MED•VET•NET NEWS

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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 !

Communications Unit

CAMPYNET II

Workpackage 8 - Identification of molecular markers of pathogenicity for *Campylobacter jejuni* (CAMPYNET II)

Introduction

Campylobacter is a small, spiral shaped genus of bacteria which can live in the intestine of a wide range of mammals and birds and which can contaminate food and the environment resulting in food poisoning in humans. It was only in the late 1970s that the association of *Campylobacter jejuni* and *C. coli* was made with human disease.

The disease

C. jejuni and *C. coli* are the most common cause of human acute bacterial enteritis worldwide. *C. jejuni* accounts for the majority (approx 90%) of human infections. Following an incubation period of 1- 7 days the infected individual will experience symptoms comprising either watery or bloody diarrhoea, often accompanied by severe abdominal pain. In most cases the disease is self-limiting. Disease may be more severe in the young, elderly or immunocompromised and although rare, infection can prove fatal in these groups. Antimicrobial therapy, comprising erythromycin or fluroquinolones, is only indicated for severe infections and concerns have arisen following increased reports of resistance to those antimicrobials in several countries.

How are campylobacters spread?

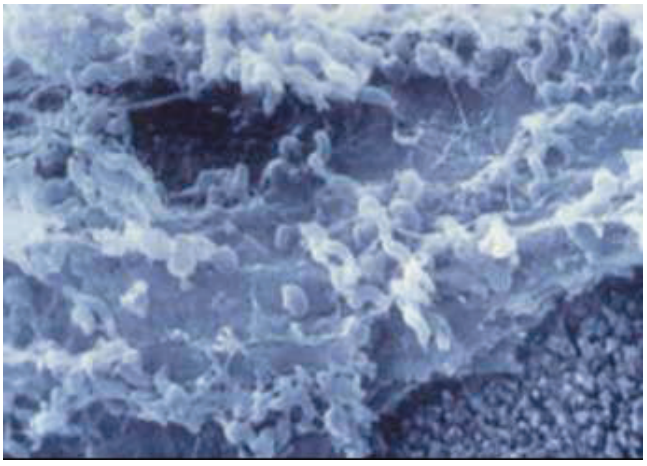
Campylobacters can be found in the intestinal tracts of wild birds and various other animals including pets. Poultry, especially broiler chickens, may carry large numbers of these bacteria without showing any signs of illness and they constitute a major source of human infection in food. Infection can also result from drinking untreated water or milk, or from direct contact with infected animals such as puppies with diarrhoea. In some parts of the UK, people have become infected as a result of drinking pasteurised milk contaminated by magpies or jackdaws pecking through the foil tops of exposed milk bottles. Although the faeces of people suffering from the disease are infective, person to person spread is uncommon.

Campylobacters in food

Any raw meat, especially offal, may be contaminated with campylobacters, but poultry is thought to be a major source; raw chicken contamined with campylobacters are commonly found in shops and supermarkets. However, the bacteria are delicate and die if exposed to air for any length of time and they are destroyed by cooking. Although they may be present in food, unlike the salmonellas, they do not multiply in food, so they seldom cause explosive outbreaks of food poisoning. On the other hand, if as it seems the number of bacteria consumed to cause illness is very small, it only needs a few organisms cross-contaminating other food (eg. salad) in the kitchen to cause infection.

Mechanism of Infection

The mechanisms by which *C. jejuni* causes disease (i.e. the virulence mechanism) in humans remains unclear, despite the availability of the genome sequence of one strain of *C. jejuni* since 2000. Two of the mechanisms thought to be involved include invasion of the cells lining the gut and toxin production. A number of genes which may be related to virulence have been identified. However, the distribution of these genes amongst strains and the roles played by these factors in virulence are not known. Considerable variation appears to exist among *C. jejuni* isolates in their ability to invade cells or express active toxin. Many schemes to classify strains of *C. jejuni* (subtyping schemes) have been applied and all of them have shown that the organism is highly diverse. The genetic diversity has also enabled the development and use of several schemes to classify strains according to their gene sequence (genotyping). However, there are few if any identified links between strain type and virulence, and this hinders accurate risk assessment. The major reason for this is the absence of suitable animal models of infection with campylobacters (campylobacteriosis). For other bacterial pathogens, virulence has been assessed using cell/tissue culture. However, these approaches have not proved useful for defining the virulence *C. jejuni*. This is partly due to a lack of standardised laboratory methods or assay systems and the use of poorly characterised bacterial strains. A well characterised strain set, of over 100 *C. jejuni* and *C.coli* isolates, was generated through CAMPYNET, a former EC-funded project set up to standardise and harmonise current molecular typing for campylobacter throughout Europe (http:/campynet.vetinst.dk).



Campylobacter jejuni colonising intestinal epithelium

Aims and Objectives of Workpackage 8

The overall objective is to extend the capacity to phenotype *Campylobacter jejuni* to include virulence-related properties using the established CAMPYNET strain set. In the first instance, a subset of the CAMPYNET strains will be used to find the most suitable and reproducible methods with which to test (assay) invasiveness and toxin activity. Once established, the CAMPYNET strains (with additional controls) will be tested for virulence potential using these assay systems. In addition, the strains will be screened for putative virulence genes, to establish the distribution of these potential markers of pathogenicity in relation to invasion and toxin production. The strain set will also be tested for antimicrobial susceptibility using an established panel of antimicrobials. The appropriate technologies will be made available to other laboratories and a web-based database will be developed to encourage data-sharing amongst participants.

Work programme

Task 1: Development of standardised virulence assays.

A one-day meeting will be held to discuss the state of knowledge and to agree the most suitable methods for virulotyping of the CAMPYNET strain set. Selected methods will be evaluated for reproducibility and ease of use in at least two independent laboratories using a subset of the CAMPYNET isolates. The effect on virulotype of subculture on selected CAMPYNET strains will also be evaluated. The requirements of each of the participating laboratories will also be determined, including whether each participant currently has access to the CAMPYNET strain set and associated molecular typing methods. The CAMPYNET strain set will be distributed to those participants who do not currently have access to these strains and who will be involved in the virulotyping.

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 toxin activity. 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 Workpackage. Ingrid Haenel of the Federal Research Institute for Animal Health, Jena, Germany updated the group on progress on *C. 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 Fimme Van Der Wal and Jeroen Dijkstra of CIDC-Lelystad (Central Institue 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 Workpackge 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 *cdt*A. 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 *tet*O gene in these plasmid DNAs is currently being determined.

PEOPLE

Dr Anne Ridley, Leader of Workpackage 8


Dr Anne Ridley

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* whilst also working 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 Threlfall on 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 areas 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.


Workpackage 8 meeting participants

PEOPLE

Philip Wheat, the new CEO for SfAM

Philip Wheat

Phil joined the Society for Applied Microbiology (SfAM) 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!).

(iv) Antimicrobial susceptibility

The following institutes are using the methods listed to test antimicrobial susceptibity:

- VLA - plate dilution MIC
- ISCIII - 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 *vir*B11, *tra*G, *cia*B, *ceu*E, *cad*F is being investigated by SVA and PZH. 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 polymorhpism (SSCP) to detect polymorphisms in many of these genes. Preliminary data suggests that while *cia*B is polymorphic, *tra*G 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 to discuss our findings, agree recommendations and toast the hard work done by all project participants.

PEOPLE

Eva Olsson Engvall - Thematic representative for the Host-Microbe Interaction thematic area.

Dr Eva Olsson Engvall

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 Bac teriology 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 are characterization, pathogenesis 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)

ADMIN BUREAU UPDATE

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 drafting the 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 SfAM 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.

Admin Bureau

PROJECT MANAGEMENT

Planning and Delivery of the Second Joint Programme of Activities (JPA2)

Much of this month has been taken up with the development of JPA2 which has to be delivered to the European Commission with our first Annual Report due in September. The Leaders of the new selected Workpackages have worked hard writing the Detailed Proposals which are now being refereed and amended for incorporation into the JPA2 and will form the basis of the subcontracts. I thank them for their hard work and patience with all my questions.
Once the JPA2 is formulated this will be agreed at the Co-ordinating Forum and Governing Board Meetings planned for September/October.
Several new features will be incorporated into the new Workpackages

- Risk analysis and register. This will comprise a list of risks or hazards which may impact on the successful achievement of each Workpackage. This will be aggregated and form the basis of an overall risk register for the network activities. Such a register is a management tool to identify crucial factors and act on them in a timely manner.
- Dissemination of information plan. This will ensure that any knowledge accrued from the Workpackage activities is spread effectively both throughout the network and to external scientists and our stakeholders such as policy makers and the general public. One major requirement will be for each Workpackage to present the results in the newsletter.
- Anticipated impact on European public Health. The Med-Vet-Net Governing Board now requires an assessment of the impact of all aspects of the project on European public health. This will be an essential factor in network sustainability in the future and is therefore very important. Such impact assessments, will provide indications of performance, such as impact on numbers of infectious intestinal disease; reduction in antimicrobial resistance; reduction in health care costs etc.
- Bioethics, Legal and Social Aspects assessment. Med-Vet-Net intends to be an active member of the EC Ethical, Legal and Social Aspects of Life Sciences and Technologies Programme (ELSA - http://www.cordis.lu/elsa/src/about.htm). Information gathered in the workpackages will be used to accurately register aspects such as animal experimentation, genetic engineering and personnel databases, in our network activities.

142nd American Veterinary Medical Association (AVMA) and 28th World Veterinary Congress 16-20 July 2005

Med-Vet-Net was one of several networks presented (by Diane Newell) in a session on Preharvest Food Safety centres, networks and consortia at the recent meeting in Minneapolis, USA. In particular the development of the EUUS-SAFEFOOD network was presented to demonstrate the role international collaboration can have in global food safety issues. The formal discussion following the presentations focussed on whether such networks are effective and how their impact can be measured. As a result of this it is hoped that Med-Vet-Net can input into the development of international performance indicators for such networked activities in the future.

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 issues 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

EXTERNAL CONGRESS

Breaking Barriers: Integrating Human and Animal Public Health against 21st Century Threats Frist Campus Center, Princeton University Princeton New Jersey USA 23 September 2005

The meeting will cover:
- Validation of the links between human and animal health/disease.
- How human and animal disease surveillance systems can be integrated into public health services and infrastructure to enhance the ability to detect and prevent human disease.
- How zoonotic diseases impact on human health. Please visit: http://www.princeton.edu/%7Eglobsec/Macy/index.html

3rd Probiotics and Prebiotics New Foods Conference Rome Italy 3-6 September 2005

There will be a very important session on the human applications. The European probiotic Association (EPA) has organised a session on animal nutrition on the afternoon of 6 September. If you interested, contact the president of the epa: Bruno Rochet - Tel. 02.97.44.86.56 - 06.03.26.97.12

New Diagnostic Technology: Applications in Animal Health & Biologics Controls. Applications in disease surveillance, molecular epidemiology and quality control tests of vaccines (OIE, IAB's, APHIS, AFSSA) 3-5 October 2005, Saint-Malo, France

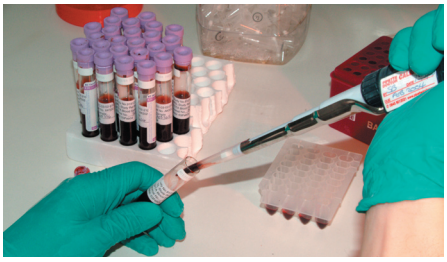
The objective of this conference is to give a presentation of the new technologies applied to the detection of agents or toxins, the diagnosis of mainly animal but also human diseases and the quality control of human and veterinary vaccines. It is intended to present the difficulties and the limits of these new techniques with their validation procedures. Practical examples will be illustrated as shown in the preliminary programme.
Please visit www.zoopole.com/ispaia/iabs2005 or contact: Genevieve Clement, Congress secretariat, ISPAIA - BP 7 - 22440 Ploufragan - France Tel : +33 2 96 78 61 30 Fax : + 33 2 96 78 61 31 genevieve.clement@zoopole.asso.fr

Communicating European Research (CER) 2005 International Conference, Brussels Exhibition Center (Heysel) 14-15 November 2005

Around 3000 participants including project co-ordinators, journalists and other communication professionals, press officers and representatives from research organisations will meet to promote mutual understanding of their respective roles, to share best practice and to define strategies to improve communication, outreach and dissemination of research results to the public and the press at a European level. Latest research results and current scientific activities will be presented to the media in press conferences and media briefings. A huge exhibition will feature selected research initiatives as well as the communication strategies of research organisations. Dissemination networks, media associations, relevant publications and editorials together with companies and service providers in the field of information and communication will present their products and services. Please visit: http://www.europa.eu.int/comm/research/conferences/2005/cer2005/index_en.html

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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