This month, MVN News provides an overview of Workpackage 8 Identification of molecular markers of pathogenicity for Campylobacter Jejuni.

The leader of Workpackage 8, Anne Ridley introduces herself, as does the Thematic Representative for the Host-Microbe Interactions thematic area, Eva Olsson Engvall.

We say big ‘Hello’ and ‘Welcome’ to the new Chief Executive Officer of the Society for Applied Microbiology (SfAM), Mr Philip Wheat.

Finally, for all who have now returned from your holidays I hope you’re refreshed and ready to resume all your good work for Med-Vet-Net!
Task 2: Virulotype CAMPYNET Isolates.
The CAMPYNET strain set includes a range of C. jejuni and C. coli isolates from a variety of sources, including epidemiologically linked strains from outbreaks. C. jejuni isolates (n=82) will be assayed, as this is this species most commonly associated with human infection. The selected assays will be used, by participating laboratories, to determine invasiveness and toxicity, in addition, the presence of self-replicating circular molecules of DNA (plasmids) and of known virulence genes, will be determined using the agreed tests. Antimicrobial susceptibility profiles will be determined, by three laboratories, using tests to detect the minimum inhibitory concentration (MIC) to a panel of seven antibiotics.

Task 3: Transfer technologies to other laboratories.
A group of ten strains will be selected on the basis of distinct phenotypes in the assays. These strains, and the standardised operating procedures for the assays, will be distributed to at least two participating laboratories to assess inter-laboratory reproducibility and ease of technology transfer. Training, if required, will be provided by expert laboratories. In addition, participants requiring expertise in the harmonised CAMPYNET genotyping techniques will be provided with the standard strain set and training if required.

Task 4: Data sharing.
The existing CAMPYNET website will be linked to the Med-Vet-Net website. The information held in the existing CAMPYNET website will be maintained and updated to include a database which will be accessible to participating laboratories. This will allow the sharing of the genotype, antimicrobial susceptibility and virulotype data generated within this project. The agreed standard methods will be placed on this site, so too will details of best practice with regard to storage and passage of strains. Data from other strains, including those used as controls in participating laboratories, and information about new techniques will be added as they become available.

Developments
Workshop
From 13-14 January 2005, a Workpackage 8 meeting was held at the University of Surrey, Guildford, UK. The meeting kicked off with an overview of campylobacter pathogenesis presented by an invited external expert, Dr Paul Everest of the University of Glasgow. This was followed by a discussion of the pros and cons of the various virulotyping assays used by our participants and those available elsewhere. At the end of this presentation, an agreement had been made with respect to the assays and genes to be used in the Workshop. Ingrid Hansen, General Research Institute for Animal Health, Jena, Germany updated the group on progress on U. jejuni toxin studies and kept us on track in our discussions. The meeting agreed that a core panel of 20 strains should be used to standardise and compare the tests applied. There has been investigation into any change to virulotype of strains during the five years since the original CAMPYNET set was distributed. Therefore, it was agreed that strains should be chosen from laboratories associated with both CAMPYNET I and CAMPYNET II. With the enthusiasm that such meetings bring, it was agreed to prepare a review of C. jejuni pathogenesis, potentially for publication in a peer-reviewed journal.

Unfortunately, within a matter of a few weeks, we found that we had been beaten to it and have revised our plans to a review for the website. Sadly, for the project, Gina Manning, Workpackage 8 Co-Leader left VLA not long after our meeting, to take up a Senior Lectureship at Nottingham Trent University.

Distribution of CAMPYNET strain set
A set of 84 C. jejuni strains were distributed to participating labs by Fiona Van Der Wal and Jeroen Dijkstra of CIDC-Lelystad (Central Institute for Disease Control), our strain coordinators. Three participants who had previously received the set and were conducting laboratory work in this Workpackage, also received the core 20 subset for investigation of strain stability. Some labs have had problems with growth of a small number of the strains, despite them being re-sent by the CIDC. Recently, DNA was sent to PZH (National Institute of Hygiene, Poland) to prevent delay to PCR assays conducted there.

Preliminary genotyping is suggesting that the core group of 20 strains has remained genomically stable, following storage for five years after distribution of the original CAMPYNET strains.

Progress of assays
An update of progress on this Workpackage was presented at the Med-Vet-Net General Scientific Meeting at the end of June, with preliminary data included in both poster and oral presentations.

(i) Toxin Production (Danish Institute for Food and Veterinary Research - DFVF and Statens Serum Institute - SSI)
Of the 20 core C. jejuni strains, two appear to be consistently negative for CDT toxin or produce negligible amounts for the four cell lines investigated to date by DFVF. However, interestingly, in results collated so far only one of these two strains appears negative for colA. HeLa cells will be recommended for the standardized assay.

(ii) Invasion (Veterinary Laboratories Agency - VLA)
INT407 cells were used to investigate 36 of the 84 CAMPYNET strains, including the core 20 strain set. No high or hyper invasive strains have been observed to date. A subset of strains assayed with Caco2 suggests that the correlation between the two cell lines is at best moderate and that refinements to bacterial cell treatment and age of the Caco2 cells are required to enhance comparability.

(iii) Plasmid content (VLA, CIDC)
The plasmid content of the core 20 strains were compared using two kit based methods, the QIAGEN Plasmid Mini Kit and the Promega Wizard SV. One of the core 20 strains appears to possess a 59 kb band with both kits, which is also present in an epidemiologically related isolate. This band appears to correspond with tetracycline resistance, and the presence of the tetO gene in these plasmid DNAs is currently being determined.

Dr Anne Ridley, Leader of Workpackage 8
Anne Ridley holds a B.Sc. in Microbiology from University of Glasgow, Scotland. She worked for six years at the Central Public Health Laboratory (now the Health Protection Agency) in Colindale, London where she studied for a Ph.D. in the Molecular Epidemiology of Listeria monocytogenes with a focus on Mycobacterium tuberculosis. She then changed direction, moving to the Royal Postgraduate Medical School in London to work on gene translocation associated with acute myeloid leukaemia for a short time, moving back to the Public Health Laboratory to work with John Thrall in molecular typing and antimicrobial resistance of Salmonella enterica. In 1998 she moved into food research to try and find out more about the food industry in the UK. She moved to the Veterinary Laboratories Agency in 1999 to work primarily on the molecular epidemiology and antibiotic resistance of campylobacters and to participate in the first CAMPYNET project.

Anne’s area of scientific expertise are molecular epidemiology and antimicrobial resistance of foodborne zoonotic organisms and is currently involved in the antimicrobial resistance in bacteria of animal origin (ARBAO-II). She has recently been part of a team working to promote broiler biosecurity to the poultry industry. In her spare time Anne runs for, and has managed cross-country and road-racing teams of, one of the top UK athletics clubs.
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DFVF).
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multiresistance.
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recorded. Nevertheless, in all cases, this does not show a similar degree of inter-strain
variation.
(iv) Antimicrobial susceptibility
The following institutes are using the methods listed to test antimicrobial susceptibility:
- VLA - plate dilution MIC
- ISCLIL - E-Test
- Bfr - broth microdilution (Sensititre)
Although all three participants have assayed most of the 84 strains, comparative analyses has only been conducted for the core set of 20 strains. Generally, there appears to be good concordance with the methods used by the three labs. However, for some antimicrobial/method combinations, for example gentamycin/agar incorporation, there was a consistent two-to-four fold difference in MIC recorded. Nevertheless, in all cases, this does not affect resistant/susceptible determination, despite lack of globally accepted breakpoints.
No resistance to the antimicrobials erythromycin or gentamycin has been observed in this panel of strains, nor is there evidence of multiresistance.
v) Detecting virulence related genes and detection of polymorphisms (SVA, PZH, DFVF).
The presence of candidate virulence associated genes virB11, trcG, ciaB, cueE, caoF in addition, detection of CDT genes is being investigated by DFVF. Preliminary results suggest that different primer pairs for the same genes can throw up different results and particular problems with weak positive results has come to light. This highlights the requirement for standardisation of such assays between laboratories and an investigation is ongoing by all laboratories concerned so that the most appropriate test is recommended. PZH are investigating use of single stranded conformational polymorphism (SSCP) to detect polymorphisms in many of these genes. Preliminary data suggests that while ciaB is polymorphic, trcG has thus far not shown a similar degree of inter-strain variation.
Data collation
There is some interesting information coming to light from this strain set, now that we are starting to compare outputs of the various tests. The data is being collated at VLA and database development is ongoing. This is slightly delayed, but will be available to participants by the end of September. Information on the techniques used in the project and a set of recommended procedures will be made available via the Med-Vet-Net website. It is hoped to be able to conduct a short wash-up meeting towards the end of the project for all participants to share findings, agree recommendations and toast the hard work done by all project participants.

Eva Olsson Engvall - Thematic representative for the Host-Microbe Interaction thematic area.
Eva is a veterinarian, with a PhD in Bacteriology from the Swedish University of Agricultural Sciences (SLU) in Uppsala Sweden. She has worked at the National Veterinary Institute (SVA) since 1985, first at the Department of Bacteriology with veterinary diagnostics, research and development of new, molecular techniques. Since 1998 she holds a position as associate professor at the Swedish Zoo nosis Center, SVA, and is currently adjunct professor in Bacteriology at SLU. She is a member of an expert panel of the Swedish research council for environment, agricultural sciences and spatial planning (Formas). Her main research interests and expertise are characterisation and epidemiology of zoonotic pathogens e.g. enterics and tick-borne bacteria. She is involved in national and Nordic Campylobacter networks and has worked on and manages own research on Campylobacter in broilers and other animals. She teaches students at SLU, and is frequently invited as speaker at meetings and courses on subjects of zoonoses. Eva is coordinator for the MVN thematic area Host-microbe interactions. She is married, has three children and lives close to Uppsala.
(Photograph by Aase Sten, SMI)

The summer period at the Administration Bureau corresponds with the preparation of two major events in the life of our network: the reporting of activities of the past year, and the preparation of the second joint programme of activities (JPA2) that will begin on 1 March 2006. All relevant documents are to be given to the EC by mid-October.
The Project Manager will administer all scientific work. This involves collecting, reviewing and finalising scientific reports provided by the first round Workpackages and scientific proposals of the second round Workpackages. At the same time the Administration Bureau will carry out the same tasks with regards the appropriate financial documents. The Administration Bureau will then collate all the scientific and financial documents into a single document which will be sent to the EC. The drafting of these documents requires close collaboration between the Project Manager, the Administration Bureau and all members involved (Institute Representatives, Financial Officers, Workpackage Leaders). In order to avoid any confusion or misunderstanding, the Administration Bureau would like to advise all participants to feel free to contact them with any queries whenever necessary.

Financial reporting of the past activities (Months 1 - 12)
Financial reporting will involve the identification of all costs incurred by Med-Vet-Net from 1 September 2004 - 31 August 2005. This will primarily involve Workpackage Leaders and Financial Officers of the Partner Institutes. Reporting Forms, which are identical to those used for the Intermediary Report last March, will be provided by the Administration Bureau. Partners will be given two weeks to fill in and return completed forms to the Administration Bureau. The next step will then be to edit and finalise financial reports with each partner institute.

Budgetary preparation of the second Joint Programme of Activities (JPA2)
During July, forms for the second budget of JPA2 were sent to Workpackage Leaders. All forms were completed and returned to the Administration Bureau, completing the first step in preparation of the budget. At a recent meeting between the Project Manager, the Co-ordinator’s Representative and the Administration Bureau, it was decided on the basis of these first drafts, that some adjustments were necessary in order for each Workpackage to be allocated a budget within the threshold value of €170,000 per 18 months.

Financial Tour 2005
The meeting of SIAM with AFSSA in late May began what we now call the Administration Bureau financial tour. This tour has continued throughout July with visits to ISS and BFR. The institutes which remain to be visited are PZH, HPA and VLA. This exercise appears to be more and more important with each visit. It allows issues to be raised and, in most cases, solved. It also improves partners’ understanding of the financial operation of the network, and demonstrates the good technical support of the Administration Bureau. This exercise has proven to be very useful and will be carried out in subsequent years.

Phil joined the Society for Applied Microbiology (SIAM) as their Chief Executive Officer in April 2005. Previously he was with Mast Laboratories, a company manufacturing and supplying products used in Microbiology laboratories, for ten years. He was Managing Director for Mast Laboratories where he directed both the manufacturing and laboratory (quality control and product development) functions of the company. During his time at Mast he studied for a Master of Business Administration degree at Sheffield Business School, Sheffield Hallam University. Before being asked to join Mast, Phil was the Laboratory Manager in the Microbiology Laboratory at the Royal Hallamshire Hospital, Sheffield. He was involved for many years in teaching and education, in particular for the Biomedical Scientist profession. He taught and organised the microbiology modules for Fellowship of Institute of Biomedical Science and Master degree levels. He has also been involved with organising the Microbe series of conferences for numerous years. He was also on the Scientific Advisory Panel for the Institute of Biomedical Science for ten years, this included the last four years as Specialist Advisor. In addition, during his stay in Sheffield, he obtained a Master in Medical Science degree by thesis from the University of Sheffield. He has over sixty scientific publications to his name. Outside work he enjoys playing squash, going to the gym, listening to classical music, keeping up with current affairs and following football (he is a long suffering Sheffield Wednesday supporter!).

Dr Eva Olsson Engvall

PHOTOGRAPH BY AASE STEN, SMI

ADMIN BUREAU UPDATE
Collaboration with New Zealand
Those of you who keep an eye on the CORDIS website will know that the EC is currently encouraging collaboration with New Zealand. In anticipation of this Med-Vet-Net (Diane Newell and Claire Cassar) were invited to attend a meeting in London with representatives from New Zealand universities and institutes. The outcome of this meeting is a commitment to develop a plan for collaboration on food safety, which will hopefully be formalised in a submission for funding to the EC in the near future. In the next few weeks Arie Havelaar will be visiting New Zealand as an invited scientist and will continue the discussions then.

Adding value!
The Treasure Hunt at the recent Med-Vet-Net Annual Meeting is now benefiting a youth charity organisation in Winchester. The questions over which many of you pondered (probably with a beer in your hand) are now being sold to keep families amused in the Winchester area this summer and a prize has been donated for the first family with all the correct answers. So far £150 has been raised for the charity.

Diane Newell

4th International Veterinary Vaccines and Diagnostics Conference (IVVDC) Oslo, Norway 25-29 June 2006
The conference provides an excellent opportunity to meet colleagues and be updated on recent progress and future perspectives in the fields of vaccinology and diagnostics. The IVVDC has become an important meeting place for regulatory authorities, pharmaceutical companies and the scientific community. An exciting scientific program has been prepared covering the various areas of vaccinology and diagnostics. Please visit: http://www.ivvdc.org/

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Contributions and suggestions are welcome. Deadline for publication is 1st of each month.

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