSSA, Maison Alforts, France</td>
<td>28/02/09</td>
<td>Month 54</td>
<td>30/04/09</td>
</tr>
<tr>
<td>WP no.</td>
<td>Milestone No.</td>
<td>Milestone name</td>
<td>Actual/forecast delivery date</td>
<td>Partner</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>---------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------------------------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>WP22</td>
<td>M22.08</td>
<td>Maintenance and further development (including development of a web-based platform) of the online dynamic database for the analysis of sequence and epidemiologic data for both <em>Cryptosporidium</em> and <em>Giardia</em></td>
<td>30/06/09 Month 58 30/06/09</td>
<td>ISS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M22.09</td>
<td>Maintenance and enlargement of repositories of <em>Cryptosporidium</em> and <em>Giardia</em> reference materials, including nucleic acids, oocysts/cysts and plasmids carrying specific gene fragments</td>
<td>30/06/09 Month 58 30/06/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M22.12</td>
<td>Collection of <em>Cryptosporidium</em> and <em>Giardia</em> field isolates from both human and animal populations from different European regions</td>
<td>30/06/09 Month 58 30/06/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M22.13</td>
<td>Generation, phylogenetic and spatial analysis of sequence data from selected animal and human parasite isolates to provide robust data useful to identify sources of infection across Europe</td>
<td>30/06/09 Month 58 30/06/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M22.14</td>
<td>Contribution to final report</td>
<td>31/08/09 Month 60 31/08/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP23</td>
<td>M23.07</td>
<td>Final meeting</td>
<td>28/02/09 Month 54 16/06/09</td>
<td>RIVM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M23.08</td>
<td>Second workshop on data integration</td>
<td>30/11/08 Month 51 30/11/08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M23.09</td>
<td>Contribution to final report</td>
<td>31/08/09 Month 60 31/08/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP24</td>
<td>M24.09</td>
<td>Training course</td>
<td>28/02/09 Month 54 28/02/09</td>
<td>RIVM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M24.10</td>
<td>Software Tool CRAFT 1.0 presented at final meeting</td>
<td>28/02/09 Month 54 28/02/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M24.11</td>
<td>Contribution to final report</td>
<td>31/08/09 Month 60 31/08/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP25</td>
<td>M25.18</td>
<td>Panel of DNA and protocols for the typing ring trial available for distribution</td>
<td>30/04/09 Month 56 10/03/09</td>
<td>AFSSA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M25.19</td>
<td>Typing ring test (MST and VNTRs) with a limited number of strains</td>
<td>30/04/09 Month 56 30/04/9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M25.20</td>
<td>Evaluation of a microarray genotyping completed</td>
<td>30/06/09 Month 58 Failed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M25.21</td>
<td>Isolation of new strains</td>
<td>30/06/09 Month 58 30/06/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M25.22</td>
<td>Analysis of serological tests for human sera</td>
<td>15/01/09 Month 53 30/06/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M25.23</td>
<td>Contribution to final report</td>
<td>31/08/09 Month 60 30/06/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP26</td>
<td>M26.16</td>
<td>Microarray analysis on extended <em>Salmonella</em> selected strains using the BfR array</td>
<td>30/11/08 Month 51 30/11/08</td>
<td>VLA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M26.17</td>
<td>Genotypic (sequencing the Q-anti-terminator gene) and phenotypic (phage induction) characterization of stx2 phages carried by <em>E. coli</em> O157:H7 EHEC strains</td>
<td>30/11/08 Month 51 30/11/08 30/09/08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M26.18</td>
<td><em>In vivo</em> testing (day-old chick model) of pathogenicity significance of selected <em>Salmonella</em> pathogenicity islands in serogroup C (such as <em>S.</em> Hadar and or <em>S.</em> Infantis strains) and in <em>S.</em> Enteritidis strains</td>
<td>31/12/08 Month 52 31/12/08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M26.19</td>
<td>Analysis of the results of long PCR on the WP26 collection and sequencing of the informative polymorphic regions and analysis at nucleotide level</td>
<td>31/12/08 Month 52 31/12/08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M26.21</td>
<td>Second analysis and comparison of collated data sets</td>
<td>28/02/09 Month 54 28/02/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M26.22</td>
<td>Identify possible genomic markers which may be hallmarks for VTEC belonging to seropathotypes of major concern for public health (A and B)</td>
<td>30/04/09 Month 56 30/04/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M26.23</td>
<td>Identify and investigate the O1-encoded information that is associated with VTEC</td>
<td>30/05/09 Month 57 30/05/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M26.24</td>
<td>Further characterize VTEC isolates from Europe, including atypical isolates using the virulotyping array</td>
<td>30/04/09 Month 56 30/04/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M26.25</td>
<td>Contribution to final report</td>
<td>31/08/09 Month 60 31/08/09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP no.</td>
<td>Milestone No.</td>
<td>Milestone name</td>
<td>Actual/forecast delivery date</td>
<td>Partner</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>---------------</td>
<td>---------------</td>
<td>-------------------------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>WP27</td>
<td>M27.06</td>
<td>Ring trial (two sessions)</td>
<td>30/09/08 Month 49</td>
<td>30/09/08</td>
<td>AFSSA</td>
</tr>
<tr>
<td>M27.08</td>
<td>Preparation of protocol for iELISA test with five Trichinella recombinant proteins</td>
<td>30/09/08 Month 49</td>
<td>30/09/08 AFSSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M27.10</td>
<td>Preparation of reference sera for ring trials for indirect test for the survey of Trichinella infection in pigs</td>
<td>30/09/08 Month 49</td>
<td>30/09/08 AFSSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M27.11</td>
<td>Agreement on protocol for Western blot test (human and pig) with five Trichinella recombinant proteins</td>
<td>30/09/08 Month 49</td>
<td>30/09/08 AFSSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M27.13</td>
<td>Completion of collection of larvae per gram (LPG) and serological test outcome</td>
<td>30/09/08 Month 49</td>
<td>30/09/08 AFSSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M27.14</td>
<td>Completion of collection and selection of human outbreak data and animal surveillance data</td>
<td>30/09/08 Month 49</td>
<td>30/09/08 AFSSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M27.15</td>
<td>Final workpackage meeting</td>
<td>30/06/09 Month 58</td>
<td>30/06/09 AFSSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M27.16</td>
<td>Completion of collection of European T. spiralis isolates</td>
<td>31/03/09 Month 55</td>
<td>31/03/09 AFSSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M27.17</td>
<td>Collection of organs and muscle samples from rats infected with high doses of Trichinella</td>
<td>28/02/09 Month 54</td>
<td>28/02/09 AFSSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M27.18</td>
<td>Contribution to final report</td>
<td>31/08/09 Month 60</td>
<td>31/08/09 AFSSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP28</td>
<td>M28.07</td>
<td>Complete updating and refining of human exposure model for Campylobacter</td>
<td>30/11/07 Month 39 Failed</td>
<td>DTU</td>
<td></td>
</tr>
<tr>
<td>M28.09</td>
<td>Complete review of existing case control studies of human campylobacteriosis and salmonellosis</td>
<td>31/03/09 Month 55</td>
<td>04/09/09 DTU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M28.10</td>
<td>Use data from food-borne disease outbreaks for source attribution of salmonellosis and campylobacteriosis</td>
<td>31/12/08 Month 52</td>
<td>04/09/09 DTU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M28.12</td>
<td>Comparison of the results obtained using different attribution methods</td>
<td>31/05/09 Month 57</td>
<td>04/09/09 DTU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M28.13</td>
<td>Contribution to final report</td>
<td>31/08/09 Month 60</td>
<td>31/08/09 DTU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP29</td>
<td>M29.07</td>
<td>Molecular identification and characterization of the integrons and plasmids conferring aminoglycoside resistance in Enterobacteriaceae</td>
<td>30/11/08 Month 51</td>
<td>30/11/08 UCM</td>
<td></td>
</tr>
<tr>
<td>M29.08</td>
<td>Evaluation of the presence of the qnr genes, that have recently emerged, in human and avian E. coli</td>
<td>31/12/08 Month 52</td>
<td>31/12/08 UCM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M29.09</td>
<td>Identification of the possible relationships between virulence genotypes, and ciprofloxacin resistance, and resistance determinants in human and avian E. coli</td>
<td>31/01/09 Month 53</td>
<td>31/01/09 UCM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M29.10</td>
<td>Establishing the presence of aminoglycoside-resistance determinants other than armA in human and animal Enterobacteriaceae</td>
<td>28/02/09 Month 54</td>
<td>28/02/09 UCM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M29.11</td>
<td>Identification of high-level aminoglycoside resistant isolates in the participating countries</td>
<td>31/03/09 Month 55</td>
<td>31/03/09 UCM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M29.12</td>
<td>Identification of the 16S rRNA methyltransferases responsible for high-level resistance to aminoglycosides</td>
<td>30/04/09 Month 56</td>
<td>30/04/09 UCM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M29.13</td>
<td>Dissemination of 16S rRNA methyltransferases in the EU in the period 2005–07</td>
<td>30/05/09 Month 57</td>
<td>30/05/09 UCM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M29.14</td>
<td>Contribution to final report</td>
<td>31/08/09 Month 60</td>
<td>31/08/09 UCM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP30</td>
<td>M30.15</td>
<td>Pan-genome array testing of selected CampyNet (CNET) strains completed</td>
<td>31/01/09 Month 53</td>
<td>31/01/09 BfR</td>
<td></td>
</tr>
<tr>
<td>M30.16</td>
<td>Genotyping of CNET strains completed</td>
<td>31/01/09 Month 53</td>
<td>31/01/09 BfR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M30.17</td>
<td>WP30 meeting (AFSSA, Ploufragan)</td>
<td>31/01/09 Month 53</td>
<td>31/01/09 BfR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M30.18</td>
<td>Selection of additional Campylobacter strains to be tested by aCGH analysis</td>
<td>31/03/09 Month 55</td>
<td>31/03/09 BfR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M30.19</td>
<td>Microarray workshop II (including WP30 subgroup meeting) at CVI</td>
<td>31/05/09 Month 57</td>
<td>31/05/09 BfR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M30.20</td>
<td>Testing of selected Campylobacter strains by selected WP members</td>
<td>30/06/09 Month 58</td>
<td>30/06/09 BfR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M30.21</td>
<td>Contribution to final report</td>
<td>31/08/09 Month 60</td>
<td>31/08/09 BfR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP No.</td>
<td>Milestone No.</td>
<td>Milestone name</td>
<td>Actual/forecast delivery date</td>
<td>Partner</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>---------------</td>
<td>----------------</td>
<td>-----------------------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>WP31</td>
<td>M31.11</td>
<td>Dissemination of results in the form of reports, scientific papers and risk assessments/codes of practice for at-risk populations</td>
<td>30/06/09 (Month 58)</td>
<td>ISS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M31.17</td>
<td>Implementation of serological assays for assessment of Hepatitis E virus (HEV) hazards related to farming and food, and investigation of HEV in conventional, free-ranged and organic farms and wildlife</td>
<td>31/12/08 (Month 52)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M31.18</td>
<td>Implementation of sensitive HEV molecular detection platforms suitable for investigation of complex matrices, and studies on HEV contamination in market food products</td>
<td>31/12/08 (Month 52)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M31.19</td>
<td>Preparation of milk sale questionnaires, extension of the human and dairy animals surveys (Poland and Hungary respectively), and collection and analysis of milk and tick samples and data</td>
<td>31/12/08 (Month 52)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M31.20</td>
<td>High affinity anti-HEV MAbs and pig sera selected for solid-phase coupling</td>
<td>30/06/09 (Month 58)</td>
<td>Failed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M31.21</td>
<td>Selection of water sources/locations geographically linked to swine farming activities</td>
<td>30/06/09 (Month 58)</td>
<td>30/06/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M31.22</td>
<td>Contribution to final report</td>
<td>31/08/09 (Month 60)</td>
<td>31/08/09</td>
<td></td>
</tr>
<tr>
<td>WP32</td>
<td>M32.07</td>
<td>Decision on possible collaboration with IID-2 study in UK and Ireland</td>
<td>30/06/09 (Month 58)</td>
<td>30/06/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M32.08</td>
<td>Recently collected population representative sera from The Netherlands (Plenti-2) tested for salmonella antibodies</td>
<td>30/09/08 (Month 49)</td>
<td>30/09/08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M32.09</td>
<td>Interim WP32 workshop</td>
<td>31/10/08 (Month 50)</td>
<td>31/10/08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M32.10</td>
<td>Analysis of sera from people exposed to Campylobacter or Salmonella, including asymptomatic persons, during at least two outbreaks</td>
<td>30/06/09 (Month 58)</td>
<td>30/06/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M32.11</td>
<td>Comparative analysis of pregnant women or blood donor sera with population-based sera from at least one country</td>
<td>01/01/09 (Month 53)</td>
<td>30/06/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M32.12</td>
<td>Presentation of WP32 interim results at European Scientific Conference on Applied Infectious Disease Epidemiology</td>
<td>31/11/08 (Month 51)</td>
<td>31/11/08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M32.13</td>
<td>Presentation of the project for the IID-2 study committee</td>
<td>31/05/09 (Month 57)</td>
<td>01/01/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M32.14</td>
<td>Serological tests of population sera from additional EU countries completed</td>
<td>30/06/09 (Month 58)</td>
<td>30/06/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M32.15</td>
<td>Final WP32 workshop</td>
<td>30/06/09 (Month 58)</td>
<td>30/06/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M32.16</td>
<td>Contribution to final report</td>
<td>31/08/09 (Month 60)</td>
<td>31/08/09</td>
<td></td>
</tr>
<tr>
<td>WP33</td>
<td>M33.06</td>
<td>Analysis of histopathology of infected tissues including IHC and confocal microscopy (tissues from experiments in Milestones 33.04 and 33.05)</td>
<td>31/01/09 (Month 53)</td>
<td>31/01/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M33.07</td>
<td>Define potential markers for induction of cell-mediated immunity that might be measurable in serum</td>
<td>31/03/09 (Month 55)</td>
<td>31/03/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M33.08</td>
<td>Undertake pilot study to determine if markers of cell-mediated immunity in sera derived from patients that suffer from Salmonella or Campylobacter infection are detectable</td>
<td>30/06/09 (Month 58)</td>
<td>Failed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M33.09</td>
<td>Contribution to final report</td>
<td>31/08/09 (Month 60)</td>
<td>31/08/09</td>
<td></td>
</tr>
<tr>
<td>WP34</td>
<td>M34.01</td>
<td>Set up online communications system and network for WP34</td>
<td>30/09/08 (Month 49)</td>
<td>30/09/08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M34.02</td>
<td>Design and initiate database of Campylobacter colonization factors for poultry</td>
<td>28/02/09 (Month 52)</td>
<td>28/02/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M34.03</td>
<td>Organise joint meeting with WP30</td>
<td>30/11/08 (Month 51)</td>
<td>30/11/08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M34.04</td>
<td>Establish a subgroup to critically evaluate the indirect poultry derived routes of Campylobacter infections</td>
<td>28/02/09 (Month 54)</td>
<td>04/06/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M34.05</td>
<td>Recommendations for sustaining accrued data from WP34</td>
<td>30/06/09 (Month 58)</td>
<td>30/06/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M34.06</td>
<td>Organise final WP34 meeting</td>
<td>31/08/09 (Month 60)</td>
<td>04/06/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M34.07</td>
<td>Complete linkage analysis of Campylobacter genes affecting colonization properties</td>
<td>31/05/09 (Month 57)</td>
<td>31/08/09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M34.08</td>
<td>Contribution to final report</td>
<td>31/08/09 (Month 60)</td>
<td>31/08/09</td>
<td></td>
</tr>
</tbody>
</table>
Appendix 1: Plan for Use and Dissemination of the Knowledge

Section 1 — Exploitable knowledge and its use

Table C1 presents a summary of the Year 5 Workpackages that have exploitable knowledge from their work.

Section 2 — Dissemination of knowledge

Table C2 provides details of press releases and publications (other than those submitted to peer-reviewed journals), posters, conference and workshop presentations and exhibits, and web sites and web pages.

Peer-reviewed publications

**Special Interest Group — Emerging and Neglected Zoonoses**


**Workpackage 1**


**Workpackage 6**


**Workpackage 21**


Table C1. Exploitable knowledge and its use.

<table>
<thead>
<tr>
<th>WP No.</th>
<th>Exploitable knowledge</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>WP3</td>
<td>- Some material from the science communication training internship has been offered as external training courses to other EC-funded projects including EPIZONE, CASCADE and COST project officers.</td>
<td>Future courses using this, and modified material will be further developed.</td>
</tr>
<tr>
<td>WP6</td>
<td>- The GIS tools and atlases will be made publicly available as a service to the European Union (EU) and research bodies.</td>
<td>Subject to future funding for maintenance.</td>
</tr>
<tr>
<td>WP21</td>
<td>- A new sensitive and specific detection method for <em>Salmonella</em> Genomic Island 1 in all bacteria species. - The strain collection at the Health Protection Agency and the Federal Institute for Risk Assessment will be sustained, and made publicly available following the publication of results. - New fingerprinting method for molecular characterization of SGI1 that can be applied to other organisms.</td>
<td>Commercialization of the provided methods has not been initiated.</td>
</tr>
<tr>
<td>WP22</td>
<td>- Data sharing at the European Union level through the establishment of an accessible, online database that can be made available to external users.</td>
<td>Commercialization of the provided methods has not been initiated.</td>
</tr>
<tr>
<td>WP23</td>
<td>- The developed software tools and models will not be commercially exploited but will be made available for external use.</td>
<td>Commercialization of the provided methods has not been initiated.</td>
</tr>
<tr>
<td>WP24</td>
<td>- The <em>Campylobacter</em> Risk Assessment Framework software tool is publicly available at <a href="http://www.rivm.nl/craf">www.rivm.nl/craf</a>.</td>
<td>Commercial exploitation is not foreseen.</td>
</tr>
<tr>
<td>WP26</td>
<td>- The array used in the project is commercially available under the brand name IDENTIBAC. - The data generated may have a number of other potential avenues for commercial exploitation. - The knowledge from the project will be used to produce new iterations of diagnostic platforms in addition to providing preliminary data for grant proposals.</td>
<td>Commercial availability of arrays that were developed in project.</td>
</tr>
<tr>
<td>WP27</td>
<td>- Various French and international companies have expressed interest in developing and producing an ELISA test (for <em>Trichinella</em> infection in pigs) using some of the recombinant proteins that have been developed.</td>
<td>Possible commercial exploitation of ELISA test.</td>
</tr>
<tr>
<td>WP32</td>
<td>- The ELISA methodology and the back-calculation model offer new dimensions for surveillance of infections with <em>Salmonella</em> and <em>Campylobacter</em>. It is aimed to establish the methodology as an additional tool for public health surveillance, and offer training in the methods to appropriate staff in EU institutions. An extension to other common bacterial infections may also be possible. - The repository of ~7,500 sera generated during the project will be maintained, and made available to other European research projects within the field of enteric infections.</td>
<td>Commercial exploitation is not envisaged.</td>
</tr>
<tr>
<td>WP33</td>
<td>- Findings have possible value for vaccine development.</td>
<td>Possible commercial development.</td>
</tr>
<tr>
<td>WP34</td>
<td>- The survey-based tool to assess the attitudes of stakeholders towards biosecurity against <em>Campylobacter</em> in poultry could be exploited by the industry and policy-makers to raise awareness and develop appropriate education programmes. - Development of a web-based newsletter on <em>Campylobacter</em> in poultry is currently in discussion with industry representatives.</td>
<td>Possible commercial development.</td>
</tr>
<tr>
<td>WP no.</td>
<td>Date</td>
<td>Type</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>WP2</td>
<td>September 2008</td>
<td>13th International Conference on Food Microbiology (Invited Speaker)</td>
</tr>
<tr>
<td></td>
<td>September 2008</td>
<td>Seminar presentation to ECDC</td>
</tr>
<tr>
<td></td>
<td>December 2008</td>
<td>Seminar presentation to network reviewers plus DG Sanco</td>
</tr>
<tr>
<td></td>
<td>February 2009</td>
<td>Health Protection Agency, UK (Invited Speaker)</td>
</tr>
<tr>
<td></td>
<td>April 2009</td>
<td>Responsible Use of Medicines in Agriculture (RUMA) Alliance Annual Meeting (Invited Speaker)</td>
</tr>
<tr>
<td></td>
<td>May 2009</td>
<td>Era-NetPathogenoMics, Como, Italy (Invited speaker)</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral)</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>DG Sanco</td>
</tr>
<tr>
<td>WP2B</td>
<td>December 2008</td>
<td>Discussion meeting — Wildlife diseases</td>
</tr>
<tr>
<td>WP3</td>
<td>All year</td>
<td>Website</td>
</tr>
<tr>
<td></td>
<td>May 09</td>
<td>Article — Parliamentary Monitor</td>
</tr>
<tr>
<td></td>
<td>Jun 09</td>
<td>Press release — Annual Scientific Meeting</td>
</tr>
<tr>
<td></td>
<td>Oct 09</td>
<td>Stakeholder report</td>
</tr>
<tr>
<td></td>
<td>Oct 09</td>
<td>Press release — Handover Meeting</td>
</tr>
<tr>
<td></td>
<td>5 times</td>
<td>Articles in SFAM Microbiologist</td>
</tr>
<tr>
<td></td>
<td>Oct 09</td>
<td>EU-AgriNet website</td>
</tr>
<tr>
<td></td>
<td>Nov 09</td>
<td>Vector — VLA Internal Magazine</td>
</tr>
<tr>
<td></td>
<td>November 2008</td>
<td>European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2008 (2 oral presentations)</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td>WP21</td>
<td>June 2009</td>
<td>European Congress of Clinical Microbiology and Infectious Disease (ECCMID) 2008 (two poster presentations)</td>
</tr>
<tr>
<td></td>
<td>June 2008</td>
<td>1st International ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Food-borne Pathogens (poster presentation)</td>
</tr>
<tr>
<td></td>
<td>October 2009</td>
<td>3rd ASM Conference on Salmonella: Biology, Pathogenesis and Prevention (poster presentation)</td>
</tr>
<tr>
<td></td>
<td>December 2008</td>
<td>RICAI (Réunion Interdisciplinaire de Chimiothérapie Anti-Infectieuse) (poster presentation)</td>
</tr>
<tr>
<td></td>
<td>September 2009</td>
<td>49th ICAAC Interscience Conference on Antimicrobial Agents and Chemotherapy (poster presentation)</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td>WP22</td>
<td>May 2009</td>
<td>2nd Workshop of the Community Reference Laboratory for Parasites (oral presentation)</td>
</tr>
<tr>
<td></td>
<td>March 2009</td>
<td>Workshop: Genotyping of parasites: molecular characterization of species and genotypes (oral presentation)</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Dutch Society of Parasitology, Spring Symposium (oral presentation)</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td>WP no.</td>
<td>Date</td>
<td>Type</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>WP23</td>
<td>November 2008</td>
<td>WHO Food-borne Epidemiology Reference Group (invited presentation)</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td>WP24</td>
<td>November 2008</td>
<td>EFSA working group on Campylobacter control (invited presentation)</td>
</tr>
<tr>
<td></td>
<td>December 2008</td>
<td>EFSA scientific colloquium (invited presentation)</td>
</tr>
<tr>
<td></td>
<td>February 2009 onwards</td>
<td>Webtool (CRAF) <a href="http://www.rivm.nl/craf">www.rivm.nl/craf</a></td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td>WP26</td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td>WP27</td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Société Française de Parasitologie (SFP) conference (1 poster, 2 presentations)</td>
</tr>
<tr>
<td></td>
<td>August 2009</td>
<td>World Association for the Advancement of Veterinary Parasitology (WAAVP) conference (96 oral, 1 poster)</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td></td>
<td>July 2009</td>
<td>International Association for Food Protection Annual Meeting 2009 (poster presentation)</td>
</tr>
<tr>
<td>WP29</td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td></td>
<td>September 2009</td>
<td>49th ICAAC Interscience Conference on Antimicrobial Agents and Chemotherapy (poster presentation)</td>
</tr>
<tr>
<td>WP30</td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td>WP31</td>
<td>October 2008</td>
<td>Conference</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td>WP32</td>
<td>November 2008</td>
<td>European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2008 (2 oral presentations)</td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td>WP33</td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
<tr>
<td>WP34</td>
<td>June 2009</td>
<td>Conference presentations at Med-Vet-Net ASM (oral and poster)</td>
</tr>
</tbody>
</table>


Workpackage 23


Workpackage 25


Workpackage 26


Workpackage 27


**Workpackage 28**


**Workpackage 29**


**Workpackage 32**


Acronyms for the Med-Vet-Net partner institutes are given on page 80.

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D</td>
<td>Three-dimension(al)</td>
</tr>
<tr>
<td>aCGH</td>
<td>Array comparative genomic hybridization</td>
</tr>
<tr>
<td>AME</td>
<td>Aminoglycoside-modifying enzymes</td>
</tr>
<tr>
<td>AMR</td>
<td>Antimicrobial resistance</td>
</tr>
<tr>
<td>ASSUt/ACSSuT</td>
<td>Ampicillin (A), chloramphenicol (C), streptomycin (S), sulphonamides (S), and tetracycline (T) resistant clone of <em>Salmonella</em></td>
</tr>
<tr>
<td>BSL</td>
<td>Biosafety level</td>
</tr>
<tr>
<td>bp</td>
<td>Base pairs</td>
</tr>
<tr>
<td>CBS</td>
<td>Center for Biological Sequence Analysis</td>
</tr>
<tr>
<td>CC</td>
<td>Clonal complex</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CEF</td>
<td>Chicken embryo fibroblast</td>
</tr>
<tr>
<td>CF</td>
<td>Med-Vet-Net Co-ordinating Forum</td>
</tr>
<tr>
<td>CFT</td>
<td>Complement fixation test</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony-forming units</td>
</tr>
<tr>
<td>CGH</td>
<td>Comparative genome hybridization</td>
</tr>
<tr>
<td>CIAs</td>
<td>Critically Important Antimicrobials</td>
</tr>
<tr>
<td>CLSI</td>
<td>Clinical and Laboratory Standards Institute</td>
</tr>
<tr>
<td>CMS</td>
<td>Content management system</td>
</tr>
<tr>
<td>CNET</td>
<td>CampyNet, Med-Vet-Net scientific project to standardize molecular typing methods for <em>Campylobacter</em></td>
</tr>
<tr>
<td>CNRS</td>
<td>National Center for Scientific Research, France</td>
</tr>
<tr>
<td>CRAF</td>
<td><em>Campylobacter</em> Risk Assessment Framework</td>
</tr>
<tr>
<td>CRL</td>
<td>Community Reference Laboratory</td>
</tr>
<tr>
<td>DALYS</td>
<td>Disability adjusted life years</td>
</tr>
<tr>
<td>DAVID</td>
<td>Database for Annotation, Visualization and Integrated Discovery</td>
</tr>
<tr>
<td>DISCON-TOOLS</td>
<td>Disease Control Tools project</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DSM</td>
<td>German Collection of Microorganisms and Cell Cultures</td>
</tr>
<tr>
<td>EADGENE</td>
<td>European Animal Disease Genomics Network for Excellence for Animal Health and Food Safety</td>
</tr>
<tr>
<td>EAEC</td>
<td>Enteraggregative <em>Escherichia coli</em></td>
</tr>
<tr>
<td>EBLV</td>
<td>European Bat Lyssavirus</td>
</tr>
<tr>
<td>EC</td>
<td>European Commission</td>
</tr>
<tr>
<td>ECDC</td>
<td>European Centre for Disease Prevention and Control</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EMCV</td>
<td><em>Encephalomyocarditis virus</em></td>
</tr>
<tr>
<td>EMIDA</td>
<td>ERA-NET An EC-funded European Research Area network on the Coordination of European Research on Emerging and Major Infectious Diseases of Livestock</td>
</tr>
<tr>
<td>EPEC</td>
<td>Enteropathogenic <em>Escherichia coli</em></td>
</tr>
<tr>
<td>EPISODE</td>
<td>Network of Excellence for Epizootic Disease Diagnosis and Control</td>
</tr>
<tr>
<td>ERA-NET</td>
<td>Networking of national programmes in European research areas</td>
</tr>
<tr>
<td>ESBls</td>
<td>Extended spectrum β-lactamases</td>
</tr>
<tr>
<td>ETEC</td>
<td>Enterotoxigenic <em>Escherichia coli</em></td>
</tr>
<tr>
<td>ETPGAH</td>
<td>European Technology Platform for Global Animal Health</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>EU-US- SAFEOFOD</td>
<td>A strategic European Union-United States research alliance on food-borne zoonoses</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration, USA</td>
</tr>
<tr>
<td>FP</td>
<td>Framework Programme (e.g. FP6, FP7)</td>
</tr>
<tr>
<td>GB</td>
<td>Med-Vet-Net Governing Board</td>
</tr>
<tr>
<td>GIS</td>
<td>Geographical information systems</td>
</tr>
<tr>
<td>GO</td>
<td>Gene Ontology terms</td>
</tr>
<tr>
<td>HEV</td>
<td><em>Hepatitis E virus</em></td>
</tr>
<tr>
<td>HRM</td>
<td>High resolution melting</td>
</tr>
<tr>
<td>HUS</td>
<td>Haemolytic urogenital syndrome</td>
</tr>
<tr>
<td>ICT</td>
<td>International Commission on Trichinellosis</td>
</tr>
<tr>
<td>IELISA</td>
<td>Indirect enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>IFA</td>
<td>Immunofluorescence assays</td>
</tr>
<tr>
<td>IgM/IgA</td>
<td>Immunoglobulin M/immunoglobulin A</td>
</tr>
<tr>
<td>IHC</td>
<td>Immuno-histochemistry</td>
</tr>
<tr>
<td>IID-2</td>
<td>Second study of Infectious Intestinal Disease in the Community</td>
</tr>
<tr>
<td>ISH</td>
<td><em>In situ</em> hybridization</td>
</tr>
<tr>
<td>JIFSAN</td>
<td>Joint Institute for Food Safety and Applied Nutrition, USA</td>
</tr>
<tr>
<td>JPA</td>
<td>Joint Programme of Activities</td>
</tr>
<tr>
<td>Kb</td>
<td>Kilo base</td>
</tr>
<tr>
<td>LEE</td>
<td>Locus of enteroctye effacement</td>
</tr>
<tr>
<td>Ipq</td>
<td>Larvae per gram</td>
</tr>
<tr>
<td>MAb</td>
<td>Monoclonal antibodies</td>
</tr>
<tr>
<td>MDR</td>
<td>Multi-drug resistant</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>MLST</td>
<td>Multi-locus sequence typing</td>
</tr>
<tr>
<td>MLVA</td>
<td>Multi-locus variable number tandem repeat analysis</td>
</tr>
<tr>
<td>MST</td>
<td>Multi-space sequence typing</td>
</tr>
<tr>
<td>MVN</td>
<td>Med-Vet-Net</td>
</tr>
<tr>
<td>NADIR</td>
<td>Network of Animal Disease Infectiology Research Facilities</td>
</tr>
<tr>
<td>NCE</td>
<td>National Centre for Epidemiology, Hungary</td>
</tr>
<tr>
<td>NCTC</td>
<td>National Collection of Type Cultures, United Kingdom</td>
</tr>
<tr>
<td>NoE</td>
<td>Network of Excellence</td>
</tr>
<tr>
<td>OI</td>
<td>Genomic O island</td>
</tr>
<tr>
<td>OIE</td>
<td>World Organisation for Animal Health</td>
</tr>
<tr>
<td>ORFs</td>
<td>Open reading frames</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulsed-field gel electrophoresis</td>
</tr>
<tr>
<td>Q-PCR</td>
<td>Quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RSS</td>
<td>Really simple syndication (news feeds)</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Real-time polymerase chain reaction</td>
</tr>
<tr>
<td>Safefood-ERA</td>
<td>Safefood European research area</td>
</tr>
<tr>
<td>ser</td>
<td>Serotype or serovar</td>
</tr>
<tr>
<td>SGI</td>
<td><em>Salmonella</em> Genomic Island</td>
</tr>
<tr>
<td>SIG</td>
<td>Special Interest Group</td>
</tr>
<tr>
<td>SME</td>
<td>Small to medium enterprise</td>
</tr>
<tr>
<td>SPF</td>
<td>Specific pathogen free</td>
</tr>
<tr>
<td>SPI</td>
<td><em>Salmonella</em> Pathogenic Island</td>
</tr>
<tr>
<td>STM</td>
<td>Short-term mission</td>
</tr>
<tr>
<td>TBEV</td>
<td>Tick-borne encephalitis virus</td>
</tr>
</tbody>
</table>
Acronyms (cont.)

Tm .............. Melting point
TPI .............. Triose phosphate isomerase
TRICHI-MED ...... Med-Vet-Net Workpackage 27 — Harmonization of Trichinella infection control methods
TTV .............. Torque teno virus
VT .............. Verocytotoxin
VT/EC .......... Verocytotoxin-producing Escherichia coli
UK .............. United Kingdom
UPGMA ........ Unweighted Pair Group Method with Arithmetic Mean
USDA .......... United States Department of Agriculture
VLP .............. Virus-like particles
VNTR .......... Variable-number tandem-repeat analysis
WHO .......... World Health Organization
WILDIST ........ Database of WiREDZ experts
WT .............. Wild-type (for example, Salmonella)
WTO .......... World Trade Organization
WiREDZ ......... Wildlife-related Emerging Diseases and Zoonoses
WP .............. Workpackage
WUR .......... Wageningen University and Research Centre, The Netherlands
ZoopNet ........ Zoonotic Protozoa Network
ZOO-VIRNET .... Med-Vet-Net Workpackage 31 — Emerging viral zoonoses

Med-Vet-Net Administration, management and communications

Med-Vet-Net Administration Bureau
Agence française de sécurité sanitaire des aliments (AFSSA)
27/31 avenue du général Leclerc
F 94700 Maisons-Alfort
France
Tel. +33 1 49 77 38 68
Fax. +33 1 49 77 27 78
Email. mvncoord@afssa.fr

Med-Vet-Net Project Management
Health Protection Agency (HPA)
61 Colindale Avenue
London NW9 5EQ
United Kingdom
Tel. +44 20 8327 6117
Fax. +44 20 8905 9929
Email. john.threlfall@hpa.org.uk
Visit. www.hpa.org.uk

Med-Vet-Net Communications Unit
Society for Applied Microbiology/Science Communications Ltd
Unit 105, Milton Keynes Business Centre
Hayley Court
Linford Wood
Milton Keynes MK14 6GD
United Kingdom
Tel. +44 1908 698 810
Email. communications@medvetnet.org
Visit. www.sfam.org.uk
Visit. www.sciencecommunications.eu
Med-Vet-Net partner institutes

Technical University of Denmark (DTU)
Anker Engelundsvej 1
Building 101A
2800 Kgs. Lyngby
Tel. +45 45 25 25 25
Fax. +45 45 88 17 99
Visit. www.dtu.dk

Statens Serum Institut (SSI)
Artillerivej 5
DK-2300 Copenhagen S
Denmark
Tel. +45 3268 3268
Fax. +45 3268 3868
Visit. www.ssi.dk

The French Food Safety Agency (AFSSA)
Agence Française de Sécurité Sanitaire des Aliments
27/31 avenue du Général Leclerc
F 94700 Maisons-Alfort
France
Tel. +33 1 49 77 13 50
Fax. +33 1 49 77 26 12

The Federal Institute for Risk Assessment (BFR)
Bundesinstitut für Risikobewertung Poststelle Thielallee 88-92
14195 Berlin
Germany
Tel. +49 30 8412 0
Fax. +49 30 8412 4741
Visit. www.bfr.bund.de

The Veterinary Medical Research Institute (VMRI)
H-1581 Budapest
PO Box 18
Hungary
Tel. +36 1 252 2455
Fax. +36 1 252 1069
Visit. www.vmri.hu

Istituto Superiore di Sanità (ISS)
Central Public Health Institute, Italy
Viale Regina Elena 299
00161 – Rome
Italy
Tel. +39 06 4990 1
Visit. www.iss.it

National Veterinary Institute (SVA)
SE-751 89 Uppsala
Sweden
Tel. +46 (0)1867 4000
Fax. +46 (0)1830 9162
Visit. www.sva.se

National Institute for Public Health and the Environment (RIVM)
PO Box 1
3720 BA Bilthoven
The Netherlands
Tel. +31 302 74 91 11
Fax. +31 302 74 29 71
Visit. www.rivm.nl

Central Veterinary Institute of Wageningen UR (CVI)
Post Box 65
8200 AB Lelystad
The Netherlands
Tel. +31 (0) 320 238800
Fax. +31 (0) 320 238668
Visit. www.cvi.wur.nl

National Institute of Hygiene (PZH)
Panstwowy Zakład Higieny
ul. Chocimska 24
00-791 Warsaw
Poland
Tel. +48 22 542 14 00
Fax. +48 22 849 74 84
Visit. www.pzh.gov.pl

Universidad Complutense de Madrid (UCM)
Ciudad Universitaria
28040 Madrid
Spain
Tel. +34 9 1452 0400
Visit. www.ucm.es

Institute of Public Health (ISCIIII)
Instituto de Salud Carlos III
C/ Sinesio Delgado, 4 al 12.
28029 Madrid
Spain
Visit. www.isciii.es

Health Protection Agency (HPA)
61 Colindale Avenue
London NW9 5EQ
United Kingdom
Tel. +44 20 8200 4400
Fax. +44 20 8200 7874
Visit. www.hpa.org.uk

Veterinary Laboratories Agency (VLA)
Newaw, Addlestone
Surrey KT15 3NB
United Kingdom
Tel. +44 1932 341 111
Fax. +44 1932 347 046
Visit. www.defra.gov.uk/corporate/vla

The Society for Applied Microbiology (SfAM)
Bedford Heights
Brickhill Drive
Bedford MK41 7PH
United Kingdom
Tel. +44 1234 326 661
Fax. +44 1234 326 678
Visit. www.sfam.org.uk
Med-Vet-Net was a European Network of Excellence that aimed to improve research on the prevention and control of zoonoses by integrating veterinary, medical and food-science research. Comprising 15 European partners and over 300 scientists, Med-Vet-Net enabled these scientists to share and enhance their knowledge and skills, and develop collaborative research projects. Med-Vet-Net officially commenced on 1 September 2004 and was funded for five years.