



# MED • VET • NET NEWS

Volume 2, Issue 3

March 2005

## HAPPY EASTER!



This month MVN News provides an overview of Workpackage 10 - Validation and standardisation of PCR-based methods for detection and quantitative risk assessment.

There is also information on the General Scientific Meeting which will be held in Winchester in June 2005 and a report from the Thematic Meeting held in Rome earlier this month.

We have two new members of staff joining the network, Claire Cassar working for Diane Newell at the VLA and Lucy Harper, new Communications Officer working for Teresa Belcher at SfAM. They will both introduce themselves and talk a little about the work they will be doing for Med-Vet-Net.

Teresa Belcher

Med-Vet-Net Communications Director

## Food-PCR 2 Overview

**Validation and standardisation of PCR-based methods for detection and quantitative risk assessment (Food-PCR 2)**

### Background

Many PCR-based methods have been developed and published for the detection and characterization of food-borne pathogens and zoonotic organisms, but few have been validated for reference purposes. The overall objective of work package 10 (WP10) is to facilitate implementation of PCR-based methods, such as real-time PCR and microarray-PCR, for both detection and verification of food-borne pathogens. This will be carried out through harmonization and standardization of methods in Europe. The target micro-organisms are the following foodborne and zoonotic bacteria: *Listeria monocytogenes*, enterohaemorrhagic *Escherichia coli* (EHEC) O:157, thermotolerant *Campylobacter* spp. and *Salmonella enterica*, and food-related viruses. The work will involve the establishment of relevant European working groups, a Polymerase Chain Reaction (PCR) database, plans for the establishment of a virtual and physical BioResource Centre, hands-on workshops for harmonisation of methodologies, collaborative ring-trials, technology transfer from research to small-to-medium enterprises (SMEs) and food-producers, and exchange of scientists and students.

Workpackage 10 will provide significant impetus to the implementation of the Zoonoses Directive by preparing guidelines for the real-time (PCR) -based detection of pathogens from farm to fork, harmonized identification of pathogens in microarray platforms, virus detection in food produce, and PCR-based detection of zoonotic bacteria in animal faeces. The first year of the project focuses on preparatory work for the establishment of research groups and collaborative trials, which will be part of technology transfer from expert to end-use laboratories.

### Facts and Figures

- The workpackage leader is Jeffrey Hoorfar (see overleaf for his biography), of Danish

Institute for Food and Veterinary Research in Denmark (DFVF).

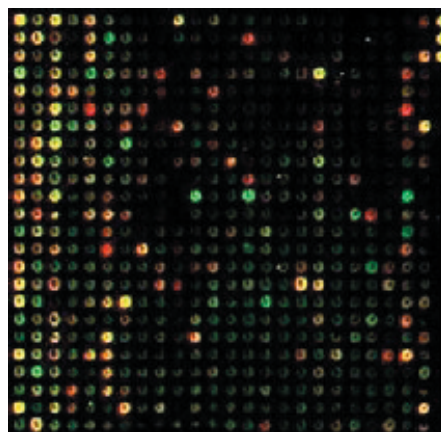
- There are 22 partners: nine main partners (funded) and 13 associated (without funding), spanning 12 countries.
- WP10 will last for 18 months: Sept. 1st 2004 to March 1st 2006,
- The budget for WP10 is 251 000 Euro, including 210 000 Euro from EC (83% funding).

### New Technologies

There are already ring-trial validated standard operating procedures for the five most important foodborne pathogens but these are limited. There is a need to continue the standardization work on a wider range of samples. Also, it is important to apply these methods to new technologies using real-time fluorescence-based PCRs and microarray platforms.

### Real-Time PCR

A simple search in the "Title/abstract" option in the Entrez database for the words "real-time & PCR" results in more than 6500 hits. This increased use of real-time PCR technology for detection and quantification of pathogens, or study of gene expression, demonstrates the strength of this technique, but also the need to come to some consensus on performance criteria and data expression. The quality of publications reporting new tests varies and inter-laboratory validation data are often missing. The requirement for standard procedures may be even more urgent for diagnostic and reference laboratories dealing with the routine detection of pathogens.



Microarray technology

Although many commercial kits are available, it can be difficult to assess their performance due to the lack of consensus criteria for strain selectivity, diagnostic accuracy, fluorescence readings, cut-off for cycle threshold (Ct), detection probability, and software incompatibilities. For example, the detection of food-borne thermotolerant campylobacters in the same PCR-based method with the identical chemistry can give a marked difference in results when carried out using different platforms. The lack of standardization among real-time PCR instrument platforms makes it difficult to optimize the same method with different instruments.

Several questions with relation to sampling and sample treatment need to be answered: are there many differences between 500 ml of chicken rinse fluid and 25g faecal sample from a food-producing animal as template for PCR? Can we use the same lysis and DNA purification procedures for all pathogens? How does copy number of the pathogen in the specimen affect methodology? Are there different PCR inhibitors in food vs. samples from the primary production? Can multiplexing or the use of internal amplification controls lower the limit of detection so these procedures cannot be universally applied? These are just a few of the questions WP10 will answer.

### Microarrays

Micro-arrays have been reported for the expression of *Salmonella* genes at the RNA level, but their potential use in diagnostic laboratories has not received sufficient attention. Workpackage 10, has established a working group to look into harmonising similar international efforts. The aim is to draft a standard guideline for a simple, single-dye approach to routinely screen *Salmonella* isolates. Isolates will be screened for the presence of genes encoding sero-group, fimbrial antigens, pathogenic properties, antibiotic resistance determinants, phage susceptibility, etc. Such an open-formula draft guideline could be used, in the first instance, by large reference laboratories to set up simple and fail-proof microarray tests for the comprehensive characterization of *Salmonella* isolates. This would be a straightforward way of identifying new outbreak strains and would allow the study of the epidemiology of *Salmonella* at the genotypic level.



*The Food-PCR 2 Group met for their second workshop in Weybridge in March 2005*

established as follows:

1. Microarray standardization: Burkhard Malorny, BfR, Germany.
2. General requirements for real-time PCR standardization: Matthias Kuhn, Congen, Germany.
3. Preparation of ring-trials and reference materials for Salmonella and Campylobacter: Dario De Medici, ISS, Italy.
4. PCR testing of animal faeces: Enne de Boer, VWA, The

## Work Programme

### Task 1: Working Group Establishment

European working groups will be established for the standardisation of real-time fluorescence-based PCR and microarrays for identification of *Salmonella enterica* and thermotolerant campylobacters. The workpackage leader will ensure harmonisation of guidelines and data expression, as recommended previously by an international panel of experts (Nature (2002), 419, 323).

### Task 2: PCR Database

The software requirements, the needs of the end-users and criteria for inclusion of open formula PCR protocols will be established and the work will be planned for an online PCR database, linked to the network website and [www.foodpcr.com](http://www.foodpcr.com)

### Task 3: Planning Ring Trials

A network of the major food analytical laboratories in the participating countries will be created. This network will be associated with workpackage participants to extend involvement in the ring-trials and promote technology transfer. A survey of real-time PCR requirements will be undertaken in order to prepare a checklist for obstacles to the implementation of automated PCR. This checklist will be discussed with external experts in order to develop technological platforms to overcome any obstacles. Collaborative ring-trial validation of

real-time PCR for detection and identification of *Salmonella enterica* and thermotolerant campylobacters, will be undertaken. The main challenge of real-time PCR ring trials is the incompatibility of different instruments from different manufacturers. Even identical models of the same brand of instruments can have different calibration readings. The involvement of external experts in the ring trial development will help to solve these problems. Participation in ring-trial development, will enable laboratories to update methods by technology transfer, assess performance, and access new reagents. However, participation in ring-trials will not entitle co-authorship of publications.

### First Meeting outcome

The preliminary work programme for the project was discussed during the first meeting which was held on September 9-10, 2004 in Copenhagen, Denmark. The participants consisted of 22 scientists from 12 countries, including the nine main partners and 13 associated (no funding) partners. Six experts were also invited to the meeting. During the first day the participants presented their institute and research activities in order to create a common understanding of the research potential of each institute. There was a friendly atmosphere at the meeting which resulted in substantial positive feedback. Liaisons were created with the European Standardization Committee (CEN) through project partners who also participate in similar efforts in Task Group 3 and Task Group 4 of CEN. Five working groups and their leaders were

Netherlands.

5. Standards for virus in produce: Wim van der Poel, RIVM, The Netherlands.

The groups decided to continue their work on the preparation of documents necessary for standardization of methods, and met again in Weybridge in March 2005.

The milestones and deliverables of the project were given high priority, and a new EU research proposal has been prepared.

A hands-on training workshop for real-time PCR and for microarray has been just held in Weybridge, UK.

The reports of the working groups has been published on the project homepage ([www.foodpcr.com](http://www.foodpcr.com)) and in the second project newsletter.

### The homepage

The Food-PCR 2 homepage [www.foodpcr.com](http://www.foodpcr.com) will have information about partners, meetings, publications, important links, guidelines etc. If you have any ideas for the webpage, please contact the webmaster Stefan Jensen, [sje@dfvf.dk](mailto:sje@dfvf.dk)

## SCIENTIFIC UPDATE

### 2nd Thematic Meeting, Rome 7-9th March

The venue for the second of the Thematic Representatives Meetings was in a fantastic location, right next door to St Peter's Square in Rome. The local organiser, Eduardo Pozio, ably assisted by Suzie babsa, had organised every aspect of the meeting perfectly. The meeting started with a visit to the roof of the conference centre, which gave a memorable view of the Vatican City and a photo-opportunity for all the delegates (see figure).

With such a religious surrounding it was not surprising that everyone worked hard to achieve the meeting goals of :

- Finalising the process for the thematic state-of-art reviews
- Drafting a five year strategic scientific plan
- Producing recommendations for the next

## PEOPLE



### Leader of Workpackage 10 - Professor Jeffrey Hoorfar

Jeffrey Hoorfar is a food microbiologist, he is 48 years old and a senior scientist with the Danish Institute for Food and Veterinary Research (DFVF) in Denmark, Department of Microbiological Food Safety. He has a Ph.D. in food science and nutrition from the Royal Veterinary and Agricultural University of Denmark (KVL) and has been researching immunology and microbiology at DFVF for the past 14 years. His main interest is the development of rapid microbiological methods and he has been coordinator of the first Food-PCR EU project under the 5th Framework Programme. He is also coordinator of several Nordic and national research projects and has published over 70 articles. He is married, has an eight year old son and lives in the North of Copenhagen, 30 min drive from his office and laboratory at DFVF.



### Workpackage plan

For the first half of the meeting the thematic groups worked individually, reassessing the outcomes from the 1st Thematic Meeting and completing outlines for the state-of-art reviews. It is anticipated that these reviews will be published on the website and, hopefully, in peer-refereed journals by April 2006. On the basis of these discussions, a five year plan for each thematic area was then drafted. This plan was then used to develop ideas for possible workpackages for funding in the next Joint Programme of Activities (JPA).

In the second half of the meeting all the delegates joined together in discussion of the proposed plans and Workpackages. A total of 32 potential Workpackage concepts were put forward by all the thematic groups. In the final session of the meeting, each of

these concepts were briefly discussed and assessed by the delegates on the basis of the following equally-weighted criteria:

- Strategic value and fit to network remit – this included fit to the strategic plan and relative importance to public and veterinary health issues
- Scientific value including originality, novelty, outputs including publications and other deliverables
- Interaction across the network including the involvement of as many partners as possible, across the medical-veterinary chain and all the thematic areas where feasible
- That the proposed workpackage fills a gap in knowledge, expertise or skills
- That the workpackage is achievable, realistic and timely

The outcomes of this assessment are still under review, however, during the

discussions it was clear that many of the projects had significant overlaps with each other and could reasonably be amalgamated to provide workpackage concepts that were more comprehensive and integrated. There were also some gaps identified in Workpackage plan.

The next step will be to present the outcome of the strategic planning and workpackage concept assessment to the Co-ordination Forum meeting on the 31st March in London. At this meeting the sub-committee on workpackage commissioning will be ratified and the process for selection of workpackages from the recommended concepts, discussed. This process will be developed over the next few months so that new workpackages for the next JPA are agreed, commissioned and negotiated by September 2005 to start in April 2006.

*Diane Newell*



*The Thematic Meeting delegates enjoyed wonderful views from their accomodation*

## ADMIN BUREAU UPDATE

### Management of the budget

Following issues relating to reimbursement of costs between partners, André Jestin and Maya Goepfert met the Financial Officers of DG-Research in Brussels in early February. We now have official confirmation from DG-Research that our Consortium is allowed to proceed with invoicing between partners. If, for example, the budget was initially misallocated between partners, we now have permission to send the funds to any partners who didn't receive their entitlement and receive reimbursement later on by the partner who holds the funds.

This process of invoicing between partners leads to maximum flexibility of the budget as requested by some partners. Unless implemented carefully, this may result in failures with regards the use of the budget. Thorough follow-up is therefore required to ensure the

budget is used correctly and to ensure consistent and appropriate reporting to the EC. To that end, the Administration Bureau will provide the framework and rules of implementation of this procedure, by publishing additional documents e.g. guidelines for transfer of funds between partners under certain conditions. A cost guide for travel and subsistence expenses will also be provided.

### Visit of the Financial Officers of VLA

Lucinda Cranham and Nigel Robinson visited the Administration Bureau this February, to brainstorm the developments of budget management that have been drafted up to now. They also aimed to prepare the intermediary financial meeting, and set the financial organisation of future meetings organised by the VLA. This visit was also an opportunity to combine ideas regarding financial management. It also encouraged the formation of collaborations between partners.

### Intermediary Financial meeting

An intermediary financial meeting will take place from the 10-24 of March as an electronic

meeting. The financial Officers of each partner institute will be requested to provide information about their use of the Med-Vet-Net budget up to now. This intermediate financial meeting will provide the opportunity for partners to target any issues in budget management and to plan any budget adjustment among partners.

### Organisation of next Co-ordinating Forum and Governing Board meetings Co-ordinating Forum in London at HPA on 31st of March

The HPA and the Administration Bureau have organised the next Co-ordinating Forum. Participants have been invited to attend the meeting and all local organisation is complete.

### Co-ordinating Forum, Advisory Panel and Governing Board in Paris at AFSSA next September

The Co-ordinating Forum has now been planned for week 39 of this year (September) and the Governing Board meeting will take place in week 40 (exact dates to follow). Both meetings will be held at AFSSA (Paris).

*Administration Bureau*



MED-VET-NET

# GENERAL SCIENTIFIC MEETING

**KING ALFRED'S COLLEGE, WINCHESTER, UK**  
Wednesday 29 June - Friday 1 July 2005

### Program:

Wed 29 - 10am registration, lunch, keynote speaker, poster session, social event

Thur 30 - keynote speakers, thematic oral presentations, posters, formal dinner

Fri 1 - communication workshop, thematic sessions, summary

**29th June**  
10.00 - 1.00 Registration

1.00 - 2.00 Lunch

2.00 - 2.15 Welcome

2.15 - 3.00 Keynote  
Lecture 1: Zoonoses research in the past: the contribution to infectious disease understanding

3.00 - 3.30 Tea and posters

3.30 - 5.30 Thematic reviews

5.30 Treasure hunt / guided tour of Winchester (optional)

7.30 B-B-Q

### 30th June

9.00 - 10.30 Progress reports WP4-6

10.30 - 11.00 Coffee and posters

11.00 - 12.30 Progress reports WP7-10

12.30 - 2.00 Lunch and posters

2.00 - 2.45 Keynote  
Lecture 2: Zoonoses research: the current global problems

2.45 - 3.15 Presentations by associate networks

3.15 - 3.45 Tea and posters

3.45 - 5.15 Presentations by associate networks

7.00 Dinner

### 1st July

9.00 - 9.45 Keynote  
Lecture 3: Zoonoses research - what are the future challenges?

9.45 - 10.45 Progress reports WP11-12

10.45 - 11.15 Coffee

11.15 - 12.15 Progress reports WP13-14

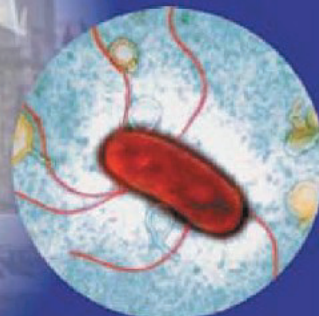
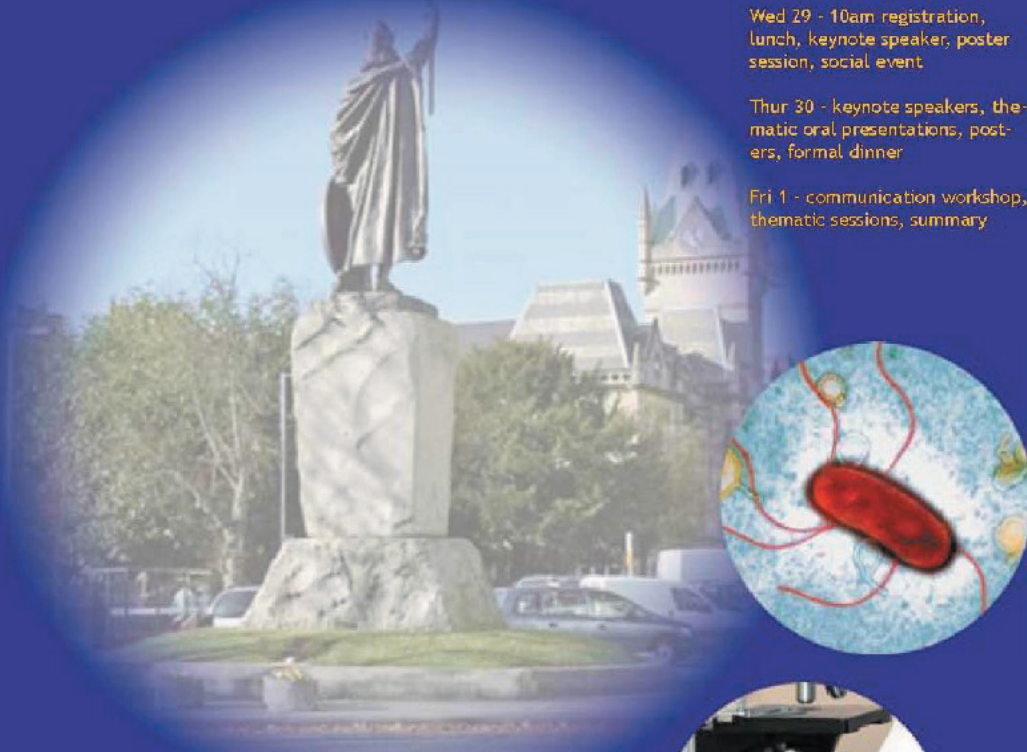
12.15 - 1.00 Meeting summary and discussion

1.00 - 2.00 Lunch and finish

This programme may be subject to alteration

### Thematic Group meeting

The Host-Microbe Interactions thematic meeting is to be held in Uppsala, Sweden on October 19th - 21st 2005.



This is a dedicated Med-Vet-Net meeting, open to Med-Vet-Net partner institutes

<http://www.medvetnet.org>



**DEADLINES**  
Online Abstract submission deadline:  
**Friday 22nd April 2005**  
Online registration deadline:  
**Friday 6th May 2005**

For further information, please send an email to: [claire@medvetnet.org](mailto:claire@medvetnet.org) or telephone +44 (0)1932 359436

## WEBSITE UPDATE

The new Med-Vet-Net website is now online. The website will provide a gateway for information on infectious diseases transmitted by animals. Current news, an overview of research topics, information on partner institutes, as well event announcements feature on the site. As work within the Network progresses, it will also provide research results and publications. A full article will follow in the April newsletter. Visit <http://www.medvetnet.org>

## INTERNAL MEETINGS

**General Scientific Meeting**  
Winchester, England June 29th - 1st July 2005  
The AGM programme is now live and registration forms and abstract submission forms will soon be available online. Remember the deadline for submission of abstracts is the **22nd April 2005** and the deadline for registration is **6th May 2005**

The preliminary programme for the meeting is as follows:



## PEOPLE

### New Members of Staff



Dr Lucy Harper

There have been two new appointments made within Med-Vet-Net in the last month.

Dr Lucy Harper is the new Communications Officer working in the Society for Applied Microbiology (SfAM) office with Teresa Belcher. After graduating from Birmingham University in 1995, Lucy worked in the Research laboratories at Birmingham Heartlands Hospital, England and later University Hospitals Birmingham NHS trust. She went on to take up a studentship at Aston University, Birmingham and she graduated with a PhD in 2003. Since then she has been working as a post-doctoral researcher at Aston University on two projects, the most recent of which investigated possible serum markers to aid in the diagnosis of infective endocarditis.

In the summer of 2004 Lucy was elected as Honorary Editor of *Microbiologist* - the quarterly magazine for SfAM. This role inspired her to leave the laboratory and take on a more communications-oriented role. She looks forward to working with all in the Med-Vet-Net network.

Dr Claire Cassar graduated in Microbiology from King's College, University of London in 1992. After completing a Masters degree in Aquatic Resource Management she went on to obtain her PhD in 1998 working in the field of food microbiology and food safety. She joined the Cellular and Molecular Sciences group at St. Georges Hospital Medical School, London as a postdoctoral researcher where she worked on multi drug resistance mechanisms in *Plasmodium falciparum* and then characterisation of factors involved in the suppression of HIV replication.

Until recently, Claire worked within the Food & Environmental Safety Department at the Veterinary Laboratories Agency where she was a Diagnostic Section manager managing a team of scientists to deliver work under zoonoses surveillance and endemic disease portfolios.

Claire's role within MedVetNet is to provide project management support to Prof. Diane Newell, the MedVetNet Project Manager.



Dr Claire Cassar

## EXTERNAL CONGRESSES

### 3rd European Meeting on Viral Zoonoses 28-31 May 2005, St. Raphaël, France

The meeting will cover research on ecology, epidemiology, virology, and control of viral zoonoses in Europe. This will include important endemic, epidemic, imported or emerging viruses such as bunyavirus, lys-savirus, flavivirus, influenza virus, coronavirus as well as less conventional agents provoking BSE. Registration and abstract deadline: 31st March 2005. ONLY ONE WEEK LEFT! Visit <http://www.euroviralzoon.com>

### Development of Practical Risk Management Strategies Based on Microbiological Risk Assessment Outputs Call for Experts

A joint FAO/WHO Expert Meeting in collaboration with the Federal Dairy Research Centre, Institute for Hygiene and Food Safety, Kiel, Germany, will convene an expert meeting in the second half of 2005 (dates to be decided) to prepare guidelines on the development of practical risk management strategies based on microbiological risk assessments. Visit: <http://www.who.int/foodsafety/micro/jemra/meetings/2005/en/> or contact: Jaap Jansen (Tel: +41 22 791 3568 - E-mail: [jansenj@who.int](mailto:jansenj@who.int))

### 4th International Conference on *Mycobacterium bovis*, 22-26 Aug 2005, Dublin, Ireland

This meeting will cover:

- M. bovis infection in a diverse wildlife community with potential for zoonotic spill-over
- Recent developments in the basic sciences: what policy-makers need to know
- Advances in our understanding of disease epidemiology, and implications for control and eradication
- Maximising the effectiveness of control and eradication options: a role for critical evaluation

tion of local programmes (case studies)

- Addressing TB at the national level (a series of national case studies)
- Varying strategies to address the wildlife reservoir

Organised by the Centre for Veterinary Epidemiology and Risk Analysis (CVERA) please visit <http://www.4icmb.org> for details

### 1st Panamerican Congress on Zoonoses, 5th Argentine Congress on Zoonoses, and 2nd Zoonoses Congress of the Province of Buenos Aires

The Argentine Congress takes place every other year. For 2006, the Argentine Congress and the 1st PanAmerican Congress will be combined to allow for the participation of a larger number of professionals from the region dedicated to research, prevention, and control of these diseases.

The Scientific Committee invites you and colleagues to submit abstracts. The deadline for their submission is 15 Nov 2005, with complete presentations submitted by 15 Mar 2006. Please contact:  
Dr Oscar P Larghi  
Fray Lamas 248  
1706 Haedo, BA, ARGENTINA  
tel: 54 11 4460 2089  
fax: 54 11 4443 2971  
[oplarghi@speedy.com.ar](mailto:oplarghi@speedy.com.ar)  
<http://www.zoonosis2006.com>

### ESV - European Society for Veterinary Virology International Conference on Animal Circoviruses and Associated Diseases

Queen's University, Belfast, Northern Ireland, UK, 11-13 September 2005  
Visit <http://www.happen.co.uk/esv2005/> for details

### The 24th ICC in Manila, Philippines : 4-6 June 2005

The meeting will be held in Manila, the Philippines. The theme of the congress is "Confronting Health Challenges, Strengthening Strategies" and will cover 5 major topic tracks:

Clinical Infections: Challenges and Strategies, New antimicrobials and rational drug use, Emerging and Re-emerging Infectious Diseases and Infections in healthcare settings. Visit: [www.psmid.org/newprogram.html](http://www.psmid.org/newprogram.html).

### The Institute of Biology - Presentation Skills one day course 12 April 2005.

This course is designed for scientists that need to be able to speak proficiently in public, whether it be presenting to a board or team or presenting a product, results or findings. Expert tuition will be offered and the course will give you the confidence you need to be able to successfully speak in public and deliver your message with conviction. Call +44 (0)20 7581 8333 ext 237 for further information

## CONTACT US

Med-Vet-Net News is published monthly by the Med-Vet-Net Communications Unit.

Editor: Teresa Belcher

Email:

[communications@medvetnet.org](mailto:communications@medvetnet.org)

Ph: +44 (0)1234 271020

Fax: +44 (0)1234 271025

Postal Address:

Society for Applied Microbiology,  
The Blore Tower,  
The Harpur Centre,  
Bedford, MK20 1TQ, UK

Contributions and suggestions are welcome.  
Deadline for publication is 20th of each month.

©Med-Vet-Net, March 2005

Med-Vet-Net is a European Network of Excellence on Zoonoses Research

Visit <http://www.medvetnet.org>