Resistance to beta-lactam antibiotics

Workpackage 9: The human health implication of emerging resistance to beta-lactam antibiotics in Salmonella and other Enterobacteriaceae from food animals

β-lactam antibiotics and how they work

The β-lactam antibiotics are the most varied and widely used of all the different groups of antimicrobials, and about 100 different β-lactam antibiotics are used clinically in the antibacterial treatment of humans and animals. The penicillins including the aminocephalosporins, carbapenems, and monobactams are all considered β-lactam antibiotics. These antibiotics all share a three carbon, one nitrogen structure known as the beta-lactam ring. An intact beta-lactam ring is required for these antibiotics to exert its bactericidal activity.

Penicillins and related antibiotics are bactericidal, destroying bacteria by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity, especially in Gram-positive organisms. β-lactam antibiotics bind to the active site of the penicillin binding proteins (PBPs), preventing the final cross-linking (transpeptidation) of the nascent peptidoglycan layer, and thus disrupting cell wall synthesis. Inhibition of PBPs may also lead to the activation of autolytic enzymes in the bacterial cell wall.

Bacterial resistance to antibiotics

Bacteria can become “resistant” to individual antibiotics by developing specific defense mechanisms which make the antibiotic ineffective. Generally there are three mechanisms that are utilized by bacteria to do this: (1) preventing the antibiotic from binding with and entering the organism, (2) producing an enzyme that inactivates the antibiotic or (3) changing the internal binding site of the antibiotic for infections in children or other cases where fluoroquinolones cannot be used. Whereas much is known about the occurrence of different β-lactamases from bacteria causing infections in humans there is a lack of knowledge about the occurrence of such enzymes in bacteria from food-producing animals.

One way in which bacteria have become resistant to beta-lactam antibiotics is by being able to express β-lactamase enzymes – an example of the second type of resistance. There are actually dozens of enzymes, produced by many different bacteria, which are capable of degrading the beta-lactam structured antibiotics.

Rapid development of resistance

In the development of resistance towards these drugs, scientists have witnessed one of the few cases of evolution occurring on a timescale of years. The TEM-1 enzyme, capable of degrading ampicillin emerged about 40 years ago. This enzyme has since evolved leading to roughly 128 different TEM β-lactamases; some with activity against almost all β-lactams. Other important classes of β-lactamases are SHV, PSE and OXA and a similar evolution can be observed in these classes. In addition, chromosomal ampC β-lactamases have relocated into plasmids and emerged in different pathogenic species where they cause resistance to virtually all β-lactams.

β-lactamases in bacteria from food-producing animals

The emergence of β-lactamases in Gram-negative bacteria is an increasing problem worldwide. It is a particular problem in Salmonella, where the use of broad-spectrum beta-lactams alone in combination with a β-lactamase inhibitor, are standard treatment in the area of zoonoses research, both internally and externally, and this newsletter provides a summary of those.

This month MVN News provides an overview of Workpackage 9 - The human health implication of emerging resistance to beta-lactam antibiotics in Salmonella and other Enterobacteriaceae from food animals.

There is also information on MVN Training Courses available to scientists involved in the Network, and the regular scientific and administration updates.

In addition, there’s a great deal happening with meetings and congresses in the area of zoonoses research, both internally and externally, and this newsletter provides a summary of those.

Teresa Belcher
Med-Vet-Net Communications Director

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The emergence of β-lactamases in Gram-negative bacteria is an increasing problem worldwide. It is a particular problem in Salmonella, where the use of broad-spectrum beta-lactams alone in combination with a β-lactamase inhibitor, are standard treatment
WP9 will also implement tools for the determination of the occurrence of different β-lactamases among Salmonella and other Enterobacteriaceae from humans and food animals. In addition, procedures for standardized characterization of plasmids will be established. A MIC-panel of β-lactam antibiotics for characterization of β-lactamases will be established. Methods for further molecular characterization will be established and reference laboratories identified. Protocols for standardized typing of plasmids will be developed and initial protocols made available through the Internet.

Creating a network of laboratories

Recognising the current lack of standard methods, WP9 will aim to initiate work that should lead to internationally standardised procedures for typing resistance plasmids from Salmonella. In addition, an international data bank for comparison of plasmid-profiles will be developed. The end goal is to create a network of laboratories that are able to detect and respond rapidly to any new emergence of β-lactam resistance. Compare the genes, plasmids and isolates to those observed in other regions and characterize new genes/enzymes. This will be ensured by the creation of a network, where diagnostic laboratories perform preliminary identification through routine diagnoses. Selected isolates are then sent to regional or national reference laboratories that characterize the isolates, monitor trends in their region and report centrally. When new interesting isolates emerge or when more detailed investigations are necessary these laboratories should have easily access to specialized reference laboratories that have the knowledge that are needed.

Workpackage tasks

Task 1. Strain collection of isolates

A meeting to discuss approaches will be undertaken by month 3. As reference strains for detection of the different genes and plasmids are not easily available, attempts will be made to collect these isolates centrally for use in all participating laboratories. Information about the strain collection will be made available through a web-based database.

Task 2. Phenotypic assays for screening and characterization.

Diagnostic laboratories use different β-lactam antibiotics for initial screening of isolates for susceptibility. During the first meeting the requirement for minimum testing during routine diagnosis will be discussed and recommendations for inclusion of β-lactam antibiotics in routine testing for all diagnostic laboratories distributed.

Susceptibility testing can provide an initial characterization of interesting β-lactam resistant isolates. A MIC-panel for determining the susceptibility to different β-lactam antibiotics at the regional/national reference laboratories will be developed and evaluated. This will provide the tool for an initial characterization of isolates.

Iso-electric focusing is often used for further phenotypic characterization of β-lactamases. The advantage of this technique is that it will enable the laboratories to detect whether more than one β-lactamase is produced by the tested isolate. The technique will be made available for all regional reference laboratories.

Task 3. Molecular characterization of β-lactamase genes

Primers will be recommended for the different gene classes (TEM, SHV, OXA, CTX, CMY). Protocols and reference strains will be made available for all laboratories on the MED-VETNET website.

Task 4. Methods for characterization of plasmids

A list of known plasmids, including their ori-T and ori-R, will be made. This will include a short description of the plasmid’s ability to spread and the content of antimicrobial resistance genes. The strains containing these plasmids will be identified and, if possible, accessed into the collection. Protocols and selected references, for publication of plasmids, will be made available in pdf-format on the website.

A meeting where the future plans for the development of standardized protocols for plasmid characterization will be arranged by month 12. Relevant key-scientist will be invited and present their data and/or opinion and a consensus of the future work out-lined.

Task 5. Identification of reference laboratories capable of further characterization

Reference laboratories, capable of providing expertise for further characterization of new genes/enzymes or during more detailed investigations, will be identified. Memorandum of Understandings for joint work will be developed at a workshop by month 15.

For more information, contact Frank Aarestrup at the Danish Institute for Food and Veterinary Research (DFVF) faa@dfvf.dk

Second Food PCR 2 Meeting

7-8 March 2005, Veterinary Laboratories Agency (VLA) Weighbridge, UK
Organised as part of MedVetNet EU Network of Excellence Programme: Day 1 - Advanced real-time PCR & microarray Workshop, Day 2 - Food PCR 2 Workgroup meetings and Food PCR plenum session and general meeting
For more information, please e-mail Stefan Jensen, sje@dfvf.dk

March Thematic Group Meeting 7-9 March 2005, Istituto Maria SS. Bambina, Rome
The next Thematic Meeting as part of WP2 will be held in Rome from 7-9 March. This meeting is to be attended by all thematic representatives.

The local organiser is Edoardo Pozio (ISS) who will provide more detailed information closer to the event.

For assistance with the booking of hotels, please contact Susy Babbsa on susan.babbsa@iss.it or fax +0039 06 49902992.

Med-Vet-Net Coordinating Forum Meet ing 31 March 2005, BPA, Collindale, UK
In accordance with the Consortium Agreement, this is one of the meetings which permits the Coordinating Forum members (Project Manager, Institutes Representatives, Thematic Co-ordinators) to undertake the Network’s management and coordination issues.

A precise agenda will be drafted and sent to attendees by the Project Manager. Attendees are invited to arrive and attend a dinner the evening before.

Med-Vet-Net Annual General Meeting 30 June – 1 July, 200, King Alfred’s College Winchester, UK
There will be three keynote lectures based around Zoonoses research past, present and future. Each Workpackage will give a 30 minute presentation of their research progress.

And Thematic Coordinators will outline the status and plans for the next Joint Activities Plan. Poster submissions will be invited from younger scientists. Entertainment will include a treasure hunt and BBQ on the first night, and a more formal dinner with a pre-dinner guest speaker on the second night.

Leader of Workpackage 9 - The human health implication of emerging resistance to beta-lactam antibiotics
Professor Frank Aarestrup
Professor Aarestrup graduated as a Doctor of Veterinary Medicine from the Royal Veterinary and Agricultural University in 1992. He obtained a PhD in 1995 and his thesis was on characterisation of Staphylococcus aureus from bovine mastitis. From 1995, he was Head of Section for antimicrobial resistance at The Danish Institute for Food and Veterinary Research (DFVF). From 2002, he was Professor at the DFVF. Frank’s research interests are mainly in relation to the development of antimicrobial resistance among bacteria from food animals and the transmission to and consequences for human health.
The current list of all the Thematic Representatives is below. Those representatives highlighted in blue are the Thematic Co-ordinators. Please note that Bob Adak from the HPA has taken over from Peter Gerner-Smidt as the Epidemiology Theme Co-ordinator.

### Developing the Network

On 24-25 January, a joint EU – US Innovative Technologies Meeting was held in Brussels. Last year I attended the same meeting in West Virginia representing Med-Vet-Net. This year I attended the meeting representing our new associate network, EU-US-SAFE-FOOD, in collaboration with our North American partner FS-CAP project managed by Jay Levine and funded by CREES, USDA. EU-US-SAFE-FOOD will fund the European end of four joint meetings on food safety issues over the next years. It will also fund European scientist exchange visits to the US and aims to organise workshops within international scientific meetings like the ASM General Meeting.

EU-US-SAFE-FOOD is the first success in our remit to extend Med-Vet-Net to external scientists. A further step was made in this direction last week by the submission of the proposal for another international associated network by Jaap Wagenaar. This proposed network (LAEU-FOODNET), submitted for EU funding to the Specific Support Action call, would generate a link between Med-Vet-Net and Latin America.

Finally, Med-Vet-Net is now reaching out eastwards. On 14-16 March I will represent the network at a joint EU-Russia conference on biotechnology. So if anyone has any on-going collaborations which they would like me to emphasis or raise during that meeting please let me know.

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### Chart of Med-Vet-Net Thematic Representatives

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<tr>
<th>Epidemiology</th>
<th>Host-Pathogen</th>
<th>Detection/Control</th>
<th>Risk Research</th>
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<td>István Tóth</td>
<td>Бела Nagy</td>
<td>Laszlo Stipkovits</td>
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**PhD Fellowships in Denmark**

Seven PhD Fellowships in Denmark now open for application!

TRAINAU (Training Risk Assessment In Non-human Antimicrobial Usage) provides multidisciplinary early-stage research training on identification, characterisation and assessment of public health risks associated with non-human use of antimicrobials.

TRAINAU is sponsored by the EC Marie Curie Early Stage Research Training programme. The Early Stage Training site consists of six inter-related research groups located at two universities, The Royal Veterinary and Agricultural University (KVL) and The Danish University of Pharmaceutical Science (DFU) and two national reference laboratories Statens Serum Institut and Danish Institute for Food and Veterinary Research. Candidates from Member and Associated States of the European Community and from Third Countries can apply for Marie Curie Host Fellowships. Danish citizens living in Denmark cannot apply. Please visit www.trainau.dk for further information.

Deadline for application is 1 May 2005.

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**Your contribution to the discussion on the Network Science Strategy and Plan**

On 8-10 March the Thematic Group will meet for the second time to discuss the scientific strategy and plans for Med-Vet-Net. At the first meeting of the Thematic Group held in September (see MVN News Dec 04) discussion focussed on developing a joint understanding of the state-of-art of each of the thematic areas (Epidemiology, Host-microbe interaction, Detection and control and Risk research) and the directions we would like to go in. The minutes of these discussions have been distributed to all the thematic representatives and the discussions will hopefully generate published reviews in these areas.

At the next meeting we will develop these concepts further leading to the planning of the scientific Workpackages for the next Joint Activities Plan (JPA). The JPA forms the basis of the next contract with the European Commission and has to be submitted in September 2005. As the JPA defines the scientific strategy and research undertaken by the network, the contribution of scientists from all the partners is essential.

By talking with the thematic representatives from your institutes, you can contribute to the Med-Vet-Net science strategy and the next Workpackage plan. Your contributions can include:

- Pointing out gaps in the networks current scientific programme
- Ensuring your specialist research topic is represented and understood
- Raising any new topics of research
- Suggesting new workpackages
- Developing new collaborative working groups to put forward novel ideas.

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Diane Newell
A key objective of Med-Vet-Net work package 2A is the spread of excellence and dissemination of scientific knowledge through training courses and workshops, and furthermore to enhance and facilitate mobility of researchers between the Med-Vet-Net institutes. In order to achieve this goal, a training sub-committee has been established. The members are Henrik C. Wegener (chair), Bogumila Litwin- ska (NHI), Christine Little (PHA), Roberto La Ragione (VLA) and Frank Aarestrup (DFVF).

The purpose of training courses within Med-Vet-Net is to develop technical capacity to the highest attainable level to investigate, detect and respond to microbial food safety hazards in national reference laboratories throughout the European Union. Furthermore, Workshops serve to facilitate and strengthen the inter-disciplinary collaboration workshops funded within the Med-Vet-Net. The network hopes to strengthen links between diverse groups such as veterinary and public health, epidemiologists and microbiologists, natural and social scientists, government and industry, regulatory agencies and reference laboratories, risk assessors and scientists.

The committee has recently made a call for proposals for different training courses among representatives. Many excellent suggestions have been produced. The committee, in consultation with the coordinating forum, have now selected the training courses and workshops, which will be supported during the first 18 months. The total number of funded training courses in Med-Vet-Net will be approximately 10. In addition, basic training courses will be developed for the EU candidate countries to develop capacity to the level of the current EU members.

It is still possible to propose topics for training courses or workshops. The proposal shall describe in general terms: 1) learning objectives/ workshop objectives, 2) participants profiles, 3) selection criteria for participants, 4) dates and venue, 5) lead course organizer, 6) list of trainers, 7) a detailed programme, 8) budget, 9) plan for making training material available on the Med-Vet-Net homepage and, 10) plans for follow-up of training and/or networking. The proposals will be evaluated by the training committee on an annual basis and a prioritized list of courses and workshops communicated to the coordinating forum for final approval.

Scientific exchange visits

Exchange of scientists between Med-Vet-Net institutions is another important element of Med-Vet-Net. Exchange visits facilitate the sharing of skills and experiences between institutions and builds personal relations, which strengthens the formal and informal scientific networks of Med-Vet-Net. All scientists are encouraged to apply for funds for short term scientific exchange visits (typically from one week to two months long). An application form can be downloaded from the Med-Vet-Net homepage, where also the procedures and selection criteria for exchange visits can also be found.

For further information about training courses, workshops or exchange visits in Med-Vet-Net contact:

Heidi Lillerøj Petersen
Danish Institute for Food and Veterinary Research
Markhøj Bygade 19
DK-2860 Søborg
Tel: +45 72 34 70 75, Email: helpe@dfvf.dk

An epidemiological calculator for estimating disease prevalence from testing of pooled samples is now available on-line at http://www.ausvet.com.au/eprev. The on-line calculator was developed by Dr Evan Sergeant from AusVet Animal Health Services, with funding from the Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease (www.abcrc.org.au).

Pooled (or group) testing is a testing strategy where samples from a number of individuals are aggregated into a single sample (or pool) and multiple such pools are then tested for the disease or agent of interest. Pooling of samples for testing provides one means of substantially reducing testing costs, without necessarily sacrificing precision of resulting prevalence or confidence interval estimates.

Pooled testing for prevalence estimation is particularly useful where disease prevalence is likely to be low and where test-costs are high, relative to sample-collection costs. Statistical techniques for estimating disease prevalence from testing of pooled samples have been developed and published in the scientific literature, but are computationally complex and therefore not widely used. This calculator provides a user-friendly interface for the implementation of a number of methods for estimating prevalence, assuming fixed or variable pool size and assuming either perfect tests or tests of imperfect and uncertain sensitivity and/or specificity. A Bayesian method is also included to allow incorporation of prior knowledge of the likely prevalence and of test performance. The calculator also includes options for estimating the required pool size and number of pools to be tested to achieve a desired level of confidence and precision when designing prevalence surveys and for simulated sampling to evaluate precision and potential bias of alternative pooling strategies.

The Pooled Prevalence Calculator provides an invaluable resource for researchers or epidemiologists undertaking disease surveillance involving prevalence estimation at the individual level (human, animal, aquatic animal, insects or plants) using pooled samples. The system also includes a comprehensive User Guide, Glossary and example analyses based on Hendra virus in fruit bats.

Evan Sergeant
AusVet Animal Health Services
evan@ausvet.com.au

EADGENE, 18-19 May 2005, Brussels

The Coordinator’s Representative, Dr Andre Jestin will give a presentation on Med-Vet-Net at EADGENE - a Network of Excellence that aims to integrate research facilities in the area of host-pathogen genomics.

Moscow, 14-16 March

Med-Vet-Net Project Manager, Prof. Diane Newell will represent the network at a joint EU-Russia conference on biotechnology.

People

Leader of Workpackage 3 and Institute Representative, SFAM

Dr Peter Silley graduated in Bacteriology from the University of Birmingham in 1977. After a period in the pharmaceutical industry with Cyanamid (GB) Limited he went to the University of Newcastle upon Tyne where he obtained his Doctorate in 1982, working in the field of rumen microbiology. He then moved to Northern Ireland where he held a joint appointment with the Department of Agriculture and a lectureship at the Queens University of Belfast and continued his research interests in gastro-intestinal microbiology. Peter subsequently joined the Glaxo group of companies working firstly in veterinary medicine as Head of Microbiology for Glaxo Animal Health and subsequently as Senior Research Leader with Glaxo Group Research working on the development of novel anti-infective compounds in human medicine.

Since 1990, Peter has been Research Scientist at Don Whitley Scientific where he has developed a highly successful contract research business largely serving the pharmaceutical industry in both Europe and the USA. Don Whitley Scientific is also extremely well known as a supplier of innovative instrumentation for the microbiology industry most notably for their range of anaerobic workstations, spiral platers and the RABIT impedance system. In January 1999, MB Consult Limited was formed to handle the increasing demand for consultancy work and Peter’s time is now split between Don Whitley Scientific and MB Consult. He is a member of a number of learned societies and currently President of the Society for Applied Microbiology (SIAM). Peter has played a prominent role within SIAM for more than 10 years and as well as being the longest serving member of the Committee he has also served as Treasurer of the Society. He holds an appointment as Honorary Visiting Senior Lecturer in the Department of Biomedical Sciences at the University of Bradford and recently acted as the IPAH representative on the European VICH delegation with respect to harmonising regulatory microbiological safety guidelines within veterinary medicine. In January 2005, he was appointed as an advisor to the CLSI (NCCLS) Subcommittee on Veterinary Antimicrobial Susceptibility Testing.

Peter’s role in Med-Vet-Net is as the leader for Workpackage 3 - ‘Spreading Excellence’, and as the Institute Representative for the Society for Applied Microbiology (SIAM).
ADMIN BUREAU UPDATE

MVN video-link SSA

The proposal for the SSA MVN video-link has been prepared and was submitted to the EC on the 8 February. We would like to thank all partners for their contribution to this document. The next step is the evaluation of the proposal by the EC and, if accepted, the subsequent negotiation process. In the case that our proposal is not adopted, the Consortium will meet again in order to decide the strategy and next actions to undertake.

Management of funds

The Administration Bureau is now working on procedures for the efficient management of the funds between partners. The allocation of funds between partners is a key factor for the framework agreement for the replacement of persons for key positions of the Network as well as management of future events like the Rome Thematic meeting, the London Co-ordinating Forum meeting in March and the Winchester Annual General Meeting next July are matters of discussion to ensure optimal organisation and implementation of meetings among the network. The Administration Bureau is working with the partners involved in these issues, particularly Diane Newell, Chairman of the Co-ordinating Forum and Thematic bodies, Henrik Caspar Wegener, responsible for the definition and organisation of trainings, and Edoardo Pozio, local organiser for the next Thematic meeting in Rome. Additionally, the Administration Bureau is also in contact with the Financial Officers from the EC to clarify the rules of management of the funds under FP6 projects, and in particular the procedures for invoicing between partners.

Organisation of upcoming meetings

Biennial Co-ordinating Forum meeting – London, March 31

The next co-ordinating Forum will take place at HPA Headquarters on the 31 March and is being co-organised by the HPA through John Threlfall for local organisation, the Administration Bureau for participants management, and Diane Newell for definition of the agenda.

Biennial Governing Board meeting – 1-8 April

A Governing Board meeting will be called following the Co-ordinating Forum meeting. It will take the form of a web-discussion on the private Med-Vet-Net web-site during one week as less conventional agents provoking BSE. Registration and abstract deadline: 15 February, 2005. Visit http://www.euroviralzoon.com

Tubital Food Congress

15-18 June 2005, Istanbul, Turkey

The “1st International Food and Nutrition Congress-Food Safety and Quality Through the Food Chain” aims to establish a platform where relevant institutions/companies will gather to discuss scientific and technological issues such as high-quality and safe production methods, processing technologies, process innovations, nutrition and health relations of foodstuffs, traceability, as well as to publicize national/international scientific and technological developments, and to share knowledge. Visit http://www.tubitalcongress2005.org or e-mail: congress@tubitalcongress2005.org

International Zoonoses Conference

15-17 June, 2005, Liverpool, UK

The Prevention and Control of Zoonoses: from Science to Policy.

The aims of the conference will be to bring together leading national practitioners and policymakers in both human and veterinary medicine with their counterparts from Europe and other areas of the world.

The themes of this conference will be:

- policy setting
- surveillance and modelling of zoonoses
- food borne zoonoses
- new and emerging zoonoses
- globalisation and zoonoses

Visit http://hpafoodzoonosesconference.org or Email to zoonosesconference@hpa.org.uk

ISAH - XLth International Society on Animal Hygiene Congress, 4-8 September 2005, Warsaw, Poland

The Congress embraces all the problems and issues concerning animal health maintenance, the production of healthy and safe food, as well as animal welfare. Registration and abstracts deadline 1 March 2005. Visit http://www.sggw.waw.pl/ish2005 or email isah2005@alpha.sggw.waw.pl

Congress IABS 2005

3-4 October 2005, Saint-Malo, France


EXTERNAL CONGRESSES

BioMicroWorld-2005

15-18 March 2005, Badajoz, Spain

1st International Conference on Environmen
tal, Industrial and Applied Microbiology “Fos
orld2005

Quantitative PCR

21-22 March 2005, La Jolle, California, USA

Real-time quantitative PCR is a highly sensi

7th International Meeting on Microbial Epidemiological Markers (IMMEM7), 11-14 May 2005, Victoria, B.C., Canada

IMMEM has a distinguished history as the ‘premier’ international meeting on strain typing and epidemiological analysis of infectious agents and diseases. This multidisciplinary meeting, co-sponsored by the ASM Confer
ces Committee and the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), will be attended by a wide variety of scientists, including microbiolo
dex.asp?bid=27725

3rd European Meeting on Viral Zoonoses

28-31 May 2005, St. Raphael, France

The meeting will cover research on ecology, epidemiology, virology, and control of viral zoonoses in Europe. This will include impor
tant endemic, epidemic, imported or emerg
ing viruses such as bunyavirus, lyssavirus, flavivirus, influenza virus, coronavirus as well

as less conventional agents provoking BSE. Registration and abstract deadline: 15 Febru

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CONTACT US

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Visit http://www.medvetnet.org