

**FINAL PROGRAMME**

**ABSTRACTS OF LECTURES AND POSTERS**

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**INTERNATIONAL CONFERENCE ON**

**RESPONSIBLE USE  
OF ANTIBIOTICS IN  
ANIMALS**

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**14-16 November 2011  
Egmond aan Zee, the Netherlands**

**International Conference on  
RESPONSIBLE USE OF ANTIBIOTICS IN ANIMALS**

**Advisory Board**

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## Welcome at the International Conference on Responsible Use of Antibiotics in Animals!

Dear participant

Antibiotics are used worldwide both in veterinary and human medicine. The widespread use has heightened concerns about the emergence of antimicrobial resistance, which impacts animal welfare, public health, food safety and environmental exposure. For example, the frequent occurrence of MRSA and the rapid increase of ESBLs have become a serious public health problem.

In recent years there has been a lot of debate about the use of antibiotics in animals. There has been much pressure to proscribe their non-therapeutic use and to remove antimicrobial growth promoters from the market. The European Union has already banned the feeding of antibiotics for growth promotion purposes. Restricting the range of antibiotics used in animals to those not regarded as important in human medicine or even prohibiting antibiotic use in animals has also been advocated as a means to reduce the spread of antimicrobial resistance.

To reduce the overall use of antibiotics in animals prudent antibiotics use is becoming increasingly promoted. Many national and international organisations, associations and federations associated with animal, human and public health have begun to develop guidelines, principles and other activities on responsible use of antibiotics. But what lies ahead?

The objectives of the **International Conference on Responsible Use of Antibiotics in Animals** are to download information and to raise key points for interactive discussions. The main value of the conference is to ensure that as many pertinent questions, rebuttals, and creative approaches regarding responsible use of antibiotics in animals are advanced as possible, with the goal that the input can serve to stimulate secondary meetings, papers, etc.

The **International Conference on Responsible Use of Antibiotics in Animals** offers high-quality speakers, ample time for discussions, and every opportunity to establish rewarding contacts. You are invited to take part in the discussions with participants from different disciplines and to meet business relations in your area. We wish you an active and fruitful meeting!

The Advisory Board

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Key to the abstracts of lectures and posters:

- abstracts of lectures and posters are grouped separately;
- the lectures are grouped according to the daily programme;
- the posters are grouped randomly.

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## PROGRAMME AT A GLANCE

### Monday 14 November 2011

13.00 - 14.45	Plenary Keynote Lectures <i>Setting the scene</i>
15.15 - 17.45	Plenary Meeting <i>Development of legislation and regulatory policies – Tour du Monde</i>
17.45 - 19.30	The Lounge Party

### Tuesday 15 November 2011

08.30 - 12.30	Parallel Session 1 <i>The focus on veterinary medical practice</i>	Parallel Session 2 <i>The focus on feed industry practice</i>
12.30 - 13.30	Poster viewing	
13.30 - 17.00	Parallel Session 3 <i>The role of monitoring antibiotic use and resistance</i>	Parallel Session 4 <i>Future research, specific approaches</i>
17.00 - 18.00	Poster viewing	
20.00	Conference dinner ( <i>reservations only</i> )	

### Wednesday 16 November 2011

08.30 - 11.00	Parallel Session 5 <i>Evidence-based use of antibiotics</i>	Parallel Session 6 <i>Food chain perspectives on antibiotic use</i>
11.30 - 12.30	Final Plenary Meeting <i>Conclusions &amp; path forward</i>	

# CONFERENCE PROGRAMME

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INTERNATIONAL CONFERENCE ON

# RESPONSIBLE USE OF ANTIBIOTICS IN ANIMALS

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**MONDAY 14 NOVEMBER 2011**

**13.00 Opening of conference**

**Plenary Keynote Lectures  
Setting the scene**

Chair: Dr. Stephen Page  
Advanced Veterinary Therapeutics, Australia

13.15 *Setting the scene – a US perspective*  
Dr. Christine Hoang, Assistant Director, Scientific Activities Division, American  
Veterinary Medical Association, USA

13.45 *Setting the scene – a European Union perspective*  
Dr. Karolina Törneke, Medical Products Agency, Sweden; Chair of Scientific Advisory  
Group for Antimicrobials, European Medicines Agency

14.15 *Future antibiotics use in animals – evolution or revolution?*  
Peter Allen, Chairman, Responsible Use of Medicines in Agriculture (RUMA)  
Alliance, UK

**14.45 Networking break & poster viewing**

## MONDAY 14 NOVEMBER 2011

### Plenary Meeting

#### Development of legislation and regulatory policies: Tour du Monde

Chair: Dr. Thomas R. Shryock  
Elanco Animal Health, USA

- 15.15 *Development of regulatory policies in Europe*  
Dr. Valérie Thomas, Chair of Anti-infectives Working Party, International Federation for Animal Health (IFAH- Europe) Belgium; Intervet Innovation, Germany
- 15.40 *The evolution of statute and regulations governing the use of antibiotics in animals – how you can make a difference*  
Dr. Nancy E. Halpern, Attorney at Law – Associate, Intellectual Property Department, Fox Rothschild LLP, USA
- 16.05 *Perspectives on the use of antibiotics in animals in Latin American*  
Dr. José Luis Rojas Martínez, National Laboratory of Veterinary Services, Ministry of Agriculture and Livestock, Costa Rica
- 16.30 *Legislation and regulatory policies on the use of antibiotics in animals and fish in Africa*  
Prof.dr. George W. Nasinyama, Deputy Director Research, Department of Veterinary Public Health and Preventive Medicine, Makerere University, Uganda
- 16.55 *Antimicrobial use and resistance in animals in Asia*  
Prof.dr. Yong Ho Park, College of Veterinary Medicine, Seoul National University, Korea
- 17.20 *Regulation of veterinary antibiotics in Australia*  
Dr. Allen Bryce, Program Manager, Veterinary Medicines Program, Australian Pesticides and Veterinary Medicines Authority, Australia

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17.45 - 19.30 **The Lounge Party**

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**TUESDAY 15 NOVEMBER 2011**

**Parallel Session 1**

**The focus on veterinary medical practice**

Chair: Peter J.G. Oostenbach, M.Sc.  
MSD Animal Health, the Netherlands

08.30 *Focus on veterinary medicine: uncertainties and demands*  
Prof.dr. Johanna Fink-Gremmels, Division Veterinary Pharmacology, Pharmacotherapy and Clinical Toxicology, Institute for Risk Assessment Sciences, Faculty of Veterinary Medicine, Utrecht University, the Netherlands

08.55 *U.S. perspectives on veterinarian oversight of antibiotics*  
Dr. Richard Carnevale, Vice President, Regulatory, Scientific and International Affairs, Animal Health Institute, USA

09.20 *Consequences of generic marketing on antibiotic consumption and the spread of resistance: facts and hypotheses*  
Prof.dr. Pierre-Louis Toutain, Laboratory of Experimental Pathophysiology and Toxicology, National Veterinary School of Toulouse, France

09.45 *Veterinary medicine use according to data sheet – exception rather than the norm?*  
Dr. Matt Dobbs, Director, Westpoint Veterinary Group, UK

**10.15 Networking break & poster viewing**

10.45 *Demonstration of efficacy for antimicrobials now and in the future*  
Dr. Gesine Hahn, Department Veterinary Drugs, Federal Office of Consumer Protection and Food Safety, Germany

11.10 *Formularies and other risk management strategies promoting responsible use*  
Dr. Annette Cleveland Nielsen, Danish Veterinary and Food Administration, Ministry of Food, Agriculture and Fisheries, Denmark

11.35 *Advertising and promotion in the context of prudent and responsible use of antimicrobials*  
Anne Chevance, French Agency for Veterinary Medicinal Products / French Agency for Food, Environmental and Occupational Health & Safety, France

12.00 *The veterinary contribution to the responsible use of antimicrobials: decoupling veterinary prescription and dispensing?*  
Prof.dr. Ludo J. Hellebrekers, President, Royal Dutch Veterinary Society and Faculty of Veterinary Medicine, Utrecht University, the Netherlands

12.30 - 13.30

**Lunch break & poster viewing**

## TUESDAY 15 NOVEMBER 2011

### Parallel Session 2

#### The focus on feed industry practice

Chair: Dr. Stephen Page

Advanced Veterinary Therapeutics, Australia

08.30 *Antimicrobial agent delivery to animals in the feed: responsible or irresponsible?*  
Dr. Stephen Page, Director, Advanced Veterinary Therapeutics, Australia

08.55 *Key management options in animal nutrition for food producing animals*  
Alexander Döring, Secretary General, European Feed Manufacturers' Federation, Belgium

09.20 *Are antibiotic residues a concern in distiller's co-products?*  
Prof.dr. Gerald D. Shurson, Department of Animal Science, University of Minnesota, USA

09.45 *Antibiotics in aquaculture: practice, needs and issues*  
Prof.dr. Peter R. Smith, Department of Microbiology, National University of Ireland, Galway, Ireland

#### 10.15 **Networking break & poster viewing**

10.45 *Manufacturing and use of medicated feeds: feed industry issues*  
Dr. Fabrice Putier, Director, Tecaliman, France

11.10 *Is there a future for medicated feed? The Dutch approach*  
Dr. Linda A.M. Stolker, RIKILT-Institute of Food Safety, Wageningen UR, the Netherlands

11.35 *Putting the vet in the Veterinary Feed Directive*  
Dr. Elizabeth Wagstrom, Chief Veterinarian, National Pork Producers Council, USA

12.00 *Antibiotic use in the UK poultry industry – past, present and future*  
Dr. Stephen Lister, Partner, Crowshall Veterinary Services, UK

12.30 - 13.30

#### **Lunch break & poster viewing**

**TUESDAY 15 NOVEMBER 2011**

**Parallel Session 3**

**The role of monitoring antibiotic use and resistance**

Chair: Prof.dr. Peter Silley

MB Consult Limited and Department of Biomedical Sciences, University of Bradford,  
UK

13.30 *DANMAP – integrated surveillance of antimicrobial consumption and resistance*

Dr. Yvonne Agersø, National Food Institute, Technical University of Denmark,  
Denmark

13.55 *Antibiotic use data: the long way from numbers to knowledge*

Nico Bondt, Market & Chains, LEI, Part of Wageningen UR, the Netherlands

14.20 *CLSI XR-08: Generation, presentation and application of antimicrobial susceptibility  
test data for bacteria of animal origin – a report*

Dr. Shabbir Simjee, Elanco Animal Health, UK

14.45 title to be confirmed

Prof.dr. J. Dewulf, Unit of Veterinary Epidemiology, Department of Reproduction,  
Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University,  
Belgium

15.15 **Networking break & poster viewing**

15.45 *Antimicrobial resistance in companion animals and horses*

Dr. Gina Pinchbeck, Department of Animal and Population Health, School of  
Veterinary Science, University of Liverpool, UK

16.10 *Challenges in harmonising resistance monitoring programmes in veterinary medicine*

Prof.dr. Peter Silley, MB Consult Limited and Department of Biomedical Sciences,  
University of Bradford, UK

16.30 Discussion / Q&A

17.00 - 18.00

**Poster viewing & drinks**

**TUESDAY 15 NOVEMBER 2011**

**Parallel Session 4**

**Future research, specific approaches**

Chair: Dr. Christine Hoang  
American Veterinary Medical Association, USA

- 13.30 *Why anti-inflammatory compounds are the solution!*  
Prof.dr. Theo A. Niewold, Nutrition and Health Unit, Faculty of Bioscience Engineering, K.U. Leuven, Belgium
- 13.55 *EU regulation of feed & feed additives – strategic opportunities for alternatives to antibiotics*  
Dr. Elinor McCartney, Director, Pen & Tec Consulting, Spain
- 14.20 *Antibiotic alternatives: vaccine, probiotic and functional metagenomic approaches*  
Dr. Heather Allen, Food Safety and Enteric Pathogens Research Unit, Agricultural Research Service, U.S. Department of Agriculture, USA
- 14.45 *Improving animal hygiene and housing conditions and measuring its effect on the use of antibiotics*  
Prof.dr. Thomas Blaha, Field Station for Epidemiology, University of Veterinary Medicine Hannover, Germany
- 15.15 **Networking break & poster viewing**
- 15.45 *Veterinary education and antibiotics*  
Dr. Virginia Fajt, Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, USA
- 16.10 *Innovation in regulatory agencies to keep pace with innovative technology*  
Dr. Thomas W. Campi, Elanco Animal Health, USA
- 16.35 *The role of future veterinary diagnostics in responsible use of antibiotics*  
Prof.dr. Enrico Bollo, Department of Animal Pathology, University of Turin, Italy

17.00 - 18.00

**Poster viewing & drinks**

## WEDNESDAY 16 NOVEMBER 2011

### Parallel Session 5 Evidence-based use of antibiotics

Chair: Dr. Annette Cleveland Nielsen  
Danish Veterinary and Food Administration, Ministry of Food, Agriculture and Fisheries, Denmark

08.30 *Antimicrobial prescribing in veterinary practice is evidence-based: true or false?*  
Dr. Ana Mateus, Centre for Emerging, Endemic and Exotic Diseases, Royal Veterinary College, University of London, UK

08.55 *Case study: Guidelines for evidence-based responsible antimicrobial treatment of pigs in Denmark*

- General outline and purpose: Dr. Annette Cleveland Nielsen
- Antimicrobial resistance data: Dr. Sven Erik Jorsal, National Veterinary Institute, Technical University of Denmark
- Clinical effects: Jens Christian Eskjær Jensen, Danish Association of the Veterinary Pharmaceutical Industry
- Pharmacokinetics: Prof.dr. Christian Friis, Department of Veterinary Disease Biology, Faculty of Life Science, University of Copenhagen
- Human health concerns – risk profiling: Dr. Yvonne Agersø, National Food Institute, Technical University of Denmark
- The swine industry's participation and use of the guidelines: Dr. Margit Andreasen, Danish Agriculture & Food Council

10.30 Discussion / Q&A

11.00 **Networking break**

11.30 - 12.30

### **Final Plenary Meeting Conclusions & path forward**

#### *Brief presentations and panel discussion*

During this three-day conference the present and future of the use antibiotics in animals have been discussed from different viewpoints. But has the path forward become clearer? What of the future? During the first part of this final plenary meeting the panelists will give brief summaries tying the keynote talks to the content throughout the conference to the 'take-home' messages. During the second part of the discussion questions from the participants will be answered.

#### Moderator

Prof.dr. Peter Silley

MB Consult Limited and University of Bradford, UK

#### Panel members

Dr. Annette Cleveland Nielsen

Danish Veterinary and Food Administration, Denmark

Dr. Christine Hoang

American Veterinary Medical Association, USA

Peter J.G. Oostenbach, M.Sc.

MSD Animal Health, the Netherlands

Dr. Stephen Page

Advanced Veterinary Therapeutics, Australia

Prof.dr. Yong Ho Park

Seoul National University, Korea

Dr. Thomas R. Shryock

Elanco Animal Health, USA

12.30 **Closing of the conference**

## WEDNESDAY 16 NOVEMBER 2011

### Parallel Session 6

#### Food chain perspectives on antibiotic use

Chair: Prof.dr. Yong Ho Park  
Seoul National University, Korea

08.30 *Responsible use – contribution of the animal health industry*

Dr. Olivier Espeisse, Chair of Antibiotics Working Group, International Federation for Animal Health (IFAH-Europe), Belgium; Director, Elanco Animal Health, Belgium

08.55 *The responsible use of antimicrobials on a global level: the view of the profession*

Dr. Tjeerd Jorna, Past-President, World Veterinary Association, the Netherlands

09.20 *Prudent use of antibiotics*

Dr. Coen H.M. Smits, Manager, Nutreco Ingredients Research Centre, Nutreco Research and Development, the Netherlands

09.45 *Demonstrable controlled prudent use of antibiotics in the food chain*

Prof.dr. Bert Urlings, Director Quality Assurance, VION Food Group, the Netherlands and Animal Nutrition Group, Wageningen University, the Netherlands

10.10 *The view of a retailer*

Dr. David Mainon, Asda Stores Ltd., UK

10.35 *Antibiotics and animals – communicating societal uncertainties to an unknown public*

Prof.dr. Lynn J. Frewer, Centre for Rural Economy, School of Agriculture, Food and rural Development, Newcastle University, UK

11.00 **Networking break**

11.30 - 12.30

### Final Plenary Meeting

#### Conclusions & path forward

##### *Brief presentations and panel discussion*

During this three-day conference the present and future of the use antibiotics in animals have been discussed from different viewpoints. But has the path forward become clearer? What of the future? During the first part of this final plenary meeting the panelists will give brief summaries tying the keynote talks to the content throughout the conference to the 'take-home' messages. During the second part of the discussion questions from the participants will be answered.

##### Moderator

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##### Panel members

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Danish Veterinary and Food Administration, Denmark

Dr. Christine Hoang

American Veterinary Medical Association, USA

Peter J.G. Oostenbach, M.Sc.

MSD Animal Health, the Netherlands

Dr. Stephen Page

Advanced Veterinary Therapeutics, Australia

Prof.dr. Yong Ho Park

Seoul National University, Korea

Dr. Thomas R. Shryock

Elanco Animal Health, USA

12.30 **Closing of the conference**



## LECTURES

### Setting the scene – a U.S. perspective

**Christine Hoang**

Scientific Activities Division, American Veterinary Medical Association, USA  
[choang@avma.org](mailto:choang@avma.org)

Antimicrobial resistance is a topic that often captures headlines around the world. Inevitably, the conversation tends to yield to a broader discussion of antimicrobial use and how best to preserve the availability and efficacy of these valuable drugs while still protecting human health, animal health, and our environment – the epitome of the ‘One Health concept’ in the responsible and judicious use of antibiotics.

For more than 40 years, antibiotics have been used in the United States to protect our food supply and improve animal health and welfare as well as improve production efficiency. Discussions on veterinary antibiotic use in the United States often revolve around varying interpretations of data and scientific evidence; differing perceptions and definitions; various proposals of how to proceed in the future; and how to implement change.

Antimicrobial use and antimicrobial resistance in animal agriculture is an extremely complex and controversial topic in itself. Yet, in the United States, the diversity of animal agriculture industries combined with the political, regulatory, and legislative structures presents unique challenges.

As the U.S. continues to build upon existing infrastructure, enhance existing programs, and launch new projects for responsible antimicrobial use, we seek collaboration with our global partners and hope to learn from the successes and challenges of others.

## Setting the scene – a European Union perspective

**Karolina Törneke**

Medical Products Agency, Sweden; Chair of Scientific Advisory Group for Antimicrobials,  
European Medicines Agency  
[karolina.torneke@mpa.se](mailto:karolina.torneke@mpa.se)

The European Union (EU) consists of 27 member states with huge differences in culture and conditions with regard to animal husbandry. This diversity implies that the EU perspective is broad and complex. Among the common features is regulation of marketing authorisations for veterinary medicinal products. Many antimicrobials<sup>1</sup>, especially newer products, are approved in several or all member states with the same indications and doses and all antimicrobials are prescription only medicines (apart from some topical products in some member states). No antimicrobials are allowed as feed additives for growth promotion purposes since 2006.

Marketing authorisation of veterinary medicinal products in EU is regulated in Directive 2001/82/EC as amended and there is a variety of (old and new) products approved, both single active components and fixed combinations with several antimicrobials. As there are both centralised and decentralised procedures for approval, the number of available products differs between countries. In addition to the original products there are nowadays a number of generics available. Besides use of approved veterinary medicinal products there is a legal option to use products off label by way of exception in particular to avoid unnecessary suffering, if no authorised product exists for the indication in that species, under the veterinarian's direct personal responsibility provided maximum residue limits (MRL-values) are set (for any species and tissue, this applies for food producing animals) for the compound.

Overarching strategies, recommendations and treatment guidelines on responsible use of antimicrobials are provided by numerous bodies, nationally and on EU level. These bodies could be governmental like the European Parliament (see Motion for a resolution on antimicrobial resistance, B7-0295/2011), the European Commission with its agencies EFSA (European Food Safety Authority; [www.efsa.europa.eu](http://www.efsa.europa.eu)) and EMA (European Medicines Agency; [www.ema.europa.eu](http://www.ema.europa.eu)), professional like Federation of Veterinarians of Europe (FVE; [www.fve.org](http://www.fve.org)) or multi-stakeholder like the European Platform for the Responsible Use of Medicines in Animals (EPRUMA; [www.epruma.eu](http://www.epruma.eu)) which has several collaborating partners representing veterinarians and farmers organisations and drug industry. Responsible use of veterinary antimicrobials in EU is not seen isolated but comprises international collaboration with bodies such as WHO, FAO, OIE and Codex Alimentarius and the Transatlantic Taskforce on Antimicrobial Resistance, bilaterally with USA. In addition, there is ongoing collaboration with human medicine involving for instance the European Centre for Disease Prevention and Control (ECDC) on EU level.

On a national level Denmark is one example of a country where development of detailed treatment guidelines has been a governmental initiative and several other countries have ongoing projects with the goal to reduce the overall use of antimicrobials to animals. Initiatives have been taken also by farmers' organisations, e.g., in Sweden where for instance growth promoters were voluntarily banned as early as in 1986. A more recent

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<sup>1</sup> Defined by OIE as a naturally occurring, semi-synthetic or synthetic substance that at *in vivo* concentrations exhibits antimicrobial activity (kill or inhibit the growth of micro-organisms). Anthelmintics and substances classed as disinfectants or antiseptics are excluded from this definition.

example is from Denmark and France where pig producers in 2010 have agreed to a temporary voluntary stop on the use of cephalosporins.

The policies and recommendations from different bodies in EU might differ in focus and comprehensiveness. At policy level the long term goal is to reduce the need for antimicrobials by improving management systems and introduce better biosecurity systems and programs for disease prevention with, e.g., all in - all out systems, hygienic measures, vaccines and probiotics. In shorter terms, discussions are ongoing on the need to reduce use, especially focussing on critically important antimicrobials such as fluoroquinolones and cephalosporins. Current recommendations from EMA/CVMP (Committee for Medicinal Products for Veterinary Use) state that such products should be used only in case of resistance to other antimicrobials and never for prevention of disease.

Most initiatives to date have been focused on foodborne antimicrobial resistance, which is estimated the most prominent risk to public health to consider. However, during recent years infections caused by MRSA (methicillin-resistant *Staphylococcus aureus*), and ESBL (extended spectrum betalactamases) carrying bacteria has increased in human medicine. Such bacteria, for which animals may be a reservoir and where spread to people could be via direct contact, constitute a new kind of hazards involving not only food producing animals but also companion animals. To date few recommendations on responsible use of antimicrobials in EU comprises companion animals but the need to focus also on non-foodborne risks has been expressed. Considering a 'One Health Concept', measures to contain antimicrobial resistance should comprise companion animals and also resistance to target animal pathogens.

With 27 member states, a diverse market and numerous recommendations and treatment guidelines available the big challenge for EU is compliance among veterinarians and farmers. Although it can be assumed that prescribers and animal owners are interested in issues related to antimicrobial resistance and would like to take their part in minimising its spread there are many counteracting factors. What factors that is and how important they are could only be speculated in, but for instance there might be economic incentives for using antimicrobials rather than reduce the density of animals or invest in management systems with better biosecurity. Cost of e.g. vaccines might be weighed against cost of antimicrobials. In addition compliance with treatment guidelines may be compromised by availability of attractive and affordable drug formulations that allow practical administration preferably combined with a short withdrawal period. There are systems in place in some countries to increase compliance with recommendations. Such systems include legal restrictions such as the Finnish legislation prohibiting off label use of certain substances and in Denmark and Sweden special legislation separate prescription and sales of antimicrobials to avoid economical incentives for prescription. Denmark has also introduced a 'yellow card system' (Government Order No. 1319 of December 1st 2010 on special provisions for the reduction of the consumption of antibiotics in pig holdings) where farmers sign a contract and allow inspections on their use of antimicrobials.

In order to be able to evaluate measures to ensure responsible use of antimicrobials, there is a need to monitor the pattern and extent of prescription and use. Such data has been collected nationally in some member states since a number of years but harmonised sales monitoring on EU level has been introduced only recently. In 2009 the project European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) started, hosted by the European Medicines Agency (EMA). Its first report (Trends in the sales of veterinary antimicrobial agents in nine European countries (years 2005-2009), available at [www.ema.europa.eu](http://www.ema.europa.eu)) historical data from countries with national monitoring systems is discussed with focus on harmonisation of reporting. As a start, ESVAC estimates and compares data based on sales figures collected by member states but for the future it would

be of great value to collect information also about prescription habits such as species and indication linked to a certain use. This is not possible on EU level today although there are national initiatives which might be expanded to several countries. For instance, Czech Republic has made a questionnaire to prescribers to explore the main drivers for prescription habits.

The ESVAC report concludes that there are obvious differences in pattern and extent of sales between the reporting countries but the data do not give any clear explanation for this difference. Similarly, the monitoring of commensal bacteria (see The Community summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks in the European Union in 2009, available at <http://www.efsa.europa.eu/en/efsajournal/doc/2154.pdf>) shows differences between countries. As ESVAC has recently started their activity no trends on EU level to be compared with resistance data are yet available. For the future such information would be highly valuable as a tool to measure impact on resistance levels of taken measures to ensure responsible use of antimicrobials. Today most reports presenting combined data for use and resistance are produced by national institutes. For instance the Danish integrated antimicrobial resistance monitoring and research program (Danmap, [www.danmap.org](http://www.danmap.org)) presents data on the impact of the ban of growth promoters.

So far the most effective initiatives to ensure responsible use of antimicrobials seem to have been made by or in collaboration with farmers' associations. To achieve a high level of compliance it is crucial that there is understanding and preferably economic incentives involved at farm level. Collaboration and common understanding between stakeholders (governmental, professional and food producers' organisations) is a prerequisite for a change in prescription and use patterns with prominent impact on future levels of antimicrobial resistance.

## Future antibiotics use in animals – evolution or revolution?

Peter Allen

Responsible Use of Medicines in Agriculture (RUMA) Alliance, UK  
[info@ruma.org.uk](mailto:info@ruma.org.uk)

The RUMA Alliance was formed in 1998 to focus co-operation between the many diverse elements of the 'farm to fork' process in promoting best practice in the use of antimicrobials in food animal production. It recognised that any legislative or behavioural changes in the light of concerns about antimicrobial resistance needed to be measured and gradual, hence the title of this paper. Balancing the protection of animal health, animal welfare, and food safety with antimicrobial resistance were the paramount considerations. On the basis of these criteria RUMA has produced and kept up to date responsible use guidelines for antimicrobials, as well as for antiparasitics, and anthelmintics, in the major farmed species, which also promote on-farm management practices which minimise the need for medicines. The thrust of RUMA has always been in the direction of policy not politics, and the discussion and communication of strategy options with National Authorities, consumers, and the media. Close links with the European Platform for RUMA (EPRUMA) give RUMA invaluable contact with relevant interests in the European Union. We believe that there would be benefit to be gained from the establishment of similar multi-disciplinary co-operation in other parts of Europe, and RUMA has presented its experience in a number of European Union (EU) member states in recent years.

Antimicrobial resistance affects us all, and we welcome Commissioner Dalli's comment in the French Parliament that antimicrobials are necessary for use in animals if the right balance can be found. The RUMA Alliance is all about identifying this balance, which requires that management of antimicrobial resistance must be firmly based on the available scientific evidence and not driven by dogma or political expediency. Like all animal medicines, antimicrobials should be used as little as possible but as much as necessary. Part of this imperative is to try to ensure that by tackling resistance we can retain the efficacy of available antimicrobials.

The RUMA Guidelines give primacy to on-farm practice which promotes health in all its aspects. They provide clear and practical guidance on use for both veterinarians and farmers, and are freely available on the RUMA website. It is difficult to gauge the impact of RUMA's guidelines, but comparatively low levels of MRSA in UK pig herds may be one positive indication. A proper 'cause and effect' study is on the long list of projects that RUMA has lined up.

What can we do? Responsible use of antimicrobials means that treatment of bacterial diseases should not always involve the newer generation of antimicrobials where older, more conventional, products are as effective. We should accept that, for example, use of fluoroquinolones for *Escherichia coli* mastitis and ceftiofur for foot rot in cattle do not represent responsible use. We must move away from the use of last resort/human critical drugs as front line treatments. This also applies to *in ovo*/day-old chick use in broilers of 3rd generation cephalosporins at the time of Marek's disease vaccination. Dairy cattle use, to avoid milk withholding, might be another example. These are valuable antimicrobials and their availability for use in animals needs to be retained. Regulation must be based on sound, scientific, risk assessment and not on inappropriate application of the precautionary principle. Use must be according to the label. The cascade should an exception. The laudable Commission initiative to provide funds for training and education on responsible

use of antimicrobials for professionals in the field of human medicines to those involved in veterinary sector should be extended.

In conclusion, we must all recognise the risks to both animals and humans from antimicrobial resistance, and work together to minimise them. Antimicrobials are essential for the treatment of farm animals to ensure the supply of safe food while maintaining animal health and welfare throughout the EU. The reflective papers of the Heads of Medicines Agencies (HMA) and European Medicines Agency (EMA) recommend that responsible use be a core part of antimicrobial resistance (AMR) control. One way to achieve this would be for all member states to introduce (if they haven't already done so) RUMA type guidelines tuned to the appropriate husbandry practices. The key to minimising resistance at grass roots level is not so much 'what' antimicrobials are used, but 'how and when' they are used. The answer to the question in the title has therefore to be 'evolution'. Any less than carefully considered and gradual process of change would be likely to have consequences damaging to the excellence we are trying to promote.

## Development of regulatory policies in Europe

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The common goal of stakeholders, including IFAH-Europe, with regard to antibiotics is to protect human health, animal health and welfare and to ensure their effectiveness now and in the future for relevant indications in all animal species. Antibiotics are developed according to regulatory requirements addressing the quality, safety and efficacy aspects of the products. In contrast to quality, the legislation regarding safety is faced with a continuous development of requirements focused on the safety of residues but more particularly on resistance development and transfer. Efficacy is recently reconsidered notably by including responsible use aspects in clinical trials.

Besides these regulatory requirements, the development of antibiotics is regularly faced with new or updated policies and position papers prepared by the different stakeholders: European Commission, Heads of Medicines Agencies (HMA), Committee for Medicinal Products for Veterinary Use (CVMP), European Food Safety Authority (EFSA), World Health Organization (WHO), etc., which influence the regulatory process. Despite the fact that regulatory bodies and other stakeholders acknowledge the need for innovation, Industry is faced with 'moving targets' particularly in relation to the legislation which leads to a lack of predictability. This greatly inhibits innovation and is leading to a situation where very few, if any, new antibiotics will be developed in the future. IFAH-Europe would therefore support clear and stable requirements for quality, safety and efficacy with decisions being science based.

With regard to the use of antibiotics the vast majority of veterinarians and farmers are reliable professionals using antibiotics responsibly and this needs to be shown.

# The evolution of statute and regulations governing the use of antibiotics in animals – how you can make a difference

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The legal debate surrounding the use of antibiotics in livestock in the United States spans decades, compounded by complex federal and, arguably, state authority governing its use. Public health concerns about antibiotic use in food animals begins with producer access to the use of approved drugs, available either by prescription, over the counter, or in medicated feed and how that use may create antibiotic resistance in human pathogens. Increasingly concerns are focusing on the potential environmental contamination from anti-bacterial resistant organisms in animal waste that end up in U.S. waterways or incorporated into fertilizer for use in crop production. This extraordinarily complex system is governed directly and indirectly through federal and state law, as well as the voluntary implementation of best management practices by livestock producers, veterinarians and the pharmaceutical and food production industries. Individuals and advocacy groups, from all vantage points, play an active role in the formation and evolution of laws governing the use of antibiotics in livestock. A clear understanding of the legislative and regulatory process provides all interested parties the opportunity to help influence the outcome.

The federal authority governing antibiotic use in food animals falls largely upon the U.S. Food and Drug Administration ('FDA'). This agency approves applications of new animal drugs for sale and regulates the manufacture and distribution of antibiotics used in animals. Antibiotics used in food-producing animals are either prescribed by veterinarians as labelled or extra-labelled drugs or added to animal feed in FDA-licensed feed mills. The FDA, responsible for 'protecting the public health by assuring the safety, effectiveness, and security of human and veterinary drugs, vaccines and other biological products, medical devices, our nation's food supply, cosmetics, dietary supplements, and products that give off radiation,'<sup>1</sup> enforces more than 45 statutes. One statute, the Federal Food, Drug and Cosmetic Act<sup>2</sup> ('FDCA'), provides authority for FDA's regulation of most foods<sup>3</sup> as well as feed, drugs, and devices used in pets, farm animals, and other animals.

New FDA laws evolve as a result of responses to health crises and political pressures. Congressional response to the concerns of consumers, industry, public interest and issue-specific advocacy groups, results in amendments to the FDCA which may not necessarily provide for scientifically sound policy. In addition to influencing congressional action, individuals and advocacy groups can provide input throughout the rulemaking process by commenting on published rule proposals or petitioning for rule-making.<sup>4</sup> Limited outside input may be permitted, without invitation, in response to FDA- issued guidance documents, informal statements or advice.

Congress empowered FDA with the authority to implement the approval of new animal drugs<sup>5</sup> and withdrawal of prior approvals pursuant to the FDCA.<sup>6</sup> The statute is administered

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<sup>1</sup> <http://www.fda.gov/AboutFDA/Transparency/Basics/ucm192695.htm>

<sup>2</sup> 21 U.S.C. § 301 *et seq.* (2011).

<sup>3</sup> U.S. Department of Agriculture ('USDA'), not FDA, has authority for most meat and poultry products.

<sup>4</sup> 21 CFR 10.30 (permits citizen petitions by an individual 'to request the Commissioner of Food and Drugs to issue, amend, or revoke a regulation or order or take or refrain from taking any other form of administrative action,' and requires an FDA response within 180 days.).

<sup>5</sup> 21 U.S.C. § 321(v) ('The term 'new animal drug' means any drug intended for use for animals other than man, including any drug intended for use in animal feed.').

by the FDA Commissioner with input from the Director of Center for Veterinary Medicine ('CVM'), using decisional law to supplement statutory interpretation. While the safety of drugs approved by FDA must be determined with regard to human health, that is not the only parameter the agency must consider.

Prior to approving a new animal drug application, FDA must determine that the drug is safe and effective for its intended use in the animal. Further, any residue that may remain in resulting food must be safe with regard to human health.<sup>7</sup> The definition of 'safe' has been the focus of serious debate. 'Safe' as used in the animal drug sections of the FDCA 'has reference to the health of man or animal.'<sup>8</sup> There are several 'safety clauses' in the statute which acknowledge the inherent risks of drug use, yet provide for their use under prescribed guidelines. The statute also references a number of considerations in addition to safety that must be considered.

Despite increasing pressure to ban the arguably 'unsafe' use of certain antibiotics in food animals, the FDA has predominantly upheld existing uses of antibiotics and medicated feeds. 'As of 2007, the U.S. FDA has withdrawn only one antibiotic, enrofloxacin, a fluoroquinolone used to cure fatal respiratory illnesses in chickens.'<sup>9</sup> FDA's continued failure to prohibit subtherapeutic antibiotic use, perceived by some to be unnecessary for animal health, has been criticized by some public health advocates. After the Obama administration identified a renewed effort to eliminate the use of subtherapeutic antibiotics in food animals, FDA took steps to begin the implementation of this policy. In 2010, FDA issued a draft guidance entitled 'The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals,' describing 'the Agency's current thinking on [the] topic,' which includes: '(1) limiting medically important antimicrobial drugs to uses in food-producing animals that are considered necessary for assuring animal health; and (2) limiting such drugs to uses in food-producing animals that include veterinary oversight or consultation.'<sup>10</sup>

In addition to FDA, many other federal agencies participate in the Interagency Task Force on Antimicrobial Resistance, 'created in 1999 to coordinate the activities of federal agencies in addressing antimicrobial resistance (AR) in recognition of the increasing importance of AR as a public health threat.'<sup>11</sup> However, despite a decade of surveillance of pathogen resistance data from human and animal populations, food processors and distributors,<sup>12</sup> CDC's efforts have failed to 'provide accurate national estimates and [have been unable]...to assess associations between [antibiotic] use and resistance.'<sup>13</sup> There is increasing concern about potential environmental contamination with antibiotic resistant bacteria from human and animal waste. The Environmental Protection Agency and US Geological Survey identify the presence of antibiotics in the environment, but regulation of waste to prevent real or perceived contamination has not yet ensued. In the future, the EPA may play an increasing role in controlling environmental exposure from animal waste through implementation of the Clean Water Act, the Safe Drinking Water Act, or a combination thereof.

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<sup>6</sup> 21 U.S.C. § 321b.

<sup>7</sup> 21 CFR 514.1(b)(8) (2002).

<sup>8</sup> Available at [http://ceres.ag.state.nj.us/exchange/aghhalp/Sent Items/April 5 doc.doc.EML/ - \\_ftn79#\\_ftn79](http://ceres.ag.state.nj.us/exchange/aghhalp/Sent%20Items/April%205%20doc.doc.EML/_ftn79#_ftn79); 21 U.S.C. § 321(u).

<sup>9</sup> Louis A. Cox, Jr. and Paolo F. Ricci, Causal Regulations vs. Political Will: Why Human Zoonotic Infections Increase Despite Precautionary Bans on Animal Antibiotics, 34 ENVTL. INT'L 459, 465 (2008).

<sup>10</sup> Available at <http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry>

<sup>11</sup> A Public Health Action Plan to Combat Antimicrobial Resistance available at <http://www.cdc.gov/drugresistance/actionplan/aractionplan.pdf>., 2011

<sup>12</sup> U.S. Gen. Accounting Office, Antibiotic Resistance: Federal Agencies Need to Better Focus Efforts to Address Risk to Humans From Antibiotic Use in Animals, Gao-04-490, at 18 (2004).

<sup>13</sup> U.S. Gen. Accounting Office, Antibiotic Resistance: Data Gaps Will Remain Despite HHS Taking Steps to Improve Monitoring, GAO-11-406, at 44 (2011).

Finally, each state retains authority over both the health and safety of its citizens and over the governance of the practice of veterinary medicine. Neither authority is necessarily circumvented by the aforementioned federal statutes. In fact, the Supreme Court rejected federal pre-emption based on the FDCA and upheld state authority in *Wyeth v. Levine*. There, FDA's labelling requirements did not pre-empt a personal injury lawsuit brought against a drug manufacturer in state court for failure to warn of the safety risk of drugs.<sup>14</sup>

Equally important, the veterinary profession is expected to have an increased role in the oversight of medicated animal feed used in food-producing animals. Veterinarians could face increased scrutiny by state and federal agencies that oversee the practice of veterinary medicine and antibiotic usage, respectively, particularly where the interests of patients and their owners do not coincide with the perceived interests of public health. Healthy animals make healthy food; for veterinarians to be effective in protecting our food supply, it is paramount they have the appropriate tools, including antibiotics, for preventing, mitigating and treating disease.<sup>15</sup>

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<sup>14</sup> *Wyeth v. Levine*, 555 U.S. 555 (2009).

<sup>15</sup> Concerning Advancements of Animal Health Within the Livestock Industry: Hearing Before the H. Subcomm. on Livestock, Dairy & Poultry, 110th Cong. 3 (2008) (testimony of Christine Hoang, Assistant Dir. American Veterinary Medical Association), available at <http://agriculture.house.gov/testimony/110/h80925/Hoang.doc>, at 3.

# Perspectives on the use of antibiotics in animals in Latin America

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In Latin America the livestock production is very important because it plays an important role in the production and exportation of products of animal origin. Thus, the availability of safe and effective veterinary products is mandatory. Veterinary medicines are vital to animal food production, with the majority of primary producers relying on chemicals to protect their animals from disease, pests, zootechnical production and also for animal welfare. Nowadays there are many veterinary medical products registered in this region.

As we know, veterinary drugs are active substances and their residues could appear in edible products of animal food as a consequence of a systemic distribution after its administration for preventive and therapeutic purposes. In addition, veterinary drugs are used in order to obtain certain zoo technical benefits as a growth promotion, enhancement of feed efficiency, synchronization of estrus, etc.

In most of the cases the active ingredients of veterinary drugs are xenobiotics. The safety evaluation of their residues in food is, in principle, identical with other xenobiotic intentionally added or unavoidably contaminating food items. National policies on the use of antimicrobials in animals must balance the possible benefits to livestock production against the medical risk and public health consequences deriving from their use.

The public concern about the abuse of the use of the veterinary drugs and the presence of drug residues in edible products of food produced by animals has rapidly grown in recent years. Consequently, in many countries the use of animal drugs has been regulated by legislation in order to avoid their residues in food. In the beginning, the basic rules of the safety evaluation of animal drugs residues cannot be considered as internationally satisfactory settled. On the contrary, proliferating regulations, with occasional disregard for science, result in further widening the gap among individual countries.

The competent authority responsible for the assessment, registration and regulation of the veterinary pharmaceuticals for each country depends on its own legislation. It could be the Ministry of Agriculture and Livestock, the Ministry of Public Health or also the Ministry of Environment. The evolution of the regulatory framework and the different regional approaches towards the harmonization of the requirements for the registration and control of veterinary products is very important for the Latin American performance of the safety of food and consumer protection.

Nowadays, countries in Latin-America are participating in all processes of international organizations such as Codex Alimentarius, OIE, etc. They are incorporating in their regulation all documents which provide guidelines on the respective responsibilities of authorities and groups involved in the registration, production, control, distribution and use of veterinary antimicrobials such as national competent authorities, veterinary pharmaceutical industry, veterinarians, and animal food producers.

According to the global concern about the antimicrobial resistance, most of our countries are trying to apply guidelines for the responsible and prudent use of antimicrobials agents in veterinary medicine. This responsible use has to consider the special productive characteristic of our region. The actual international requirements for the animal production

and livestock trade, and also the need for active registration and control systems for veterinary products becomes an unavoidable issue for the veterinary authorities.

Regarding the potential public health consequences of the transmission of resistant bacteria through the food chain, the objectives of the authorities of the countries in risk management at the animal production level are to assure the efficient production of safe and wholesome food of animal origin for human consumption and to reduce potential public health risks associated with farming practices at local and regional levels. This approach must take into account the following principles: good agricultural practices, good veterinary practices, good manufacturing practices, HACCP (Hazard Analysis and Critical Control Points) and guidelines on prudent antimicrobial use.

Official authorities generally consider, when they register, control and use antibiotics, the followings items: (i) protecting public health, assuring the safety of food of animal origin for human consumption; (ii) ensuring the ethical commitment and economic need to maintain healthy animals; (iii) maintain the efficacy of antimicrobial agents and ensure the rational use of antimicrobials in animals with the purpose of optimizing both their efficacy and safety in animals; (iv) prevent or reduce as much as possible the transfer of resistant bacteria or resistance determinants into the animal populations and from animals to man; (v) prevent contamination of animal derived food with antimicrobial residues exceeding the established maximum residue limits.

# Legislation and regulatory policies on the use of antibiotics in animals and fish in Africa

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Antimicrobial resistance is increasingly reported among bacteria that contribute to most of the human diseases on the African continent and other developing countries. These include the multidrug resistant pathogens such as *Mycobacterium tuberculosis*, typhoid *Salmonella*, diarrheagenic *Escherichia coli*, vancomycin-resistant *enterococci*, methicillin-resistant *Staphylococcus aureus*, invasive non-typhoidal *Salmonella*, penicillin-resistant *Streptococcus pneumoniae*, resistance to anti-malarial and to anti-HIV drugs. The main reason advanced for the observed antimicrobial resistance is misuse of antimicrobials in the treatment of human infections and their use in animal and fish production. This problem has been compounded by the regulatory framework in African countries. The challenge in African countries as elsewhere in the developing world has been weak and/or inadequate implementation of policies and guidelines where they exist.

Although policies and guidelines on antimicrobial use in humans exist in many countries in Africa, this may not true for antibiotic use in animal and fish production on the continent. There are international guidelines provided by the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO), however, adoption and use by governments in Africa is, by and large, not evident.

This presentation reviews the regulatory framework and policies on the use of antibiotics in animal production in Africa and highlights the challenges faced in the implementation of the policies where they exist. Measures to strengthen the use of antibiotics in animal and fish production are proposed.

# Antimicrobial use and resistance in animals in Asia

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Pathogens resistant to various antimicrobial agents have emerged as a major concern of human and veterinary medicine. The emergence of antimicrobial resistant bacteria may be attributed to overuse and misuse of antimicrobial agents in humans and animals. Selection of antimicrobial resistant zoonotic bacteria in animals can lead to transmission of these bacteria to people by the consumption of contaminated animal products or by direct animal contact. Here, we summarize the current status on the antimicrobial usage and resistance trends, as well as zoonotic resistant bacteria in animals and animal products in Korea.

Trends in antimicrobial consumption and resistance. In Korea, antimicrobial consumption and resistance in animals have been much higher than those in other countries. Because of the growing concern of the impact of antimicrobials used in animals on human health and food safety, feed additive antimicrobials had been banned since 1995 and only the ionophores were allowed as from July 2011. We assessed the impacts of the phase out of feed additives on usage of antimicrobials and resistance in bacteria of animal origin. The overall consumption of antimicrobials in food producing animals and fisheries decreased by 33%, from 1,553 tons in 2005 to 1,047 tons in 2010. This decreased consumption covers a 66% decrease in feed additives while in antimicrobial consumption prescribed by farmers themselves, the consumption remain unchanged. Of the total consumption, antimicrobial consumption in pigs accounted for 54-57%, followed by chickens (18-24%) and cattle (6-8%). Consumption of antimicrobial agents that are used for feed additives namely, tetracycline, penicillin, and sulfonamides has gradually decreased, while therapeutic use of some antimicrobials such as macrolides and phenicols has increased. The trends of antimicrobial resistance among indicator *Escherichia coli* isolated from healthy animals, on the whole, showed much higher prevalence of resistance in isolates from pigs and chickens than that from cattle. The most frequently observed resistance in isolates from animal samples was to tetracycline, followed by resistance to streptomycin and ampicillin. This may be the reflection of the more abusive use of these antimicrobials for treatment in pigs or chickens. However, resistance trends of tetracycline and streptomycin decreased with the decrease in consumption of these antimicrobials in pigs and chickens. Furthermore, the resistance against critically important antimicrobials such as third-generation cephalosporin and fluoroquinolones was increased.

Methicillin-resistant *Staphylococcus aureus* (MRSA). Recent reports have documented MRSA infections in animals and it is now considered as one of the most important zoonotic pathogens. In Korea, MRSA has been isolated from various non-human sources such as animals, raw meat, and bovine milk. In a recent study, MRSA were isolated from various raw meat samples including beef (1.0%), pork (0.3%), and chicken meat (0.3%). All the MRSA isolates from beef and pork were Pantone-Valentine leukocidin-negative, SCC<sub>mec</sub> type IVa strain with sequence type 72. In mastitic milk, about 4.2% (17/402) *Staphylococcus aureus* were methicillin resistant. Genotyping of these 17 MRSA isolated from each cow, revealed two types of MRSA, SCC<sub>mec</sub> IVa-t324-ST72 (n=11) and SCC<sub>mec</sub> IV-untypable-ST72 (n=3). Among the pig isolates, prevalences of MRSA were 3.2% (21/657) and 22.7% (15/66) in pigs and farms, respectively. Two different types were found among these 21 MRSA isolates: 17 strains of livestock-associated type (LA; ST398 or ST541/*spa* t034) and 4 strains of human-associated type (HA; ST72/*spa* t664 or t2461). Our data provide evidence for the existence

of not only LA types (ST398 and ST541) but also HA type (ST72) MRSA in animals and animal products in Korea. This was the first report on LA-MRSA ST398 in commercial pigs in Asian countries. The MRSA clones reported in pigs in the Asian countries differed from those of the EU countries and North America. The MRSA clonal complex (CC) 9 was predominantly isolated from swine in China and Malaysia while ST221 type MRSA was reported in Japan. The presence of human MRSA clones in animal and animal products observed in this study suggests an additional reservoir for human MRSA infection, and vice versa.

CTX-M producing *Enterobacteriaceae*. Extended-spectrum  $\beta$ -lactamase (ESBL)-mediated resistance is of considerable importance in both human and veterinary medicine. During the past couple of decades CTX-M type ESBLs or cefotaximases have been increasingly reported in many countries of the world. In a study done in Korea, a total of 408 *E. coli* were isolated from sick farm animals and pets during 2003–2006. Of these, four strains showed resistance to third-generation cephalosporins. The *bla*<sub>CTX-M-14</sub> gene was encountered in three *E. coli* strains, each of which were isolated from two cows and a dog, respectively, and *bla*<sub>CTX-M-15</sub> was identified in an *E. coli* isolated from a pig. Among the *Salmonella* isolates from humans and animals, twenty of 1279 non-typhoid *Salmonella* isolated from food animals and humans produced CTX-M-type extended-spectrum  $\beta$ -lactamase. All expressed CTX-M-15 except two which co-expressed CTX-M-14 and TEM-1. The *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-14</sub> genes were disseminated by a large conjugative IncFII and IncI1-ly plasmid, respectively. These results suggest that a combination of clonal and horizontal transmission is spreading *bla*<sub>CTX-M</sub> genes among NTS strains in Korea. In Asian countries including Korea, *bla*<sub>CTX-M-14</sub> and *bla*<sub>CTX-M-15</sub> are prevalent in animals and animal products.

Overall, consumption of antimicrobials in animals gradually decreased following the discontinuation of feed additives. However, we should pay attention to the negative effects of banning of feed additives such as increase of therapeutic antimicrobials, animal diseases, cost of production etc. Although the prevalence of zoonotic resistant bacteria in animals or food products is lower than that in human, resistant genotypes similar to or identical with those of the human isolates were also found in non-human sources in Korea. Where resistance is present among zoonotic bacteria, there is a possibility of transmission of those bacteria between humans and animals. To reduce and prevent the spread of resistant bacteria, responsible and prudent antimicrobial use along with integrated monitoring of antimicrobial resistance in both humans and animals is needed.

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## Regulation of veterinary antibiotics in Australia

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Antimicrobials are essential medical tools used to treat bacterial infections in animals and humans. The number of effective antibiotics available for therapeutic purposes is limited, and few new ones are being developed. Indiscriminate use of antibiotics in animals and humans could result in the selection of resistant bacteria. These can be transferred from animals to humans directly and through human contact with edible commodities from treated animals. Transfer may also occur from humans to animals. Once bacterial pathogens become resistant, the consequence is the loss of valuable effective antibiotics available for successful treatments in the future. For these reasons, the use of antibiotics needs continued careful management.

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is responsible for the regulation of veterinary medicines, including antimicrobial products, in Australia up to the point of retail sale. The APVMA rigorously assesses antimicrobials proposed for sale in Australia and determines how they can be used prudently.

In making regulatory decisions, the APVMA is guided by a raft of national and international requirements and protocols. Amongst other things, the APVMA must consider the probability of development of resistance to antibiotic products, the potential implications for animals requiring treatment, the possibility of infection in susceptible people with antibiotic resistant pathogens arising from the proposed use of antibiotics in animals, and the consequences for treatment of human disease.

Australia has over the years adopted a highly conservative approach towards the regulation of antimicrobial products. This approach has led to restricted use of cephalosporins and a prohibition on the use of fluoroquinolones in food-producing animals.

Australia's rigorous approach to controlling the amounts and types of antibiotics used in food animal industries has led to lower levels of resistance than are found in many other countries. Recent surveys, for example, have not only demonstrated a very low frequency of antibiotics detected in food but have found that resistance to critically important human antibiotics is non-existent or very low. Scientific opinion, however, indicates that this status is fragile and maintaining it will require ongoing vigilance, surveillance and commitment.

## Focus on veterinary medicine: uncertainties and demands

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The use of antibiotics in veterinary medicine is challenged in the light of a global emergence of antimicrobial resistance. Criticism focuses on the use of antibiotics in farm animals, where despite the long-standing mandate for prudent use, the overall consumption of antibiotics has not declined in the last decade. An analysis of the factors that determine a high use identifies an association with modern animal husbandry practices, changes in feed composition and the availability of new feed components, as well as the steep increase in the costs for feed materials at a global level. Monitoring of these important changes in animal farming should be used to identify genetic susceptibilities and altered nutritional demands for optimal productivity and health of livestock.

The remaining unavoidable therapeutic use of antibiotics requires insight in infection biology and epidemiology. While traditionally the selection of an antibiotic in a clinical situation was based merely on susceptibility testing, conducted *ex vivo*, recent refined selection criteria include the time-course of an infection, the inoculum size, population variability in drug kinetics, and the risk for the emerge of chronic infections. Moreover, onset and duration of treatment need to consider any added value of a concomitant application of anti-inflammatory agents and disease modifiers. The use of standard formularies guiding the selection of the optimal antibiotics is widely advocated but should address the entire therapeutic approach. Together with the registration of animal-daily-doses and days-in-treatment, the outcome of intervention strategies can be monitored and used for epidemiological studies.

Traditionally, preventive medicine was translated simply as the need for refined vaccination protocols and preventive use of antibiotics. The increasing availability of feed additives and feed components that modulate and improve the immune competence of animals requires a change in mind-set and a critical appraisal of the tools applied in animal health care.

## **US perspectives on veterinarian oversight of antibiotics**

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Antibiotics used in food and companion animals in the United States are regulated by the Food and Drug Administration of the Department of Health and Human Services. The Department is located in the Executive Branch of the government reporting to the President. While the Department enforces the federal or national laws of the United States, the US Congress establishes the laws upon which the Executive branch implements. In this regard Congress more than 40 years ago determined how animal drug including antibiotics were to be regulated. Laws were enacted to align the review and approval process with that used for human medicines with some exceptions noting the differences in veterinary and human medicine, the rural nature of food animal production and the availability of trained veterinary personnel to manage the geographical concentration of livestock and poultry. Over the years there have been a number of amendments to the law affecting how antimicrobial are regulated and most notably newer provisions for applying veterinary control to antimicrobials used in feed.

The presentation will review the history of animal drug and antibiotic regulation in the United States with regard to the availability of medicines with and without veterinary prescribing and the changes that are being contemplated by the FDA to restrict the feeding of certain 'critically important' antibiotics on the order of a licensed veterinarian only.

# Consequences of generic marketing on antibiotic consumption and the spread of resistance: facts and hypotheses

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The sustainable use of antibiotics in animals involves not only good end-user practices (prescribing veterinarians, farmers) but also regulatory bodies in charge of developing public policy. Currently the generic drug manufacturers only had to establish the bioequivalence of the active ingredients of the original drug to obtain the approval, thus obviating the need to conduct clinical trials. The impact of this policy was manifold with both desirable and undesirable effects. In human medicine, generic drugs are generally much cheaper than their branded counterparts, which is good news for the consumers and healthcare services because generic substitution cuts health-care costs. The bad news for antibiotic resistance is that competition between generics and also between generics and branded antibiotics (usually forced to lower their prices in order to remain competitive against the cheaper generic versions), leads to more aggressive promotion for the use of antibiotics both in human and veterinary medicine.

Flooding the market with different generics and/or 'me-too' branded drugs is not without consequences on overall antibiotic consumption, a major driving factor contributing to the acceleration of the emergence and spread of resistance (Finch 2010). In addition this competition can favour the promotion of poor veterinary practices to gain some market share.

In human medicine, generic antibiotics account for about 80% of prescriptions (Finch, 2010) and in an EU survey it was shown that in human medicine, there is a relationship between antibiotic consumption and the number of trade names of oral antibacterial agents (Monnet *et al.*, 2005). More recently the consequences of the introduction of generic versions of ciprofloxacin on the Danish market were investigated over a period of 10 years from 1995 to 2005 (Jensen *et al.*, 2010). It was shown that the number of marketed versions of ciprofloxacin increased from 3 to 10 and that in parallel the median price per define daily dose was halved. More importantly, it was shown the total consumption of oral ciprofloxacin was increased by a factor of 3. During the same period, the frequency of ciprofloxacin resistance increased by 200%.

In veterinary medicine, we now have the first evidence that the introduction of generics on the market has influenced antibiotic consumption. In France (Chauvin, 2009) investigated the impact of generic introduction on antimicrobial use in poultry production. Exposure data from about 7,000 chicken and 5,500 turkey flocks were analyzed to check whether the introduction of generics had led to an increase in exposure. The data showed a marked increase in fluoroquinolone use: up by mean values of 30% in turkey production and 50% in broiler chicken production. It was concluded that generic introduction may influence the patterns of antimicrobial use in animal production.

The relationship between the use of fluoroquinolones and the extent of antibiotic resistance in veterinary medicine has been reported by Hellmann (2005). The level of resistance was compared between countries with cheap generics available (10% of reference) leading to a high use of fluoroquinolones in poultry and pigs, against countries (Germany, UK, Denmark) with high price products, and well controlled prescribing since the launch of quinolone. It was

shown that the level of resistance was fundamentally higher for *Salmonella* and *Escherichia coli* in chickens in Spain vs. Denmark

Another possible concern with antibiotic generics is the fact that some generic and brand-name drugs for intravenous administration were shown to be very different in terms of efficacy despite apparent pharmaceutical equivalence. This is currently a matter of discussion due to a series of articles from the same Colombian group (O. Vesga ) showing that pharmaceutical equivalence for several antibiotics including vancomycin (Vesga *et al.*, 2010), oxacillin (Rodriguez *et al.*, 2010) and gentamicin (Zualaga *et al.*, 2010) does not imply *in vivo* therapeutic equivalence when using the neutropenic mouse thigh infection model. However, other authors are challenging these views saying that these generics do not fulfil the quality standard for that pharmaceutical product (e.g., purity of content) (Silva *et al.*, 2010; Diaz *et al.*, 2011)

Another issue with the marketing of multiple generics is the fact that two generics that have actually demonstrated their bioequivalence to the pioneer formulation can be themselves not be bioequivalent and currently there is no information on the substitutability of generics between them.

Another possible undesirable consequence of the promotion of generics is the encouragement to use old rather than more recent antibiotics. Traditionally, from a public health perspective, veterinarians are encouraged not to employ newer drugs, but rather to use the older antibiotics. The recommendation as to whether to choose older rather than newer antibiotics was challenged in human medicine on an epidemiological basis (Amyes *et al.*, 2007) and appeared to be flawed for quinolones, cephalosporins and carbapenems. For these three antibiotic classes, it was observed that the less active drugs could be even worse at hastening the spread of resistance more than active drugs in the same class. No equivalent data exist in veterinary medicine but the question deserves at least some attention. It is not certain that old antibiotics, with very poor oral bioavailability such as tetracyclins in pigs, marketed with historical dosage regimens, guarantee a more prudent use of antibiotics than the recourse to more innovative antibiotics properly developed in terms of formulation (to improve compliance) and using the current state-of-the-art dosage regimens optimized by taking into account the antibiotics' PK/PD.

More difficult to document is the influence of generic promotion on some malpractices such as the unnecessary prescription of antibiotics for undocumented prophylactic reasons, or worse, on practices such as *in ovo* or day-of-hatch subcutaneous antibiotic administration or the systematic use of antibiotics in piglets when clipping teeth because the use of blunted clippers crush teeth and lead more often to articular infection.

For a drug company, generics are disproportionately cheap to market in comparison to innovative compounds. On a short-term basis and to render shareholders happy, market generics and other off-patent medicines is very appealing, especially in emerging markets. This was recently acknowledged by the world's biggest drug company to explain why it might sell or spin off its animal health unit, the current leader of the veterinary market. On a long-term basis, it is more likely that research and development of innovative products is the ultimate source of the economical value that the pharmaceutical industry creates. For antibiotics, no truly innovative product has been marketed for decades in veterinary medicine. This is unfortunate because antibiotics specifically designed for food-producing animals could be one of the best options to minimize the emergence of antibiotic resistance associated with animal treatments. The main drawback of veterinary antibiotics in terms of public health is their lack of pharmacokinetic selectivity. Most veterinary antibiotics gain access to the gut, impacting negatively on the commensal flora, whatever the targeted systemic biophase (lung, udder etc) and whatever the route of administration (oral or others).

However, there are several options to render veterinary antibiotics more selective or/and to neutralize their adverse effect on gut flora and also on the environmental flora.

Currently, rather than to be engaged in a long-term and risky R & D programs, many veterinary drug companies prefer to copy each other and to expand their market share by influencing veterinary prescriptions. This is done thanks to available large financial incentives to promote the sale of generics. In several EU countries, most prescription drug products are directly sold by veterinarians to the end-users. In the case of Denmark, Aarestrup *et al.* (2010) concluded that the reduction in antimicrobial consumption observed from 1994 to 1995, was due to the limitation of sales incentives paid to veterinarians imposed by Danish authorities and was decisive in the reduction of antibiotic consumption in that country. In France the annual refunds given by generic suppliers when the negotiated annual drug turnover is achieved, can reach up to 80% of the sale price for antibiotics. This potential conflict of interest between prescriptions and drug sales may impede the stewardship role of veterinarians with respect to public health.

The business model for human and veterinary drugs is fundamentally different with respect to both the final payer and ethical issues. For veterinary medicine, it is neither a patient at risk nor a public health system that pays for drugs but a farmer who will pass the cost on to the final consumer by adjusting the price of the marketed product. There is no crucial ethical motivation to promote generics for veterinary medicine. For veterinary medicine, the key issue for antibiotic use is public health and preservation of a rare resource by limiting the speed of the development of antibiotic resistance that is increased with the magnitude of antibiotic use. In this respect, generic promotion in veterinary medicine is not consistent with this general objective and it is the opinion of the author that veterinary antibiotics should be expensive with a strictly regulated market, rather than cheap and freely available drugs.

It is not the intention of this communication to challenge the very principle of generics but rather to draw attention to the fact that to slavishly adopt all human regulations in veterinary medicine may be counterproductive for public health. Regulatory agencies play a critical role in the licensing of new antimicrobial agents and it is difficult to understand why the same authorities recommend the prudent use of antibiotics for veterinary antibiotics, while in the mean time, they encourage the marketing authorization of antibiotics by decreasing some regulatory hurdles for generics. This is the case for the EMA when in their new guidelines on bioequivalence, they consider that the demonstration of bioequivalence for a new generic is enough to substantiate an extrapolation of a withdrawal period between two formulations, i.e., that no residue data are required to confirm the withdrawal period of a generic if there is no local residue. Such a decision is neither scientifically correct nor acceptable from a public health perspective.

## **Veterinary medicine use according to data sheet – exception rather than the norm?**

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The role of the veterinary profession in livestock production is changing rapidly, with the role of the farm vet developing from the reactive livestock practitioner to a proactive animal health consultant. Even as consultants, livestock veterinary surgeons rely on many tools in this developing role promoting the health and welfare in food producing animals. These tools include: (i) production data and the ability to identify health planning priorities; (ii) surveillance data with the analysis of animal health risks; (iii) management programmes to optimise health and production; and (iv) modern animal medicines used according to best practice to promote positive health.

Medicines are a key tool and these medicines must be safe, efficacious and avoid contamination of the food chain. The role of the modern farm vet is to prescribe these medicines and instruct on their administration in such a way that both animal health and public health are maintained. When considering antimicrobial use, the livestock practitioner is seeking to achieve a clinical cure in a timely manner with the minimum impact on public health, either through the contamination of the food chain by medicine residues or by avoiding the emergence of antimicrobial resistance by pathogenic and commensal organisms. The gold standard is to achieve a bacteriological cure with minimal host toxicity and the earliest opportunity of meat and milk re-entering the food chain.

The prescribing vet must understand the pharmacodynamic principles of the antimicrobial they are prescribing, including the effect of the host status, pathogen type and medicine properties. The clinician must understand the health status of the patient, including immune status, and identify the target pathogen including any known resistance issues. They must understand the antimicrobial method of action, e.g., bacteriostatic, bacteriocidal, mode of killing, e.g., time or concentration dependent killing, and the spectrum of activity, i.e., is the organism known, suspected or historically susceptible. Furthermore the prescribing vet must consider the pharmacokinetics of the antimicrobial to be used, including the route of administration, the volume to be administered, medicine distribution in the target species, half life and clearance rates, distribution and elimination characteristics, including any barriers to penetration and the impact of disease on the action of the medicine.

As if these challenges were not enough, the prescribing vet must further more understand the products and preparation(s) available that may be suitable to prescribe. This includes an in depth knowledge into the data sheet for the product and particularly any limitations therein, specifically, limitations in target species, dose rates, routes of administration, frequency of administration, length of course of treatment and the withdrawal times for re-entering the food chain. The prescribing vet must also understand the will of the regulators of the industry and specifically any additional administrative requirements. These may include the use of the product limited to veterinary administration only, the use of the product as a second line treatment only and any additional concerns for public health such as the transfer of antimicrobial resistance.

The prescribing vet will also take notice of any available research relating to the product or the condition being treated. The requirements for the application of 'best practice' can change regularly as the research into and understanding of disease principles changes.

These changes can occur regularly, as new and novel information on a product, disease or control programme become available. Lastly and probably most importantly, the prescribing veterinary surgeon must address the concerns of the livestock keeper or farmer. The keeper is almost certainly the customer and in many situations the person responsible for administering the product. Addressing compliance in this matter is crucial to the success of treatment. Furthermore in production animal systems, there is the ever present juxtaposition of ensuring a response to therapy with the cost of treatment and the immediate concern of returning the animal to the food chain.

Understanding the scope and impact of all these considerations, the prescribing vet must make difficult decisions in the prescribing process. Knowledge of the host species, disease process, target pathogen are crucial as are the pharmacokinetic and dynamic properties of the medicine. The producer may often wish the animal to return to the food chain at the earliest opportunity.

The product datasheet is a crucial part of ensuring the medicine is prescribed in a manner it was intended. However following datasheet guidelines may not be acceptable as: (i) there may not be a product licensed for the species concerned, any other food producing species or the required route of administration may not be appropriate; (ii) research may indicate that an alternative treatment protocol e.g. dose or route of administration, is optimal; and (iii) the administration of products or combinations of products not strictly according to datasheet may lead to extensions in withdrawal periods. To help overcome these challenges the practicing vet applies the cascade. The cascade provides the guiding structure for medicines in veterinary practice and how the prescribers choices should be influenced. However simple the cascade principle appears, its application in clinical practice is both poorly understood and often does not allow the application of 'best practice' treatments. The pragmatic result of this is the prescription and administration of medicines, including antimicrobials, outside of the datasheet. The frequency of this deviation is poorly understood but in many circumstances constitutes the 'normal' approach to the administration of the product rather than an exceptional use. The strict application of the cascade is poorly understood cross the industry and this will lead to an escalating impact on animal and public health.

If we are to ensure the requirements of the patient, the needs of the client and the objectives of the wider society are met, prescribing vets urgently require a more thorough understanding of the cascade and the principles for keeping the animal and public health safe. To reduce the reliance on the cascade the pharmaceutical companies must, as a priority, be allowed to continually update the datasheets incorporating the most up to date best practice protocols and treatment programmes. This will help practitioners in their decision making on farm and help safeguard the use of medicines and antimicrobials in food producing animals for the future.

# Demonstration of efficacy for antimicrobials now and in the future

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According to European legislation no veterinary medicinal product must be placed on the market unless the efficacy and safety for animal and human health and for the environment are proved according to the provisions of Council Directive 2001/82/EC as amended. These legal requirements are supplemented by a set of scientific and regulatory guidelines. For veterinary antimicrobials, a specific scientific guideline outlining the data requirements for the demonstration of efficacy has been in force since 2003 (Guideline for the demonstration of efficacy of veterinary medicinal products containing antimicrobial substances, EMEA/CVMP/627/01-Final). However, since its development, the scientific knowledge and the political environment have changed and a revision is overdue.

Antimicrobial veterinary medicinal products are essential for the treatment and prevention of bacterial diseases in animals to protect animal and public health and animal welfare. At present, no sufficient therapeutic alternatives do exist in veterinary medicine. However, antimicrobial treatment of animals selects for resistant bacteria, leading to treatment failures of bacterial infections. This may also compromise human health by transfer of resistant bacteria from animals to humans through contact or the food chain. National resistance monitoring programmes in target and food-borne pathogens indicate that resistance rates for many pathogens appear to be relatively stable. However, for certain animal pathogens, e.g. *Escherichia coli* infections in calves, piglets and poultry, high resistance rates of 50% or more against conventional antimicrobials such as ampicillin, tetracycline and sulphonamide/trimethoprim (Germvet, resistance monitoring 2008) have been found. In addition, there is evidence of an increase in resistance against newer antimicrobials such as fluoroquinolones and cephalosporins. The presence and frequency of multiresistant bacteria, ESBL (extended spectrum beta-lactamases) producing bacteria and MRSA (methicillin-resistant *Staphylococcus aureus*), do certainly not only raise concern in veterinary medicine but also in human medicine due to potential transfer. Treatment options in man against diseases associated with these often multiresistant pathogens are limited as the same classes of antimicrobials as in veterinary medicine are used.

Hence, third and fourth generation cephalosporins and (fluoro)quinolones are the antimicrobial classes which are in the focus of antimicrobial policy, and it is common agreement that substances of these classes should not be used for the routine treatment of animals.

Due to this, the principle goal in Europe is promoting prudent use of antimicrobials in animals to limit the development of resistance, and to minimise the antimicrobial consumption in animals. This goal is addressed in the EMA/CVMP five-year strategy on antimicrobials. In line with CVMP's updated strategy on antimicrobials 2011-2015 (EMA/CVMP/287420/2010) the Efficacy Working Party of this committee published a concept paper for consultation in April 2011 (EMA/CVMP/EWP/760764/2010) which emphasizes the need to update the current guideline on efficacy.

According to this concept paper, the main focus of the revision will be on the update of the existing guideline regarding current scientific knowledge on areas relating to the efficacy of antimicrobials, prudent use and development of resistance.

In the following, some of the problem statements outlined in the concept paper are dealt with.

As part of the risk management strategies of CVMP, advice on prudent use is routinely included in the product literature for third and fourth generation cephalosporins and (fluoro)quinolones, applied for marketing authorization. However, it appears no longer acceptable to recommend the use as 'second-line', i.e., 'reserved for the treatment of clinical conditions which have responded poorly, or are expected to respond poorly, to more narrow spectrum antimicrobials', while the demonstration of efficacy is based on pivotal clinical field studies demonstrating non-inferiority with a 'first-line' antimicrobial. Hence, a major topic for revision of the guideline will be the development of more guidance for 'second-line' antimicrobials intended for use in animals. In this context clear guidance will be needed on which or based on which criteria substances or product types must be considered as 'second-line' and how this should be reflected in the indication or other sections of the product literature. In addition, it is intended to provide guidance how prudent use of antimicrobials could better be reflected in the design of efficacy studies in support of these claims and to exclude clinical conditions which could be treated with a more conventional antimicrobial.

The systemic preventive use of antimicrobials in intensively reared animals (e.g., poultry, swine) is another area of concern, because apparently there is still no common understanding of what is considered preventive use. Many antimicrobials are authorized for the 'treatment and prevention' of a given bacterial disease, and both terms should always be read in combination. According to good veterinary practice it may be necessary in the case of an outbreak of a bacterial disease in a herd or flock not only to treat clinically diseased animals but also other (in contact) animals of the same group or flock at the same time to prevent them from developing clinical signs and to prevent further spread of the disease. Experience of clinical assessors shows that clinical efficacy studies on preventive use are not always conclusive as, e.g., the conditions of an outbreak of a disease are not properly defined and/ or the results cannot properly be interpreted due to inappropriate control methods. Therefore, more guidance is considered helpful with regard to the design of studies to support such a claim. In particular, further clarification is deemed necessary under which conditions such preventive treatment would be acceptable, taking into account resistance containment, field practices and differences between infection types. However, there may be also some discussion in relation to the question whether additional field studies to demonstrate a preventative claim as required by competent authorities would indeed be necessary for an antimicrobial for which the therapeutic efficacy has already adequately been demonstrated.

There is a need for more detailed guidance for the selection of appropriate control methods in laboratory and field studies. Often, clinical studies are designed as non-inferiority studies, using a reference product authorized for the same indication for comparison. Such non-inferiority data are difficult to interpret in terms of internal validity, and when a substantial cure rate is expected during the course of the study. Next to a positive control, an untreated or placebo group may be necessary to confirm the efficacy and clinical relevance of the treatment in such situations. In addition, negative control groups are considered necessary to confirm the risk of infection in studies attempting to confirm a preventative claim.

According to the rules the efficacy of an antimicrobial must be demonstrated for each claimed indication, i.e., clinical disease condition and associated bacterial pathogens. However, experience shows that clinical field studies sometimes lack of precise clinical and bacteriological diagnoses and the disease status in a group or herd is not always evident from the data provided. For example, clinical signs of the disease are only mild, the number of pathogens isolated from diseased animals before treatment initiation is low, sampling

methods may be inappropriate (e.g., nasal swabs), or too few animals in relation to the study population have been sacrificed for post mortem examination to confirm the diagnosis. Therefore, more guidance is considered useful on how etiological diagnosis can be confirmed appropriately and how the disease status in a herd should be established.

The conditions of post-treatment bacteriological examination and susceptibility testing as recommended in the current guideline appear not sufficiently clear, e.g., to be performed in laboratory and/or field studies, in what proportion of animals, sampling methods and how to interpret the resulting re-isolation rate or the possible decrease in susceptibility. More guidance should be provided on this including exploring the possibility of new diagnostic methods including molecular techniques.

For any new antimicrobial to be placed on the market the antimicrobial spectrum of activity needs to be defined. For this purpose, usually data of MIC survey of target pathogens obtained from diseased animals in EU member states is provided. It is considered useful to provide more precise guidance concerning a representative number of epidemiologically unrelated strains per pathogen, the number of different geographical regions and livestock product types that need to be considered and the criteria to select these. As to the potential selection for resistance, more guidance is intended on the interpretation criteria used to determine the level of resistance. In the case PK/PD data or clinical breakpoints are referred to these should be defined based on data.

PK-PD data is a valuable tool to support the dosing regimen for antimicrobial products for systemic use, and, if properly established prospectively, help to reduce *in vivo* experiments in target animals. Sometimes, the provided data is inconclusive or difficult to interpret, because the relevance of PK-data such as concentrations in plasma and tissues or body fluids to the relevant infection is not well established. It is considered helpful to clarify the requirements for such data to be regarded as conclusive, and to specify under which conditions part or all dose determination studies on animals could be replaced by PK-PD data. It is also intended to explore the possibility to use PK-PD data to support the dosing regimen for locally acting antimicrobials, such as intramammary, intrauterine applications or topical use.

As a conclusion, the revision of the guideline is intended to amend the requirements for the demonstration of efficacy of antimicrobials applied for marketing authorization according to current scientific knowledge, while minimising the development, maintenance and spread of resistance at the same time. Yet, the efficacy of antimicrobials, once marketed, can only be maintained if the responsible use of antimicrobials is consequently followed in veterinary practice.

## Formularies and other risk management strategies promoting responsible use

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Since 1994, Denmark has had an ongoing risk management strategy for optimisation of antimicrobial (AM) consumption and reduction of antimicrobial resistance (AMR), aiming at ensuring food safety and the future treatment possibilities for humans without jeopardizing animal health. The 'Danish model' results from a one health perspective with a strong interdisciplinary cooperation between the human and veterinary health authorities. The Danish formularies consist of: monitoring, solely legislation based risk management, science based risk assessment and data based risk management and risk communication – on legislation basis naturally. The VetStat database monitors AM use in production-animals. AMR is monitored through the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP). From 1994 to 2004 risk management was done on the country level using science based risk assessment and solely legislation based risk management. Since 2005 and 2010, respectively, a new concept of data based risk management and communication on the vet and farmer level is used. From VetStat, consumption and prescription patterns within animal species and age-groups are calculated on the vet level. Graphical displays are elaborated for use by DVFA's supervision-team in their direct risk communication with the vets, comparing the individual vet to the country level and supervising the vet on responsible use. The latter is supported by DVFA's evidence based treatment guidelines. The yellow card is the first strategy on the farmer level. National threshold values from population data forms the basis for the yellow card. AM usage in age-groups of swine on the herd level, in relation to numbers of animals, is calculated and used to define the national threshold values. From these the yellow card limits within age-groups are set annually. A new risk communication tool, designed in VetStat, is used to show the farmer and his vet, the herd AM usage in age-groups of swine in relation to the yellow card limits. This enables the farmer and vet to follow the herd usage and take action towards a responsible use, if the usage approaches the yellow card limits.

Formularies on the country level in the nineties resulted in a 44% decrease in the therapeutical usage. They were: stop of growth promotors, no preventive AM treatments, one-to-one herd health contracts between farmer and vet, decoupling veterinary prescription and dispensing and transferring of pharmacy discounts from vets to farmers. Strategic use of fluoroquinolones from 2002 resulted in zero usage. These strategies were done without VetStat. On the vet level, the results are mainly changing of prescription patterns following the guidelines on responsible use. On the farmer level, the yellow card has resulted in a 27 % decrease of the consumption in swine since January 2010 and until July 2011.

Monitoring is crucial for risk assessment, management and communication. Country level strategies, using risk assessment and solely legislation based risk management, can be done without a detailed monitoring. Economic matters make large changes, shown from decoupling vets prescription and dispensing in 1994 and farmer costs in today's yellow card. Data based risk management with focus on the top risk vets and farmers and direct risk communication, is key for long term changes of attitudes. Today's Danish results show that the vet is responsible for the choice of AM treatment, but the farmer is responsible for the consumption. Multitarget formularies, strategic use of AM and new strategies regularly, optimize use and AMR.

# Advertising and promotion in the context of prudent and responsible use of antimicrobials

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Competent authorities have an ever important regulatory role in guaranteeing the responsible use of antimicrobials. This role includes the control of advertising and promotion of antimicrobials. To achieve an adequate level of control, competent authorities use a number of official sources including legislation and guidelines. Where controls of advertising and promotion of antimicrobials and other veterinary medicinal products (VMP) are absent or incomplete, this sometimes leads to a biased non-professional presentation of the product that distorts its scientific attributes.

Who is targeted by the advertising of antimicrobials? The regulation regarding advertising is very different from a country to another. The monitoring of advertising remains anyway the responsibility of the National Competent Authorities. At international level, according to the OIE guideline about responsible and prudent use of antimicrobial agents in veterinary medicine (chapter 6.9): 'All advertising of antimicrobials should be controlled by a code of advertising standards, and the relevant authorities must ensure that the advertising of antimicrobial products is restricted to authorized professionals, according to national legislation in each country'. At European level, it is common to restrict the promotion of prescription products to only the veterinary surgeons and pharmacists, but in some countries antimicrobials may be advertised to users with or without codes of practice setting the standard for promotions. The legislation (Directive EC 2001/82) mentions that Member States shall prohibit the advertising to the general public of VMP that are available on veterinary prescription only. However, the directive does not furthermore define the concept of 'general public' and the interpretation is variable between member states. Advertising to professional keepers of animals are allowed in some member states.

What content for advertising of antimicrobials? At international level, the OIE guideline about responsible and prudent use of antimicrobial agents made it clear that the relevant authorities must ensure that the advertising of antimicrobial products complies with the marketing authorization granted, in particular regarding the content of the summary of product characteristics. In several countries, according to the national legislation, advertising materials can be reviewed by the authorities and are subjected to controls. The conformity with the marketing authorization is checked as well as the messages given. For example, the advertising doesn't have to create the impression that the consultation with a veterinarian is not necessary. At European level, the International Federation for Animal Health-Europe, IFAH-Europe has adopted the European Code of Good Practice which establishes some recommendations concerning all methods of promotion.

A need for harmonization. For the moment, there is no harmonization of the regulations regarding advertising activities. At international level, the World Health Organization (WHO) has published in 2001, the Global Strategy for Containment of Antimicrobial Resistance. The aim of the strategy is to provide a framework of interventions to stimulate the prevention of infections, to slow the emergence of resistance and to reduce the spread of resistant microorganisms. The Global Strategy also includes recommendations for interventions to reduce the overuse and misuse of antibiotics in food animals for the protection of human health based on the WHO Global Principles for the Containment of antimicrobial resistance

in Animals intended for Food (WHO, 2000). Among the general recommendations, several concern interventions on the pharmaceutical promotions (including advertising on the internet). The Heads of European Medicines Agencies (HMA) is a network of the Heads of the National Competent Authorities whose organizations are responsible for the regulation of Medicinal Products for human and veterinary use in the European Economic Area. The HMA supports a proposal regarding advertising for the revised EU legislation. This proposal introduces restrictions to the advertising of antimicrobials, so that no advert can promote an antimicrobial on the basis of factors which are not consistent with responsible use of antimicrobials. For example, undue prominence should not be given to withdrawal periods in adverts for antimicrobials. In addition all adverts for antimicrobials should be required to include a strap-line concerning the responsible use of antimicrobials.

Influence of advertising and promotion on sales. The close relationship between drug advertising and drug sales is supposed. Actually, in some countries, the content of advertisements appears to be a key source of information on antimicrobial agents. In veterinary medicine, in the Czech Republic, a recent questionnaire on 2000 practitioners shows that the price influences the antimicrobials prescription for 24 % of the veterinarians, the advertising influences 5% of the veterinarians. Discounts and other types of promotions (such as 'buy one, get one free') are common practices. These practices lead to a decrease of the price of the VMPs for the veterinarians and are economic incentives that encourage inappropriate antimicrobial use.

In conclusion, government controls on drug promotional activities and compliance of the pharmaceutical industry with both legislation and agreed codes of practice are important factors for an appropriate antimicrobial use. There is clearly a need for greater effort to ensure that veterinary health professionals receive accurate information regarding the efficacy, the safety of antimicrobials agents and of the problems of antimicrobial resistance. The next revised EU legislation on veterinary medicine products may propose recommendations on advertising.

## **The veterinary contribution to the responsible use of antimicrobials: decoupling veterinary prescription and dispensing?**

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It no longer needs elaboration that the risks associated with antimicrobial use in animals are primarily related to human health. It is therefore that veterinary profession should, and does, take its responsibility and be instrumental in reducing antimicrobial usage in animals. It is with this effort and goal in mind, that the RNVA developed an automated system of full documentation of all antimicrobial prescriptions, was supportive of the initiation and subsequent functioning of the national medicine agency, the Animal Drug Authority ([www.diergeneesmiddelenautoriteit.nl](http://www.diergeneesmiddelenautoriteit.nl)) overseeing antimicrobial use in animals, and undertook private initiatives together with its members, to reduce and substantiate the use of antimicrobials in the production of food stuffs of animal origin.

Since underlying cause of antimicrobials use in the animal sector is complex and involves next to the veterinarian many other parties, including the primary producers, the meat industry, the retail and ultimately the consumers, the RNVA seeks to approach the issue in a more holistic way. The primary and ultimate goal is described by the repositioning of the veterinary health professional as the central health advisor of the animal owner or keeper. The veterinarian is to be positioned as the primary care provider directing animal welfare and health, while guarding and supporting human health. This independent health care provider will not only provide in-depth advice to the animal keeper, but will also address the worries of the general public with respect to animal welfare and human health. For this reason, the advice provided by the veterinarian will no longer be discretionary for the animal keeper, and proper documentation of the advice and subsequent follow-up by the keeper will provide assurances that aspects such as animal welfare and health, and human health, are adequately addressed in the production process.

To this date, this approach has been presented to the veterinary profession in The Netherlands and has received ample support. The remaining challenge lies in organizing the necessary concerted action towards 'free riders' both in the veterinary field as well as in the field of 'primary producers'.

By organizing capacity building within the veterinary profession and at the same time providing assurances via an internal quality assurance system within our own organization, we feel that this approach does more towards the goal of a reduced antimicrobial use and subsequent limiting resistance, than decoupling prescription and sales of drugs by the veterinarian. The entire animal production system needs to undergo a paradigm shift in order to improve baseline health status and increase animal welfare standards. Only then will a sustainable animal stewardship go hand-in-hand with an adequate level of protection of human health, thus providing all participants in the sector with a continuation or renewal of their License-to-Practise, i.e., License-to-Produce.

# Antimicrobial agent delivery to animals in the feed: responsible or irresponsible?

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Antimicrobial agents are defined as chemical compounds that kill or inhibit the growth of microorganisms which include bacteria, protozoa, fungi and viruses. With the exception of the use of antimicrobial agents for the control of coccidiosis, principally in broiler chickens but also less commonly in young pigs, sheep and cattle, the dominant form of antimicrobial agent delivery in feed applies to the use of antibacterial agents in the management of the health, welfare and productivity of aquaculture species, poultry, pigs and cattle.

There are a variety of routes of administration of antimicrobial agents used commonly in animals. For example, local and superficial infections (including otitis, conjunctivitis, dermatitis, skin wounds) may be treated by local application of antimicrobial preparations; mastitis may be treated by the intramammary route. Systemic infections may be treated by any of various parenteral routes – for example by subcutaneous, intramuscular and intravenous injection. There are a large number of dosage forms of antimicrobial agents that have been specifically developed for enteral or oral administration. Amongst the variety of preparations for individual animal administration are boluses (quick release and sustained release), tablets, capsules, pastes and liquids (solutions and suspensions). For administration to large numbers of animals, antimicrobial agents can be formulated for administration in drinking water or for delivery within or on feed that may be liquid or in the form of crumbles, pellets, or a dry mixed total ration. Under intensive livestock production systems of poultry, pigs or ruminants where thousands to tens of thousands animals may be in the same airspace it is frequently not possible to identify and administer antimicrobial agents to individual animals and consequently means of mass medication via water or feed are often favoured and selected.

The reasons for treating animals with antimicrobial agents in feed may be for prophylaxis, metaphylaxis, treatment or to enhance production efficiency (via improved growth rate or rate of conversion of feed to body mass).

There are a number of critical questions that arise and that must be addressed when considering whether or not antimicrobial agent delivery in feed is responsible or irresponsible. Not the least of these considerations is what in fact is meant by responsible use and its opposite, irresponsible use. These concepts will be explored further in the presentation. When any chemical agent is administered to humans or to animals, be it a mineral, a vitamin, another nutritional agent or a medicine or a placebo, there is always a need to consider the balance of associated benefits and risks. Clearly the benefits must outweigh the risks in order to support a decision to administer a product and it could be considered irresponsible if the balance is not in favour of benefits. But which entity should benefit from the use? Is it an individual treated animal; is it the group of concurrently treated animals? Should the benefits accrue to the farmer? Or is the beneficiary or possible maleficiary quite distinct from the treated animals? In the case of antimicrobial resistance, which may be transmitted via affected microorganisms or their resistance determinants from one animal to another (including to humans), it is possible that the focus of attention when considering whether or not the use is justified, judicious, prudent or responsible may in fact not be the group of animals consuming the medicated feed but the population of unexposed animals or humans.

What if the use of the antimicrobial agent in feed was completely consistent with the directions set out in the label of the product that was used? And in approving the directions for use, what if the relevant regulatory agency had based its decision on a microbial resistance risk assessment? If a particular use pattern is approved for use in one regulatory jurisdiction but not in another or even prohibited in another, is the use still responsible in either of the jurisdictions?

These questions and others will be reviewed in the presentation and it will be observed that care must be taken with any use of an antimicrobial agent, irrespective of the route of administration. Irrespective of whether the antimicrobial agent is administered by topical, parenteral or enteral routes, all uses of antimicrobial agents are concurrently subtherapeutic, therapeutic and supratherapeutic.

## **Key management options in animal nutrition for food producing animals**

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The author will provide an introductory overview on perceived benefits and some challenges to in-feed medication and recall FEFAC's actions in the uprun to the European Union (EU) wide phasing out of AGPs in 2006.

In the main part of the presentation, the author will set out the principles and targets for a balanced nutrition as a critical factor for optimising the management of gut health which enables to minimise the need for the use of therapeutic antimicrobials. This includes an outlook to alternatives to antibiotics and R&D objectives for new technologies.

In the final part of his presentation, the author will draw some preliminary conclusions on the independent impact study for the review of the EU Directive on medicated feed 90/167/EC carried out by the European Commission and the industry viewpoint on the future role of medicated feed in EU livestock production systems.

## Are antibiotic residues a concern in distillers co-products?

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The US ethanol industry is expected to produce over 35 million MT of distillers co-products from 207 dry-grind fuel ethanol plants in 2011. Nearly all of these corn co-products are fed to livestock and poultry in domestic and export markets. Predominant corn co-products produced from the dry-grind ethanol plants include wet and dried distillers grains, wet, modified wet, and dried distillers grains with solubles, and condensed distillers solubles. Bacterial contamination during fermentation in the ethanol production process is an ongoing challenge. Lactic acid producing bacteria (*Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella*) are the most common contaminants [1]. These bacteria compete with yeast for sugars and micronutrients during fermentation, reducing ethanol yield by 1 to 5% [2], and resulting in lower quality distillers co-products. To manage this problem, antibiotics have been used to control bacterial infections for many years. Virginiamycin and penicillin have been the most commonly used antibiotics when added to fermenters in very small quantities relative to their respective usage rates in animal feeds. In the US, the Food and Drug Administration (FDA) has regulatory authority for all drugs, additives, and ingredients used in feeds for food animal production, including additives used in the production of distillers co-products. In November, 1993, the FDA's Center for Veterinary Medicine issued a 'letter of no objection' for the use of virginiamycin in ethanol and distillers co-product production. Currently, there is no regulatory monitoring or enforcement of antimicrobial residues in distillers co-products produced by fuel ethanol plants. No data have been published regarding the extent of use of antibiotics, occurrence of antibiotic residues, and the extent of any residual antimicrobial activity in corn co-products from the use of antibiotics in dry-grind ethanol industry. Therefore, the objectives of this study were to (i) collect and evaluate wet and dried distillers co-products samples from multiple geographical locations and dry-grind ethanol plants in the US for the presence of virginiamycin, penicillin, erythromycin, tetracycline, and tylosin residues, and (ii) determine the extent of any antimicrobial activity of samples using sentinel bacteria strains of *Escherichia coli* O157:H7 and *Listeria monocytogenes*.

Preliminary data are reported for the first two quarter sampling periods. Sixty-three samples (36 wet and 27 dried) of the 78 collected during the first two quarterly sampling periods have been analyzed for tetracycline, tylosin, erythromycin, and penicillin residues. None of the samples contained detectable concentrations of tetracycline, but 5 samples (7.9%) contained tylosin residues, 30 samples (47.6%) contained erythromycin, and all 63 samples contained penicillin residues (100%). However, antibiotic residue concentrations were extremely low with mean concentrations (dry matter basis) for dried samples of 0.002 µg/g for tylosin, 0.069 µg/g for erythromycin, and 0.020 µg/g for penicillin. Dried distillers grains samples had greater ( $P < 0.0001$ ) tylosin concentrations (0.002 µg/g) than wet distillers grains samples (0.0001 µg/g). No differences in penicillin or erythromycin residue concentrations were observed between type of distillers grain. Also, there were no distillers type × state and distillers type × sampling period interactions for penicillin or erythromycin. All 78 samples collected during the first two quarterly sampling periods have been analyzed for virginiamycin residues, and only two samples had detectable concentrations (>0.3 ppm)

using Phibro's FDA-approved bioassay. One sample contained 0.6 µg/g and the other contained 0.5 µg/g virginiamycin. No differences were observed between distillers grains sample type and there were no interactions.

Currently, only 24 of the 78 samples from the first two quarters of sampling have been evaluated for antibiotic activity. Of these samples, extract from only one sample was found to have any inhibitory properties for *E. coli*, but not *L. monocytogenes* growth. The extract inhibited *E. coli* at dilutions of  $10^{-4}$  and  $10^{-5}$ . The MIC for this sample was determined to be  $10^{-4}$ , because no bacteria grew in the  $10^{-4}$  dilution mixture. All of the other samples tested for antibiotic residue activity showed no bacterial inhibition, and produced plates with too many colonies to count for both *E. coli* and *L. monocytogenes*. Therefore, no MIC could be determined for these samples.

The preliminary results of this study indicate that extremely low concentrations of penicillin, erythromycin, and tylosin residues can be detected by HPLC procedures in wet and dried distillers co-products, but not tetracycline. Less than 3% of the samples tested thus far contained low (0.5 to 0.6 µg/g), but detectable concentrations of virginiamycin residues using the FDA-approved bioassay. However, it appears that there is minimal, if any, concern of residues having inhibitory properties when using strains of *E. coli* and *L. monocytogenes* as sentinel bacteria. It is likely that the majority of antibiotic residues in distillers grains are inactivated during the distillers grains production process, and detectable antibiotic residues have no effect on sentinel bacteria chosen to test their antimicrobial activity.

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# Antibiotics in aquaculture: practice, needs and issues

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Scientists with primarily a European experience tend to view aquaculture as a relatively small industry and assume that antibiotic use in this industry can be regulated by an extension of the systems developed for land-based agriculture. In reality global aquaculture is a huge, diverse and rapidly expanding activity. Total production was recently estimated to be 60 million tonnes and it has been expanding at 6% per annum for the last 20 years. Aquaculture now represents 50% of global fisheries with approximately 40% of production being traded internationally. Globally most (>75%) aquaculture activity is concentrated in China and S E Asia.

Aquaculture is very diverse. Over 400 aquatic species are known to be farmed but approximately 25 of these, belonging to many different genera and even phyla, account for the bulk of the production. Fish farms vary from small units rearing low-value fish operated by peasant, subsistence farmers to large, technically sophisticated units operated by multi-national companies that tend to produce high-value fish. It is estimated that approximately 80% of aquaculture production derives from small farmers.

The environments in which aquaculture is carried out also show extensive diversity. Many differing production and husbandry systems are employed and they may use fresh water, brackish water or seawater and, importantly when poikilothermic animals are concerned, may encounter temperatures from 0-30°C. The socio-political contexts within which it operates also vary widely. These variations have major implications for antibiotic use in aquaculture. Some countries have developed and maintain a rigorous regulatory environment whilst others, the majority in production terms, have not. Only approximately 5% of world aquaculture is carried out in countries that can be considered to have a well-developed scientific infrastructure. Much production occurs in countries that experience major difficulties in developing the expertise required to form and enforce regulations, collect and collate data, provide on-farm technical advice or to generate the research data required by their local industry. It has been argued that the inadequate diagnostic and on-farm advisory services available to most aquaculture operatives is one of the most important factors contributing to the excessive and irrational application of antibiotics in this industry.

The geographical imbalance between production and scientific expertise also has major implications for our understanding of antibiotic use in this industry. Currently we have no accurate idea as to the volume or even the classes of antibiotics used in global aquaculture. Very crude estimates might suggest that a figure for global use of 1,000-6,000 tonnes might be offered. In contrast to land-based agriculture, there is a very small number of products with Market Authorisations for aquaculture applications. In some countries (mainly in northern Europe or northern America) a very limited number of products (3 or 4) have been licensed but for the majority of countries, many with significant production, no products at all have been licensed. Current international attempts to produce prudent use guidelines are seriously hampered by this lack of Market Authorisations.

It will be argued that there are three main areas, optimising treatment regimen, assessing bacterial resistance and performing risk analysis, where scientifically developed nations can contribute to improvements in the use of antibiotics in global aquaculture.

The majority of antibiotic treatments in aquaculture involve the presentation of medicated feed to populations that contain some infected or dying individuals. However we have no knowledge of how these metaphylactic treatments achieve their therapeutic success. Partly as a consequence, no sophisticated pharmacokinetic/pharmacodynamic (PK/PD) models of these treatments have been developed. This, in turn, means that progress has been slow in the rational development of optimised treatment regimen.

Recently some progress has been made towards developing and harmonising standard laboratory protocols for susceptibility testing of aquatic bacteria. Very much less progress has been made in developing criteria for interpreting the data these tests generate. For theoretical and practical reasons it is argued that it will not be possible, in the near future, to set clinical breakpoints from either PK/PD data or clinical outcome data. Efforts are, therefore, being focused on developing epidemiological cut-off values. Currently there is a debate as to whether these can be formulated internationally as universal, laboratory-independent but protocol and species-specific values, or whether the lack of precision of susceptibility testing and the importance of low-level resistances requires the use of laboratory-specific values. At present interpretive criteria relevant to standardised test protocols are available for only one single bacterial species

Quantitative or qualitative assessments of the risk to human therapies of antibiotic use in aquaculture have proved difficult. It is generally accepted, in the absence of extensive data, that the risks mediated via aquacultural products consumed by humans is less than that associated with the products of land based agriculture. However, antibiotic use in aquaculture has been shown to result in the selection of resistant bacteria and resistance determinants in the aquatic environment. Antibiotic use in human medicine, land-based agriculture and aquaculture may all contribute to a potential reservoir of resistance determinants in the 'environment'. Currently we lack an evidence-based understanding of the significance of any such 'environmental' reservoir for the frequencies of resistance in human pathogens and of the relative importance of the three areas of antibiotic use in creating and maintaining the reservoir. It is argued that some model of these interactions is necessary before we can decide what form of data should be collected to progress our assessment of the risks represented by aquacultural use of antibiotics.

# Manufacturing and use of medicated feeds: feed industry issues

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Tecaliman is a private technical research centre in France based in Nantes created 30 years ago by and for the feed compounders. One of its three research axes (research programmes) is to improve feed safety and quality.

The feed industry's aim is to produce feed with a good nutritional equilibrium according to the animals' needs for growing. To this end, feed compounders manipulate grains, powders and small quantities of liquids and steam. These manipulations are essentially carried out with mechanical conveyors, tubes and silos. Because of the powders' behaviour, some deposits may occur in this type of feed mills and they may be transferred from batch to batch. This carry-over can be called cross-contamination if one of the components is not tolerated in subsequent batches. However, production at the industrial level is not carried out without default and quality. If carry-over could be considered as default in some cases (when a component is not acceptable), there are quality systems for the management of production such as HACCP, ISO, GMP, automation, traceability, etc.

The production of medicated feeds is one of the services provided by the feed industry to animal production. The major quality aims of this production are good homogeneity, conformity of the recovery level and a low level of carry-over in batches subsequent to the medicated feed batches. Even if carry-over is unavoidable, it is possible to achieve very low levels through the management of carry-over in a feed mill.

To control carry-over feed compounders can manage the active products used, the feed, the process and the processing. However, to measure the effect of each action, feed compounders need a good and right method of measurement. As the regulation is the same in all European Union member states the expected level of control should be the same, too. Nevertheless, in all member states the control of medicated feed is higher than that of standard feed. For example, the measurement tools adopted 10 years ago in France referred to the production of four batches on the same line: two containing a tracer like a medicine molecule and subsequently two without any formulation. Samples were taken at the silos' intake before the pelleting lines. Now, after 10 years, approx. 200 tests following these tools are yearly done in France. During this period, Tecaliman has developed an external tracer method of measurement to facilitate trials and to decrease the costs. Actually, more than 70% of the trials made in France use external tracers.

Two examples of solutions to reduce carry-over will be presented. Firstly, carry-over is strongly linked with the dust behaviour of powders, whatever their composition. Thus, it is possible to choose low dust behaviour products in order to reduce the risk in a mill. The second example deals with the length of the process (the number of apparatus) before the mixer. It has been shown that reduction of this length can significantly reduce the level of carry-over. As shown in France the level of carry-over in the mills can decrease year by year. This improvement has been followed up by many European countries and it should be generalized. The carry-over levels only concern the first batch right after the production of the medicated feed batch. Risk evaluation has shown that the 'chance' for a pig to be in contact with a veterinary product generated by carry-over, is a maximum of 2 times during its lifetime.

To continue the reduction of the levels of carry-over many solutions could be adopted such as for example: (i) using the same method to evaluate carry-over levels for all mills over the years; (ii) qualifying the medicines on the market for use in feed mills, especially on low particle dust behaviour, and buying the medicines with the lowest carry-over behaviour; (iii) producing medicated feed by series; and (iv) changing some process parameters (i.e., the length of the premix conveying before the mixer) and processing (i.e., flushing) in the mills.

## Is there a future for medicated feed? The Dutch approach

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Sustainable and integrated management practices in animal husbandry have an absolute priority in government strategies and international policies. Disease control and animal welfare need to be achieved with responsibility, especially concerning the use of antibiotics. Authorities, producers and consumers became aware not only of the toxicological effects of the antibiotic residues in food, but also of their effects on the increase of antimicrobial resistance. In an attempt to reduce the antimicrobial resistance in zoonotic pathogens, the European Commission (EC) has prohibited the use of antibiotic feed additives as growth promoters since January 2006. However, the therapeutic use of antibiotics (e.g., tetracyclines) in veterinary practices has increased in the Netherlands since that date.

Medicated feed is still the most common way of oral administration of antimicrobials in the Netherlands and/or in European Union (EU). In the Netherlands 2% of all produced feed for pigs is medicated. However the use of medicated feed has some disadvantage like the inhomogeneity in the antibiotic concentration – depending on the production process – and the unavoidable cross-contamination or carry-over problem.

This study focused on the problem of unavoidable carry-over of antibiotics by the production of medicated feed. In 2008 21 feed mills were visited and 140 different samples of feed – flushing feed samples, collected after production of medicated feed – were collected and analysed. The samples were analysed by using liquid chromatographic separation and mass spectrometric detection technique. With this technique low levels of antibiotics (0.1-1 mg/kg) are detected and identified. The second part of the study focused more on the effect of the sampling approach. Factors like mill construction, place of sampling, number of samples taken and how these factors have influence on the detected levels of carry-over were studied. For this part of the study four feed mills were visited and flushing feeds of medicated feed containing oxytetracycline were sampled. For each flushing feed approximately 20 samples were collected. The samples were analysed for oxytetracycline. The results measured were evaluated and also compared with the levels of carry-over measured by protein-manganate/microtracer approaches (see Dutch Product Board Animal Feed, GMP+ Certification Scheme Animal Feed Sector 2006). During the study all kind of information from the factory was collected like the amount of medicated feed and flushing feed produced and the final destination of the flushing feed. This information was used for the evaluation.

The first part of the study in which 140 samples of flushing feed for pigs were collected and analysed shows that in 87% of all samples residues of antibiotics were detected (Table 1). It is remarkable that the concentrations measured are in the same range as the banned antimicrobial growth promoters (AMGBs). Tylosin was banned in 2006 for use at concentration levels ranging from 5 to 40 mg/kg and the most frequently detected antibiotic, oxytetracycline, was banned in 1975 for use at concentration levels ranging from 5 to 50 mg/kg.

Acknowledging the importance of the control on antibiotics in animal feed the Dutch office of Risk Assessment issued a recommendation to set a limit of 2.5% of the lower therapeutic

dose on the cross-contamination of the medicated feed in the production lines (nVWA, 2010).

In this study 36 of the samples analysed (26%) contained levels of antibiotics above the proposed maximum carry-over concentration (>2.5%).

Table 1. Antibiotics detected in flushing feed, frequency and concentration range.

Antibiotic	No. of samples the antibiotic was detected*	Concentration range (mg/kg)
Oxytetracycline	46	0.8-154
Sulphamethoxazole	17	0.4-23
Tilmicosin	18	0.1-38
Sulphadiazine	16	1-63
Amoxicilline	11	0.1-8.9
Tylosine	7	0.6-6.0
Lincomycine	4	1.1-2.9
Doxycycline	2	0.5-17
Tiamulin	2	0.6-1.1
Total	122	

\*140 samples were collected

In the Netherlands there is a detailed national monitoring scheme of usage of antibiotics in animal husbandry ([www.fidin.nl](http://www.fidin.nl)). Animal feed is produced according to national guidelines for good manufacturing practices and carry-over in medicated feed is registered separately ([www.gmpplus.org](http://www.gmpplus.org)). According to these guidelines the first flushing charges may not be used as feed for laying hens, milk cows and as the final feed before slaughtering.

It takes approximately 1.5 weeks for a batch of animal feed to be fed, therefore exposure of the animal to 'contaminated' feed is set at 1.5 weeks. Based on the levels of antibiotics found in the flushing feed and the exposure of 1.5 weeks a very rough estimation is made regarding the exposure of pigs and calves to antibiotics due to carry-over. This shows that 11% of the piglets, 12% of the meat pigs in their 'starter feed' period and for example 100% of lactating sows at least once a year are exposed to feed containing antibiotics due to carry-over.

To get a more detailed picture of the exact level of exposure of animals to contaminated feed due to carry-over it is necessary to have more information regarding the contamination of the flushing feed. For example for the exposure – and for proper sampling of the feed – it is important if the antibiotic is homogeneously divided in the feed or if there are places of high and low contamination.

The second part of the study focused on this issue. At different feed mills flushing feeds were sampled after the production of the medicated feed. This time the focus was on the antibiotic oxytetracycline and the samples were taken every 3-6 minutes during the production of the flushing feed batch. A maximum of 20 samples was taken. All samples were analysed for oxytetracycline to study the homogeneity of the batch. Figure 1 presents the results obtained for one (representative) flushing feed. From the results it is concluded that the first part of the flushing feed is much higher contaminated than the last part. Furthermore, it is obvious that during the first 20 minutes of the production the flushing feed contains concentrations of oxytetracycline >2.5% carry-over. From the results presented in Figure 1 it is also concluded that the sampling approach has to take into account that the flushing feed batch is inhomogeneous.

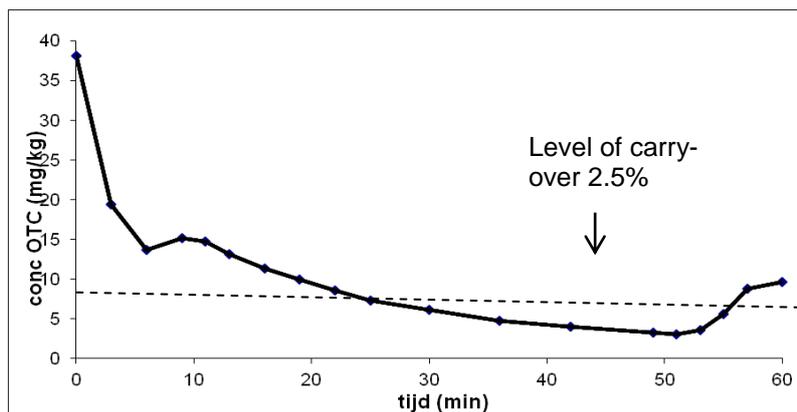


Figure 1. Concentration of oxytetracycline in flushing feed versus production time; dotted line shows concentration corresponding with 2.5% carry-over.

At this moment it is not possible to draw final conclusions about how to prevent or even reduce the carry-over of antibiotics after the production of medicated animal feed. It is obvious that a lot of factors have influence on it, e.g., the construction of the feed mill, type and concentration of medicated feed, amount of medicated feed produced in combination with the flushing feed charge. Because of this it is difficult to obtain and keep a maximum carry-over level of 2.5% which is proposed as the maximum acceptable carry-over. Furthermore, no correlation is found between the carry-over measured versus the feed mill carry-over measured by protein-manganate/microtracer approach. These observations together with the knowledge of the increasing problem of antibiotic resistance motivated the NEVEDI (the Dutch Feed Industry Association) to announce that they will voluntarily stop the production of medicated feed in 2011. They are the first in Europe. If this means that in the future in the Netherlands no medicated feed will be produced and if this really will result in antibiotic free feed, then this will be – together with the alternatives for medicated feed and new risks – the topic for further research.

#### Acknowledgements

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## Putting the vet in the Veterinary Feed Directive

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Antimicrobials in the United States are regulated by the Food and Drug Administration (FDA). During the FDA approval process the agency makes a determination of the appropriate level of risk management for the compound. One risk management option employed by the agency is marketing status limitations requiring veterinary oversight. Currently, antimicrobial drugs may be marketed as requiring a veterinary prescription (Rx), over-the-counter (OTC), or veterinary feed directive (VFD) products. Both Rx and VFD status require an order from a veterinarian to obtain the antimicrobial.

Antimicrobials delivered in animal feeds are available either as OTC or VFD. Prior to 1996, all antimicrobials delivered in animal feeds were marketed at OTC. In 1996, with the approval of tilmicosin phosphate for swine, the FDA developed the VFD mechanism for marketing status limitation of in-feed antimicrobials. The new mechanism was considered necessary because state pharmacy boards, in most states, regulate pharmacies and some veterinary distributors and additionally because the pharmacy boards had no regulatory authority over feed mills. In addition, to tilmicosin, one other antimicrobial for use in swine and catfish has been approved under the VFD.

Currently, the following information is required in the VFD order for the order to be valid:

- veterinarian's name and address, telephone number and if the VFD is faxed, facsimile number ;
- client's name, address, telephone number and if the VFD is faxed, facsimile number;
- identification and number of animals to be treated/fed the medicated feed, including identification of the species of animals, and the location of the animals;
- date of treatment, and, if different, date of issuing the VFD drug;
- approved or index listed indications for use;
- name of the animal drug;
- level of animal drug in the feed, and the amount of feed required to treat the animals;
- feeding instructions with the withdrawal time;
- any special instructions and cautionary statements necessary for the use of the drug in conformance with the approval;
- expiration date of the VFD;
- number of refills (reorders) if necessary and permitted by the approval;
- veterinarian's license number and the name of the state issuing the license;
- the statement: 'Extra-label use, (i.e., use of this VFD feed in a manner other than as provided for in the VFD drug approval) is strictly prohibited'; and
- any other information required by the VFD drug approval regulation.

The original VFD order must be maintained by the veterinarian, client and feed mill for 24 months.

Since the VFD mechanism has been limited to two compounds available for use in swine and one in catfish, the impact of this regulatory mechanism to producers and swine veterinarians has been minimal. However, in 2010 the FDA issued an Advanced Notice of Proposed Rule-Making on VFD. They also published a draft guidance (Guidance for Industry #209) stating that 'the use of medically important antimicrobial drugs in food-producing

animals should be limited to those uses that include veterinary oversight of consultation'. The expected timeline for publication of a final Guidance #209 and the issuance of draft language for a revision to the VFD is fourth quarter of 2011. With the expected move of most in-feed antimicrobials for all species of food producing animals to VFD the impact to producers, veterinarians and commercial feed mills is expected to be significant.

# Antibiotic use in the UK poultry industry – past, present and future

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The discovery of antibiotics, their mechanism of action and their role and application to livestock disease control has grown alongside the development of the global poultry industry in the generation since the end of the Second World War. In that time period antibiotic use in poultry flocks has had an important part to play in the successful growth of the industry through the control of infectious disease and the promotion of efficient production. In the most part this has been achieved through the responsible use of available antibiotics, prescribed and administered by the veterinary profession.

Despite this veterinary control, in more recent times consumers have voiced concern over the use and abuse of antibiotics and its contribution to infectious drug resistance. Indeed this concern has expanded to a range of issues and potential misconceptions. In relation to the poultry industry these misconceptions have included: (i) the suspected constant drip-feeding of antibiotics to poultry in a totally uncontrolled manner; (ii) the use of antibiotics leading to the production of antibiotic resistant super bugs, damaging to human health; (iii) over-use of antibiotics inevitably leading to the presence of harmful residues in the meat and eggs we eat; (iv) antibiotic use contributing to poor animal welfare by allowing birds to be reared in less than optimal conditions; and (v) antibiotics being used for growth promotion and rapid growth rather than to treat disease.

Antibiotic resistance, as defined as the ability of bacteria to survive and flourish in the presence of an antibiotic, is likely to be an inevitable outcome of the use of antibiotics. This form of natural selection occurs as much in animal use of antibiotics as it does in human use. This phenomenon and the spectre of transferable multi-resistance indicate the need for responsible and restricted use of such products in poultry and humans, to help safeguard animal and human health.

Clearly, anything that can be done to reduce the use of antibiotics in animal production will reduce the likelihood of antibiotic resistance development, as indeed it will in human medicine too. The poultry industry is well aware of the role played by husbandry, management, nutrition and environmental control in the incidence, severity and magnitude of disease challenges. The role of effective biosecurity and the use of structured vaccination programmes will reduce the dependency on antibiotic use.

One of the most important aspects of ensuring targeted therapy is accurate and prompt diagnosis of disease challenges. This requires effective communication between producers and their veterinarian with rapid identification of signs of ill health and professional investigation as to the cause of any morbidity or mortality. This in turn requires access to specialist poultry veterinarians, and a range of rapid and effective diagnostic tools, developed at centres of academic excellence. Structured monitoring plans can then be devised to allow early identification of health issues and help assess the relative contribution of infectious, management and environmental factors. This can help prevent any dependency on unnecessary medication strategies and should be incorporated into the practical implementation of effective veterinary health and welfare plans.

Where antibiotic use is considered necessary under veterinary direction the aim is to use this targeted therapy early in the disease process and more accurate diagnosis will allow therapy

aimed specifically at the organism being treated enabling use of narrow spectrum antibiotics. One major aspect of this responsible use is that following accurate and prompt diagnosis there should be pre-treatment sensitivity testing. Pre-treatment testing is the norm in poultry therapeutics ensuring that the most appropriate antibiotic is selected.

Despite calls to reduce antibiotic use, there is clearly an ongoing need for antibiotic use in poultry production to assist in the control of the health and welfare impacts of disease and to contribute to improved food safety. To allow this to continue there is an equally clear need to promote responsible use by farmers with clear and unambiguous instructions given by the prescribing veterinarian.

Responsible use of medicines guidelines have been produced by a number of industry bodies in many countries over recent years to promote judicious use. One of the leaders in this process has been the Responsible Use of Medicines in Agriculture (RUMA) Alliance in the UK. This alliance was established in 1998 as a cooperative and independent body encouraging best practice in the use of antimicrobials in farmed livestock. RUMA has identified responsible use guidelines for most food producing species. Their guidelines for the responsible use of antimicrobials in poultry production serve as a useful template for others to follow.

To safeguard the availability of necessary and effective antibiotic therapy all those involved in the supply, procurement and usage of these products should act responsibly in their use. This will help to ensure the health and welfare of poultry flocks whilst reducing the likelihood of the development of antibiotic resistance, reduce the likelihood of unwanted residues in foodstuffs and prolong the ability to use antibiotics effectively. These aims can best be achieved by:

- reduced dependence on antibiotics to control disease;
- continued availability of effective diagnostic techniques supported by well funded centres of research and academic excellence;
- continued availability and targeted use of effective vaccines;
- employing best practice in all aspects of disease biosecurity;
- better hygiene procedures and terminal cleansing and disinfection;
- consideration of the role of alternative products i.e. non-antibiotic products to control disease;
- judicious use of antibiotics where they are considered essential on veterinary advice;
- monitoring antibiotic use and the development of resistance as an early warning system for problems with specific classes of antibiotic and specific pathogens; and
- actively communicating with consumers, and as importantly retailers, on the need for antibiotics and the proven ability of the industry to use them appropriately.

## **DANMAP – integrated surveillance of antimicrobial consumption and resistance**

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The objectives of DANMAP are: (i) to monitor the consumption of antimicrobial agents for food animals and humans; (ii) to monitor the occurrence of antimicrobial resistance in bacteria isolated from food animals, food of animal origin and humans; (iii) to study associations between antimicrobial consumption and antimicrobial resistance; and (iv) to identify routes of transmission and areas for further research studies.

The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme, DANMAP, was established in 1995 on the initiative of the Danish Ministry of Health and the Danish Ministry of Food, Agriculture and Fisheries, as a coordinated national surveillance and research programme for antimicrobial consumption and antimicrobial resistance in bacteria from animals, food and humans. The participants in the programme are Statens Serum Institut, the National Veterinary Institute, the National Food Institute, and the Danish Medicines Agency. The DANMAP programme is funded jointly by the Ministry of Health and the Ministry of Science, Technology and Innovation.

The monitoring of antimicrobial resistance is based on three categories of bacteria: Human and animal pathogens, zoonotic bacteria, and indicator bacteria. Human and animal pathogens are included because these cause infections and they reflect primarily resistance caused by use of antimicrobial agents in the respective reservoirs. Zoonotic bacteria are included because they can develop resistance in the animal reservoir, which may subsequently compromise treatment effect when causing infection in humans. Indicator bacteria are included due to their ubiquitous nature in animals, food and humans and their ability to readily develop antimicrobial resistance in response to selective pressure in both reservoirs. The surveillance methods have developed over the year and consists now of phenotypic testing of antimicrobial resistance supplemented with genotypic testing and bacterial typing techniques.

Human consumption data are obtained from the Danish Medicines Agency (DMA) (<http://www.laegemiddelstyrelsen.dk>). The DMA has the legal responsibility for monitoring the consumption of all human medicinal products. Since 2001, animal consumption data have been obtained from the VetStat database. In Denmark, all therapeutic drugs are prescription-only and VetStat collects data on all medicines prescribed by veterinarians for use in animals.

The report is published once a year and describes the annual consumption of antimicrobial agents and the occurrence of resistance in different reservoirs in Denmark. Results from the monitoring program as well as from selected research projects are presented in overview tables and figures. In the Appendices, detailed tables of antimicrobial consumption in animals and humans and specific MIC distributions are presented, along with a list of abbreviations, explanations of terminology and description of materials and methods. A list of DANMAP publications in the international scientific literature in 2010 is also included. The DANMAP report is available at [www.danmap.org](http://www.danmap.org).

# Antibiotic use data: the long way from numbers to knowledge

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Knowledge about exposure required. Because of the effects of antibiotic usage in food animals on antimicrobial exposure and consequently the development of antimicrobial resistance, it is important to gain quantitative insight into the use of veterinary antibiotics. However, it is difficult to find appropriate usage data, with sufficient detail. In the coming years the insight into antibiotic use will still be primarily based on national total sales data, at least in most countries. Could the true level of exposure to veterinary antimicrobial agents be estimated on the basis of such overall usage data?

Only overall data available. Recent publications express the antibiotic usage per country in mg of active substance sold per kg of animal produced or present [1,2], which seems to be a quite rough indicator. Nevertheless the calculated 'mg per kg' will generally be interpreted as true level of antibiotic exposure in a country. This is an oversimplification, because the 'mg per kg' mainly reflects the animal demographics: some countries have relatively large pig or poultry populations, with relatively high antibiotic use, while other countries have mainly dairy cattle or extensively held animals like suckling cows and sheep. Besides, the differences in 'mg per kg' also reflect different ways in which antibiotics are used, i.e., differences in dosage.

Short- and long-term solutions. Obviously a country comparison based on total sales figures runs the risk of serious misinterpretations. However, it is possible to reduce such misinterpretations by using more adequate methods to analyse and present the data. Differences in dosages have to be taken into account [3] and, more importantly, also differences in farm animal demographics. In the long term total sales data provide inadequate information for solid risk-assessments. Therefore it will be necessary to collect more detailed information about the use per animal species, expressed as number of defined daily doses per animal per year [4,5]. Adopting this approach offers an opportunity to obtain true insight into the relationship between antibiotic usage and resistance. Moreover, the unit 'daily dose' conforms to international developments in this field and developments in the human health sector. Detailed data collection of this nature will improve the feasibility to compare for example the antibiotic use in different EU member states in similar livestock systems.

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## **CLSI XR-08: Generation, presentation and application of antimicrobial susceptibility test data for bacteria of animal origin – a report**

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Veterinary monitoring programmes consist mainly of collecting food-borne and commensal bacteria in food-producing animals at slaughter and determining their susceptibility against a panel of antibiotics relevant for human medicine. The data generated are part of the risk analysis for potential food-borne transmission of resistance. There are many veterinary surveillance systems for antimicrobial susceptibility currently being undertaken throughout Europe. However, there has been little success in attempting to harmonise the respective approaches, thus making it difficult to compare data across countries.

One of the main issues is that surveillance schemes do not all define resistance in the same way. This means that it is not possible to simply compare resistant rates from different surveillance schemes as they are not measuring the same parameter. Indeed, even within national surveillance schemes, methods of analysis have changed over time such that the percentage resistance values may not be comparable. There are two fundamental reasons for this being the case: (i) the trend for 'resistance' to be defined by the epidemiological cut-off value rather than by the long-established clinical breakpoint; and (ii) no standardisation on how to define the epidemiological or wild-type cut-off value.

Clinical and Laboratory Standards Institute (CLSI) report X08-R 'Generation, presentation and application of antimicrobial susceptibility test data for bacteria of animal origin – a report' offers guidance on areas in which harmonization can be achieved in national veterinary antimicrobial surveillance programs, with the intent of facilitating comparisons of data among various national surveillance programs. CLSI veterinary antimicrobial susceptibility testing (VAST) methods are used to generate minimal inhibitory concentrations or zones of inhibition, and the laboratory interprets that information into a category of susceptible, intermediate, or resistant. The veterinarian uses this information to make an informed decision in the selection of an appropriate antimicrobial for animal treatment. However, various surveillance programs or projects use the data for many other purposes, including the drafting of risk assessments (subsequently used for risk management) or to determine the success of intervention policies. These programs include multiple national programs, several multinational programs, product-specific programs, and purpose-specific regional or local programs. Currently, there is a lack of standardized methodology describing how the data from these programs are presented in the reports and discussed with regard to the specific program objective. In keeping with the intent of CLSI document M39, this document seeks to bring the CLSI VAST perspective to these programs and projects by means of a comprehensive report that can help form the basis for a global consensus.

This report provides guidance on aspects of AMR surveillance programs ranging from sample collection, AST methodology, data presentation, and data interpretation, including situations in which CLSI-approved veterinary-specific clinical breakpoints are not established. Efforts are made to highlight areas in which laboratories deviate from CLSI methodology and the subsequent misinterpretation of data that can occur. Comparisons are made among some of the more established veterinary AMR surveillance programs and among human AMR surveillance programs, along with indications of the usefulness of certain points of human AMR programs for veterinary programs.

This report provides a review of current applications of susceptibility test data generated using CLSI methodology for bacteria of animal origin and recommendations for summarizing, presenting, and applying the data. More specifically, the report provides an overview of the CLSI veterinary antimicrobial susceptibility testing (VAST) approach to the use of reference methodology, quality control (QC), and establishment and use of clinical breakpoints and epidemiological cut-off values (ECVs). Recommendations for the presentation of minimal inhibitory concentrations (MICs) or zone inhibition data in frequency histograms and scatter plots are provided, in addition to recommendations for the use of ECVs and/or CLSI clinical breakpoints. A review of various applications of surveillance programs is provided, with clarification of descriptive summary statistics of MIC frequency histograms (e.g., MIC<sub>50</sub>, MIC<sub>90</sub>, geometric mean), and recommended standardized approaches.

Finally, consideration is given to the intended use of any antimicrobial resistance (AMR) surveillance program. The usual goal in collecting antimicrobial susceptibility data is to detect the early emergence of resistance for a given bacterial species/antimicrobial combination that may be used for the following purposes:

- provide a basis for policy recommendations for animal and public health;
- generate data that may guide the design of further studies;
- provide information for prescribing practices and prudent-use recommendations;
- determine the prevalence or trend in prevalence of reduced susceptibility (or resistance) to a certain antimicrobial in a defined population;
- detect emergence of AMR (e.g., particular phenotypes);
- identify the need for potential intervention;
- assess the impact of intervention(s); and
- identify the emergence of new mechanisms of resistance.

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For abstract, see Appendix I

# Antimicrobial resistance in companion animals and horses

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Antimicrobial resistance is a public health threat impacting both human and animal health. Resistance trends and mechanisms have been studied extensively in humans and food producing animals, however humans and companion animals, such as dogs and horses, are often in close contact and there is the risk of transfer of bacteria, from one to the other. Antimicrobials are widely used to treat a variety of clinical conditions in companion animals and increased resistance has led to problems in the treatment of some infections in animals.

The aims of work at University of Liverpool over the last 5 years has been to characterise the prevalence and risk factors for resistance, both in the community and in hospitalised animals, in *Escherichia coli* and staphylococci. Further work has identified genes responsible for antimicrobial resistance and current work has aimed to describe antimicrobial prescribing practices in UK veterinary practice and evaluate factors associated with prescribing.

Resistance in faecal *E. coli*. A nationwide cross-sectional study of 692 horses in the general equine community of mainland Great Britain revealed an overall prevalence of faecal carriage of *E. coli* with resistance to any antimicrobial of 69.5%; a prevalence of extended-spectrum beta-lactamase producing *E. coli* of 6.3% and of multidrug resistance *E. coli* of 37.6%. Identified risk factors for carriage of resistance included recent hospitalisation, contact with specific non-equid animals, the type of premises, the surrounding land use, and antimicrobial treatment in the previous 10 days. Being stabled on the same yard as a recently hospitalised horse was identified as a risk factor for increased risk of carriage of ESBL-producing *E. coli*. There was also spatial variation in risk identified for carriage of resistant and multidrug-resistant *E. coli*, with higher risk in southern and eastern regions of the UK.

Treatment within the previous ten days with the fluoroquinolone enrofloxacin, was unsurprisingly a risk factor for ciprofloxacin and nalidixic acid resistance. However, treatment with any type of antimicrobial in the last ten days was a risk for trimethoprim resistance. Trimethoprim (cotrimoxazole in combination with sulfamethoxazole) was the most widely prescribed antimicrobial of horses in this study.

A longitudinal cohort study of 103 horses admitted to a referral equine hospital with faecal samples collected on hospital admission and subsequently every two days until discharge showed higher levels of resistance with a prevalence of faecal carriage of *E. coli* with resistance to any antimicrobial of 70.2%, and a within horse prevalence of 91.3%. There was a high prevalence of ampicillin (50%), tetracycline (51%) and trimethoprim (65%) resistance. Multidrug-resistant *E. coli* (resistant to three or more antimicrobial classes) were identified in 47.7% of samples and ESBL-producing *E. coli* were recovered from 131 samples (27.3%), from a total of 55 horses (53.4%).

The day the sample was obtained was significant, with increased risk for samples taken on day 2 or later. For all outcomes except ESBL-mediated resistance, having had antimicrobial treatment in the seven days prior to a sample also significantly increased the risk of resistance. The defined daily dose of cotrimoxazole in the hospital in the 24-48 hours prior to a sample was also a significant risk factor for many resistance outcomes.

In a similarly designed nationwide study of 580 dogs 44.7% of samples had a least one resistant *E. coli* present in faecal samples and multi-drug resistant *E. coli* was isolated from 18.4% of dogs. The most common resistances observed were to ampicillin (37.7% of dogs), tetracycline (29.8%) and trimethoprim (23.6%). ESBL producing *E. coli* were isolated from 4.1% of dogs and an AmpC  $\beta$ -lactamase producing *E. coli* from 7.1% of dogs.

Although the *E. coli* recovered from these studies were not disease causing and are likely to represent commensal strains, these may have the potential to cause disease via virulence determinants, or through inoculation of non-gastrointestinal sites, such as post-surgical wounds. In addition, their resistance determinants may be transferred to other pathogenic bacteria. The multidrug-resistant *E. coli* isolates identified in this study would prove refractory to treatment by most of the antimicrobials currently available for use in veterinary medicine if involved in infection. In particular, the prevalence of carriage of ESBL-producing *E. coli* identified in this study was higher than expected.

Resistance in staphylococcal species. There is the potential for dogs and horses to act as a reservoir for both MRSA and other resistant staphylococci. Genetic characterisation of MRSA isolated from dogs suggests transmission between humans and pets, since they carry the same strains that are prevalent in humans. However the main MRSA strain types found in horses appear to be largely restricted to the species, and less commonly encountered in humans and other animals, unless they have contact with horses.

A large cross sectional study of 678 nasal swabs were collected from horses located on 525 stable yards randomly selected across the UK. The overall prevalence of nasal carriage of MRSA was 0.6% and 29% were found to carry methicillin-resistant coagulase-negative *Staphylococcus* species. An identically designed study in small animals yielded 724 nasal samples from 87 randomly selected veterinary practices. MRSA was isolated from 1% of dogs, all of which were shown to be of the same sequence type as the dominant healthcare associated strain (EMRSA-15, ST22). No methicillin-resistant *Staphylococcus pseud-intermedius* were isolated and 40 dogs (5.5%) were found to carry methicillin-resistant coagulase-negative *Staphylococcus* species.

Knowledge of risk factors that may contribute to antimicrobial-resistance from companion animals is necessary for development of preventive measures to limit occurrence, for example where guidelines on antimicrobial usage may be useful, and will guide future targeted surveillance for resistance strains that may be of risk to humans.

Antimicrobial use. Two methods of determining antimicrobial prescribing have been used. Firstly postal questionnaires including four clinical scenarios were sent to randomly selected veterinary surgeons that treat horses and dogs. Data were collected on the clinician, their practice and their sources of information regarding antimicrobials and their use. Secondly the indication-based use of antimicrobials in animals treated by veterinary surgeons was evaluated through the completion of a prescription logs over a period of 5 days. The antimicrobial used, presenting complaint and total number of animals under consultation was documented.

For small animal veterinarians 3.5% of clinicians reported that their practice had an antimicrobial use policy. Most veterinarians (96.1%) reported that they could prescribe antimicrobials completely at their discretion. Overall, 2.3% of prescriptions were not licensed for use in dogs or cats in the UK and 5.4% of prescriptions were under the recommended dose and 20.0% were over the recommended dose. Prescriptions for fluoroquinolones and 2nd and 3rd generation cephalosporins accounted for 5.9% and 4.6 %, respectively of the total number of prescriptions. Broad-spectrum penicillins were the most commonly prescribed antimicrobials.

Prescriptions logs showed that 25.9% (n=1179/4559) of all dogs attended by clinicians received antimicrobials. Penicillins (especially amoxicillin/clavulanic acid 15.4%) were the most commonly prescribed antimicrobials. Fluoroquinolones and 3rd generation cephalosporins accounted for 5.6% and 1.3% of prescriptions respectively. 8.2% (n=84/1029) of dogs were under dosed. Dogs with skin, oral/dental or uro-genital tract complaints were more likely to be under-dosed.

For equine practitioners less than 1% of practices had an antimicrobial use policy. Trimethoprim-sulphonamides were most commonly prescribed in each clinical scenario. Eleven percent of prescriptions were for antimicrobial drugs un-licensed for use in horses in the UK. Five percent of prescriptions for licensed antimicrobials were under the recommended dose rate. Fluoroquinolones and 3rd and 4th generation cephalosporins accounted for 1% and 3% of prescriptions respectively, however veterinary surgeons working at referral practices were more likely to prescribe 3rd and 4th generation cephalosporins and fluoroquinolones and off-license antimicrobials.

Broad-spectrum drugs were the most commonly prescribed antimicrobials in both species which goes against recommendations to use narrow-spectrum drugs where possible. Surprisingly few practices had antimicrobial use policies. These have been shown to result in more prudent use of antimicrobials in human and small animal hospitals, including a general reduction in the quantity of antimicrobials used and an increase in the relative use of first-line compared to third-line drugs. Use of antimicrobial guidelines by UK veterinary practices could result in more prudent use hence preserving the effectiveness of important drugs.

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# Challenges in harmonising resistance monitoring programmes in veterinary medicine

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Veterinary monitoring programmes consist mainly of collecting food-borne and commensal bacteria in food-producing animals at slaughter and determining their susceptibility against a panel of antibiotics relevant for human medicine. The data generated can be used as part of the risk analysis for potential food-borne transmission of resistance. There are many veterinary surveillance systems for antimicrobial susceptibility currently being undertaken, however, there has been little success in attempting to harmonise the respective approaches, thus making it difficult to compare data across countries.

Data will be considered from surveillance systems in Denmark (DANMAP), The Netherlands (MARAN), Spain (VAV) and Sweden (SVARM) as well as the European Antimicrobial Susceptibility Surveillance in Animals (EASSA). Minimum inhibitory concentration (MIC) testing is performed in all cases, except for VAV where disk diffusion is used for some antibiotics. The choice of reviewed surveillance schemes was in part based on the availability of data for several years in order to identify trends as well as choosing countries with what are perceived to be relatively low use antibiotic consumption rates (Denmark and Sweden) versus relatively high use rates (The Netherlands and Spain). However, hard data on use rates, particularly in respective animal species, in all countries are not available although the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project is now generating some very useful data in this area.

At the outset it is important to emphasise that all of the reviewed surveillance systems have merit, especially when considering resistance trends within the countries in which the surveillance has been instigated. However, there are considerable challenges when making horizontal comparisons. The EASSA surveillance programme specifically addresses trends across countries, and data from EASSA will be considered alongside that from the national systems.

The major challenge when analysing data across surveillance systems is a lack of harmonisation in sampling, susceptibility testing methods, choice of antimicrobial test drugs, and such basic terms as defining resistance. All of these factors can confound data interpretation even when analysing data vertically within a country, but in horizontal analysis, across countries, it can become rather difficult. Franklin *et al.* (2001) published a guideline on the harmonisation of national antimicrobial resistance monitoring programmes in animals and animal-derived foods on behalf of the Office International des Epizooties (OIE). The objective of the guideline was to allow the generation of comparable data from national monitoring systems in order to compare the situations at the national and international levels. Other stakeholders have also drawn attention to the need for harmonisation and standardisation. For instance, the European Food Safety Authority (EFSA) has issued several reports in which the need to harmonise national surveillance in Europe has been described, most notably in the most recently published zoonoses report. Similarly, the European Centre for Disease Prevention and Control (ECDC), EFSA, European Medicines Agency (EMA) and Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) Joint Opinion on antimicrobial resistance have continued to emphasise the need for harmonisation. The issue in question is that all surveillance schemes do not define resistance in the same way. This means that it is not possible to simply compare resistant

rates from different surveillance schemes as they are not measuring the same parameter. Indeed, even within national surveillance schemes, methods of analysis have changed over time such that the percentage resistance values may not be comparable. There are two fundamental reasons for this being the case: (i) the trend for 'resistance' to be defined by the epidemiological cut-off value rather than by the long-established clinical breakpoint; and (ii) no standardisation on how to define the epidemiological or wild-type cut-off value.

Whilst the use of epidemiological cut-off values might be important for the detection of decreased susceptibility, it is inappropriate to use this value to determine the percentage clinical resistance. Additionally, whilst it is intuitive that decreased antimicrobial susceptibility may in time lead to clinical resistance, the hypothesis has not been tested.

DANMAP, MARAN and SVARM use epidemiological cut-off values to determine resistance, but they do not use the same values in all cases. VAV uses a combination of epidemiological cut-off values and clinical breakpoints. SVARM in 2008 made clear that whilst it uses epidemiological cut-off values to determine resistance it should be understood that this does not always imply clinical resistance. A change from clinical breakpoints to epidemiological cut-off values when determining the percentage resistance does matter depending, of course, on the antimicrobial class and bacteria of interest. One example that illustrates the point relates to *Salmonella* and fluoroquinolones. In MARAN 2004, ciprofloxacin resistance in all *Salmonella* (n=2195) was reported to be 0.3% applying a clinical breakpoint of >2 mg/l. In MARAN 2005, ciprofloxacin-resistance in all *Salmonella* (n=2238) was reported to be 10.1% as the epidemiological cut-off value of >0.06 mg/l was used, yet there was no change in the population susceptibility distribution. The reader may be misled into believing that clinical resistance has increased to 10.1%. Here, the differentiation between decreased susceptibility and clinical resistance is important. As an alternative to the indication of percent decreased susceptibility and percent clinical resistance, use of '% non-wild-type' may avoid misunderstandings and can be supplemented with the '% clinical resistance'.

Besides definition of resistance, other aspects of surveillance systems also need to be considered for harmonisation. One of them is the susceptibility testing method, which should be quantitative instead of qualitative, with inclusion of quality controls to allow the validation of results. All national surveillance programmes do not yet apply quantitative methods. In addition, it is important to make the point that, with regard to *Salmonella*, isolates do not have the same origin; DANMAP, MARAN and SVARM include *Salmonella* from subclinical and clinical infections in animals (i.e., which might be under treatment), whereas VAV and EASSA only collect *Salmonella* from healthy animals at slaughter. It must be further realised that MARAN pools all *Salmonella* from animal and human sources.

Surveillance data, when appropriately standardised and systematically collected, are useful for risk assessment to food safety, but the susceptibility of isolates from diseased animals may not appropriately reflect that of isolates from healthy animals at slaughter. It is also important to make reference not only to the percent decreased susceptibility and/or percent resistance but also to indicate the total number of strains screened (n) or the absolute numbers of decreased susceptible/clinically resistant strains. In many cases, the percent resistance is reported despite the 'n value' being very small; again, this can confuse objective analysis of the data.

Analysis of the data highlights the usefulness of using both epidemiological cut-off values and clinical resistance breakpoints for the purpose of detection of decreased susceptibility and development of clinical resistance, respectively. It can be concluded that harmonisation in resistance monitoring programmes is needed since there is potential for data to be appropriately used within risk analysis, providing the opportunity to implement appropriate risk management steps as a response to the public health issues arising from changes in antibiotic resistance in food-borne pathogens and commensal organisms.

# Why anti-inflammatory compounds are the solution!

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The increasing concerns about the use of antibiotics in animal production are mainly driven by the fear for inducing bacterial resistance against antibiotics. Whether or not this poses a real risk for humans is still hotly debated. Anyway, one could ask why there is such reluctance in the field to decrease the prophylactic and therapeutic use of antibiotics. The simple answer is that antibiotics are very cost effective promoters of growth and health in production animals. Therefore, farmers would only be willing to reduce dependence on antibiotics if effective alternatives are available.

The search for alternatives is hampered by misconceptions about the exact physiological mechanisms behind the growth promoting effects of antibiotics. Hitherto, the growth promoting effects of antimicrobial growth promoters (AGPs) were attributed to their antibiotic properties. This is highly unlikely for various reasons, the main one being the sub therapeutic concentrations used. Indeed, alternatives based on the antibiotic theory such as probiotics are not very successful. AGPs work much more likely as growth promoters by direct inhibition of the intestinal inflammatory response. This also explains why non-antibiotic anti-inflammatory compounds like acetylsalicylic acid have a similar growth promoting effect, although they require higher dosages (e.g., aspirin and paracetamol). In any case, it implies that alternatives to AGP and antibiotics should be anti-inflammatory compounds. The obvious advantage is that the argument of inducing bacterial resistance will become irrelevant. However, there is public resistance against the perceived abuse of not only antibiotics but also other medicines in animal production. This means that alternatives to antibiotics should preferably not be registered pharmaceuticals, because legislative action can be foreseen. In the longer term, most viable options could come from plants and plant extracts. Plants are perceived as green, and contain a plethora of candidates. Anti-inflammatory compounds can easily be selected by using simple *in vitro* assays, and subsequently tested *in vivo*. At least one compound has been successfully selected this way. It is expected that this and similar compounds will help to considerably reduce antibiotic use in animal production while maintaining profitability.

# EU regulation of feed and feed additives – strategic opportunities for alternatives to antibiotics

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The European Union (EU) completed its ban on antibiotic growth promoters (AGPs) in 2006 (Regulation EC No 1831/2003), although Sweden and Denmark had implemented largely voluntary bans in the 1990s. In fact, EU legislation affecting the food chain, has been substantially overhauled between 2000-2010, following the various food 'scandals' of the 1990s, notably the BSE crisis (Bovine Spongiform Encephalitis, 'mad cows'). Other public perceptions driving EU food chain legislation included those in relation to GMOs (Genetically Modified Organisms) and GMMs (Genetically Modified Micro-organisms) (Regulations EC No 1829/2003 and No 1830/2003); dioxins and PCBs (polychlorinated biphenyls), and, of course, the use of antimicrobials in food animals, and the dissemination of antibiotic resistance in the food chain and more generally from animals to humans (e.g., companion animals to owners and handlers).

The EU ban on AGPs has resulted in increased use of medicated feeds and veterinary antibiotics in animals, as illustrated by the DANMAP statistics from the mid-1990s onwards. However it should be taken into account that increased veterinary antibiotic use in Denmark has tracked increases in the production of food animal products. The EU AGP ban has also driven innovation in veterinary antibiotics, for example the development of long-acting injectable products for farm and other animals. Currently, in the EU, coccidiostats are the only 'classic' antimicrobials allowed as feed additives. Whereas veterinary antibiotics in the form of injectables and medicated feeds are still heavily used in the EU, there are a number of feed ingredients and feed additives that can help reduce dependence on antibiotics.

Currently, zinc oxide (ZnO) at 3,000 mg/kg may be used in some EU countries as a veterinary product to prevent diarrhoea in piglets. For example in Spain and Denmark, two of the largest pig-producing Member States in the European Union, most weaned piglets are fed diets containing 3,000 mg/kg, despite obvious environmental concerns. Porcine plasma was banned in the EU at the height of the 'mad cow' crisis, but is now permitted for pigs and poultry (since September 2005), and commonly used to provide highly digestible nutrients and antibodies to piglets in the period immediately post-weaning.

The core feed additive regulation (Regulation EC No 1831/2003) classifies feed additives into five categories: (1) technological; (2) sensory; (3) nutritional; (4) zootechnical; and (5) coccidiostats and histomonostats.

Innovative non-antibiotic products in most, if not all of categories 1-4 may help reduce the use of veterinary antibiotics. Additionally, a new feed regulation (Regulation EC NO 767/2009) allows limited claims in relation to gut function, physiology and nutrition, and this has also stimulated innovation in functional feed ingredients that may help reduce antibiotic use in animals.

Some additional examples of novel feed ingredients and feed additives are presented to demonstrate how such products may support animal health, improve gut function and directly or indirectly reduce the need for prophylactic and therapeutic veterinary antibiotics.

## Antibiotic alternatives: vaccine, probiotic, and functional metagenomic approaches

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Certain antibiotics are fed to livestock and poultry for disease treatment, disease prevention, or improved feed efficiency. Ongoing discussions in the US about limiting the use of antibiotics in agricultural animals are controversial. Both sides cite the need for more data and for effective alternatives. Work in our lab addresses both needs in the context of food animals: how do growth-promoting antibiotics affect the gut microbiota, and what novel techniques will mitigate the carriage of human pathogens?

The most efficacious alternatives for growth-promoting antibiotics will mimic the antibiotic effects on the host and its microbiota. However, the comprehensive effects of antibiotics are poorly defined. We used metagenomics and 16S rRNA gene sequence analyses to assess the impact of in-feed ASP250 (chlortetracycline, sulfamethazine, and penicillin) on the gut microbial community of swine. The results show measurable and repeatable shifts in certain members of the community, such as decreases in *Streptococcus* sp. and increases in *Escherichia* sp. with antibiotic treatment. Additionally, microbial functions relating to energy production and conversion increased in medicated animals. These data will inform future studies using pre- or probiotics to elicit comparable responses.

An additional function of antimicrobial alternatives is for disease treatment and prevention, including the elimination of bacteria that are commensals in animals but pathogenic in humans. Food-producing animals can be asymptomatic carriers of human foodborne pathogens such as *Salmonella* spp., *Escherichia coli* O157:H7, and *Campylobacter* spp. Vaccines are a promising approach to improve animal health, reduce antimicrobial use, and enhance food safety. An overview of our development of vaccines against *Salmonella* spp. in swine, *E. coli* O157:H7 in cattle, and *Campylobacter* spp. in turkeys will be presented. Pre- and probiotics are additional tools in the antibiotic alternative arsenal. We are currently developing the swine intestinal isolate *Mitsuokella jalaludinii* as a probiotic because work from our lab and others has shown it to be an effective inhibitor of *Salmonella* invasion in tissue culture. Finally, functional metagenomic experiments are underway for the identification of novel phage lysins and inhibitors of *Salmonella* invasion, and preliminary results will be reported.

# Improving animal hygiene and housing conditions and measuring its effect on the use of antibiotics

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The development of sulphonamides by Gerhard Domagk and the discovery of penicillin by Alexander Fleming have been celebrated as milestones in mankind's attempts to reduce premature death and pain and suffering due to disease. There are no hard data about the percentage of the causes of death prior to the establishment of any state health system, but it can be argued that infectious diseases have been the major death causes for thousands of years. This is definitively true for the centuries when, due to an increase of human populations moving and commingling throughout Europe and to the start of urbanisation without any coordinated sanitation, the main epidemics that killed millions of people were plague, cholera and typhoid. These bacterial epidemics had an exponentially higher death toll than any viral epidemic. There are no hard data to prove or disprove, if not many a million of the many millions of deaths due to the 1918 Hongkong flu epidemic were due to secondary bacterial infections that maybe dramatically increased the number of fatalities. There are speculations that the number of people that lost their lives in wars due to the direct effect of a weapon is manifold smaller than the number of wounded people that later died due to the bacterial infections caused by their being wounded. In other words the fact that mankind suddenly was able to cure bacterial infections and to fight life-threatening bacteria (chemotherapeutics/ antibiotics and disinfectants) was celebrated as man's victory over infectious disease.

Whereas in the early years of the availability of sulphonamides and antibiotics, only life-threatening infections of humans were treated, but soon more and more application areas were added: less harmful infections in humans, bacterial diseases in animals, more and more non-fatal diseases in humans up to the treatment of just 'annoying' infections such as common colds, growth promotion ('non-therapeutic use') in food animals, and the routine prophylactic and 'metaphylactic' use in large scale food animal production units. This development of expanding the use of antimicrobial substances would have remained being welcomed and undisputed, if there were not the phenomenon of acquired bacterial resistance in bacteria species that naturally are highly sensible to certain antimicrobial substances.

The initial hope that the threat of bacterial pathogens to human health had been banned by the use of antimicrobial substances was shattered. However, exactly this experience led, to increased research activities into the mechanisms of the development of bacterial resistance through either the acquisition of a plasmid or a single point mutation. The major step in developing a general understanding that maintaining the efficacy of whichever antimicrobial is the way we use them in human and veterinary medicine was that it was comprehended that any use of any antimicrobial compound leads to a selection pressure in the targeted (and the simultaneously occurring) bacterial populations towards bacteria that are less vulnerable (sensitive) and withstand the bactericidal or bacteriostatic mechanisms, and that it is necessary to develop intelligent strategies to minimise the magnitude of the selection pressure. This understanding was also supported by the relatively late realisation that bacterial populations are by far faster to adapt to antimicrobial compounds than humans are able to find or develop and produce new compounds.

The most important step in guiding medical and veterinary users of antimicrobial substances

as treatment of bacterial infections was the development of the concept of the 'prudent use of antibiotics', which is defined as applying antimicrobials in a way that leads to the highest possible health effect in humans or animals and to the lowest possible resistance in the bacteria that are exposed to the compound. WHO and FAO, but also many national medical and veterinary associations, chambers and international organisations such as FVE for veterinary medicine have issued guidelines on the prudent use of antibiotics. These guidelines for the prudent use of antibiotics are nowadays broadly accepted in the medical and veterinary professions and they have definitively led to a higher degree of compliance with practices that are known to reduce the development of bacterial resistance (including a better supervision of the application of antibiotics in feed and water by the farmers!).

However, to which degree the magnitude of bacterial resistance in veterinary medicine has been influenced by the principles of the prudent use of antibiotics is more or less unknown, since the existing data on bacterial resistance are hardly comparable not only from country to country, but also over time. The decision of the EU to command the EFSA (based on Article 33 of the Regulation EC No 178/2002) to carry out a standardised collection and analysis of data on zoonoses, antimicrobial resistance and food-borne outbreaks, is a major precondition for a meaningful measurement of the development of bacterial resistance specially in bacteria that cause zoonoses and foodborne diseases in humans. Unfortunately, the period of time, which EFSA needs to harmonise this data collection and to demonstrate make sound decisions on which measures which country needs to take, is longer than we need quiet down the still growing criticism with the use of antibiotics in food animals.

The still growing societal concerns expressed by public health authorities and organisations such as WHO, CDC and EFSA, is nowadays increasingly taken up by NGOs targeting modern animal production systems. And their 'cause' is, unfortunately, supported by an increasingly public discussion about multi-resistant *Salmonella* strains, MRSA in food producing animals (LaMRSA), and ESBL. Only one quote from the Global Edition of the New York Times (23 March, 2011) illustrates the general perception of the deficiencies in the use of antibiotics in general, but also in food producing animals: '...But antibiotics are frequently misused – overprescribed or incorrectly taken by patients, and recklessly fed to farm animals'.

As responsible researchers used to asking for sound data-based analyses we tend to ignore statements that lack reasonable evidence (show me the data that prove that the use of ... has led to...). However, in the light of our consensus on the fact that any use of antimicrobial substances leads to bacterial resistance as well in the targeted bacterial strains as in all other bacteria that are exposed to the antimicrobial in question, we need to agree on the demand for reducing today's amount of antibiotics and chemotherapeutics used in general in food animals as much as possible as long as we achieve the necessary health effect in case of acute infections. But here we need to accept that the rules for the prudent use of antibiotics are not able address the reliance of animal production on the routine use of antimicrobials. If bacterial disease is occurring in any animal population, it is the ethical duty of veterinarians to apply antimicrobial substances, of course following the rules of their prudent use. It has been ignored for too long that field veterinarians have not the legal 'power' to enforce their recommendations on how to improve animal health in food animal herds and flocks.

Especially in countries, where veterinarians are the only source of drugs for the farmers, it is widely believed that this fact is the major reason for a comparatively high consumption of antimicrobial substances in food animals. At first glance this seems to be supported by the relatively low amount of antibiotics (measured in mg/kg meat produced) in Norway, Sweden and Finland. However, Austria has the same low consumption, and it should not be forgotten, that the Scandinavian countries have a long tradition of animal health schemes including

long-standing and successful Salmonella monitoring and reduction programmes. And, the wide range from about 50 mg/kg meat produced in Ireland and Denmark up to more than 250 mg/kg in Greece and the USA shows that there must be other factors that much more influence the quantities of antimicrobial substances that are used in food animal populations than just the way how farmers are supplied with drugs.

Not only well-proven experiences of every single veterinary practitioner, but also a growing number of scientific papers on the huge variability of the amount of antimicrobials used in food animals at the herd and flock level, tell us that the animal-health awareness of farmers and their management skills determine the health status of the herd or flock in question, which in turn, determines the necessary amount of antibiotics applied or prescribed by the veterinarian.

The results of our research projects on testing the so-called 'Animal Treatment Index' and the Danish comparison of the use of antimicrobials per pig herd by measuring the Animal Daily Doses (ADD) prove that there is a need to revise the main stream attempts to force veterinarians to use less antimicrobials (which is twisting the arm of the wrong people) into first measuring the amount of antibiotics at farm level. Using a benchmarking system such as the 'Yellow Card Initiative', introduced last year in Denmark by the Danish Veterinary and Food Administration, will lead to concerted actions to measurably increase the health of the food animal populations, which 'automatically' will lead to a reduction of routinely administered antimicrobials in food animals.

Concluding, the paper speculates that there will be no significant reduction in the amount of antimicrobials used in food animals, unless farmers and veterinarians find new approaches to investing money in the health of herds and flocks, i.e., paying veterinary services for maintaining the animals' health rather than for curing their diseases.

# Veterinary education and antibiotics

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The phrase 'veterinary education and antibiotics' is used here to broadly refer to any educational effort that impacts all parties involved in using antibiotics in animals. Several related questions, outlined below, should be discussed by the interested parties at this conference and elsewhere, as we talk about the responsible use of antibiotics in animals.

Who needs educating (and how do we know they need educating)? The obvious answer to this question is veterinarians and veterinarians-in-training. But how do we know that learning about antibiotics actually has effects on use patterns which change resistance prevalence? Does educating veterinarians change the burden of resistance?

What should they be learning? Anecdotes about medical practices by individual veterinarians do not add up to a data-supported need for better education. An expert-based assessment of the educational needs along should drive what veterinarians and veterinary students learn about antibiotics. In addition, it would be far-reaching to suggest that changes in resistance patterns directly indicate a need for education, so other approaches are needed to evaluate the learning needs of practitioners.

How should they be learning? Currently available educational resources include veterinary (and human) textbooks in pharmacology, medicine, and infectious disease, published expert reviews, presentations, primary scientific literature, and web-based resources. There are few published data, however, on the success of the use of any of these resources (outcomes assessments): are veterinarians and veterinary students learning what they need to be learning about antibiotics?

When should they be learning? In an unpublished survey, we asked veterinary instructors about how many hours students should receive on antibiotics and antibiotic resistance, and it was clear that antibiotics do not dominate the time spent in veterinary school. In many countries, veterinarians must attend continuing education in order to maintain licensure. In the U.S., these requirements may be mandated at the state level, although veterinarians accredited to perform certain federal duties are going to be required to complete certain educational modules periodically. At the state level, there are few mandates as to what those hours of education must cover, only how many hours are needed. Because of the variety of veterinary practice, it would be difficult to mandate particular topics.

How will we know they are learning? The most direct question to ask is: does a lack of change in prevalence of resistance mean education is not occurring? Most current outcomes assessment of learning in veterinary education is limited to broad skills. Is it possible to measure prescribing behaviour at the clinic level to determine if educational needs are being met? This is certainly a question worth discussing, and it has occasionally occurred in human medicine, although the driving force is often the reduction in costs and days in the hospital rather than anything directly related to antibiotic resistance.

# Innovation in regulatory agencies to keep pace with innovative technology

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Regulatory agencies worldwide are tasked with reviewing dossiers and approving new molecular entities when data demonstrate a favourable benefit:risk ratio. This requires a thorough review of available data to ensure the product in question is effective for its intended use, safe to the target species and the environment with an acceptable safety margin, and if indicated for use in a food producing species, safe for humans consuming the edible products of treated animals. Regulatory reviewers must also ensure the product can be manufactured in a consistent manner with appropriate controls and that a label can be written to ensure the safe and correct use of the drug. For many products, guidance documents are often available to assist sponsor companies with study design, protocol development, data evaluation and final report formatting. They are also an important source of information on how an agency currently evaluates medicines within specific therapeutic areas. Regulatory agencies will also generally provide information on the construction of dossiers and instructions for submission in their respective geographies.

Under the umbrella of VICH, regulatory agencies across the globe have collaborated to produce guidance documents that provide industry with information that may allow the building of dossiers with greater geographic regulatory acceptance. There are several good examples of how VICH has increased the global reach of regulatory dossiers; one such area is in the evaluation of new antimicrobials (VICH GL27 and GL36). While the VICH concept and the issuance of regulatory guidance documents are generally favourable to industry for known therapeutic products, they are, by definition, always behind the curve with respect to emerging technology. For example, GL27 and GL36 are based on known chemical classes with known mechanisms of action. Because they cannot predict from where technological advances will come, they cannot adequately address new areas of research. How then, can industry properly evaluate new technological advancements to design an appropriate development plan with a high likelihood of regulatory success?

The short answer to the question above is, 'they can't'. The longer and more complete answer is that they must work closely with regulatory agencies to determine the most appropriate approach for the specific technology. This is complicated because not all regulatory agencies have an approach that allows for technological discussions prior to dossier submission. In Europe, the European Medicines Agency has the provision for paid scientific advice. When there is pre-existent knowledge about the technology in question, sponsors can write questions specific enough so that the resulting advice is sufficiently specific and relevant to the technology. When the technology is emergent, it is less likely that specific questions can be written. In those instances, only general concepts can be addressed, leaving only general answers to be provided. The result can be increased ambiguity rather than increased clarity.

Another approach worth noting, although early in concept and practice, is the approach undertaken by the U.S. FDA Center for Veterinary Medicine (CVM). Around March of 2010, CVM began to address emerging technology (i.e., innovation). The reasons, stated by Dr. Steven Vaughn at the DIA conference in London, March 2011 were, 'To engage in the development and evaluation of new animal drugs, especially new innovative technologies, to meet the demand for increased safe, affordable and abundant food production'. During the

meeting, he further asserted, 'The products of the future will not fit the current paradigm. Products of the future will deploy new technologies for which ONADE (Office of New Animal Drug Evaluation) has not considered the critical safety and effectiveness standards for evaluation'. Therefore, working within the ONADE Dr. Vaughn worked to (i) create a cross-divisional advisory group to imaginatively and creatively develop new ways of embracing innovation, IVET; (ii) visit the pharmaceutical companies and other R&D groups to talk about bringing new innovation through the animal drug evaluation process; (iii) establish a new innovation working group to replace the multi-cycle review teams at AHI; (iv) establish technology teams to tackle new technologies; and (v) work with sponsors earlier, before the pre-submission conference.

Successful implementation of the Innovation Initiative above can be defined as employing new scientific approaches for proving safety and effectiveness, providing predictability in regulatory decisions, avoiding the lure of increasing scientific rigor beyond that which is necessary and addressing ambiguity. The resultant theoretical 'win' for sponsors could be two-fold: development of new approaches and engagement to enable a new technology to be met with the same predictability and seamless regulatory process that a traditional animal drug meets at CVM and the removal of barriers that prevent a pharmaceutical company from filling their pipeline with new innovative products.

The technology team concept is shown in Figure 1. The basic premise is that well in advance of a pre-submission conference, the sponsor would have the opportunity to teach CVM about the technology and CVM could then identify risk questions that would need to be addressed as part of the project development plan. By addressing these questions early, the sponsor would have a more predictable regulatory process moving forward. The IVET approach is more general in nature and with AHI is working to establish best practices for evaluating innovative technologies. Consideration is being given to processes for decisions on what is innovative, training programs for review staff, processes for information management and improving tech team/sponsor interactions.

The presentation will focus on the IVET/Tech Team approach as a potential regional or global approach for regulating innovative technologies. It will address the concepts, what has been working well and what could be improved.

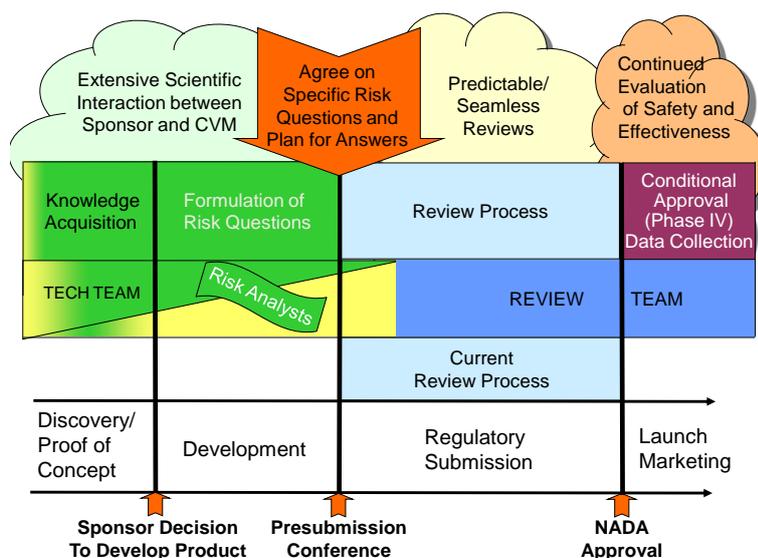


Figure 1. Technology Team concept. From Dr. Steven Vaughn, with permission.

# The role of future veterinary diagnostics in responsible use of antibiotics

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A confident diagnosis of animal diseases can sometimes be made on the basis of clinical signs or symptoms, but an accurate diagnosis usually requires specific diagnostic tests, which may be performed in point-of care veterinary clinics, but often involve access to a diagnostic laboratory.

Improved, quality assured diagnostics are important for disease control in animals; they provide a basis for appropriate treatment of animal patients, for monitoring diseases and for the enhancement of disease-surveillance capacity. Early diagnosis and treatment of diseases not only reduces the risk of the animal patient developing long-term complications, but for some diseases a prompt treatment will also reduce further transmission of the disease to other animals and to humans, and has important implications for a responsible use of drugs, including antibiotics. Good quality diagnostic tests that are adequate for their purpose and provide accurate results are therefore of paramount importance in reducing the burden of infectious diseases in animals and controlling the possibility of transmission to humans.

The clinical veterinarian is often confused, in choosing an immunodiagnostic test, by the lack of regulatory controls on the quality of diagnostics for veterinary purposes. Often the selection of a test is based only on information provided in the product insert or on published data that often originate from inadequate or flawed study designs. To be useful, diagnostic methods must be accurate, simple and affordable. They must also provide results in time to institute effective control measures, particularly treatment. Therefore, several parameters shall be taken into considerations when choosing a diagnostic test: sensitivity, specificity, positive and negative predictive values, ease of use, condition of use and storage, and shelf life. In the field of veterinary diagnostics, there is an increasing need for simple, rapid, point-of-care immunodiagnostic tests that do not require special training and dedicated technical personnel.

The analysis of biological molecules has become increasingly important over the past few years, due in part to the potential threat that viruses, bacteria and toxins pose as warfare agents to animal and human health. Consequently, the science and engineering of the detection, identification and quantification of small quantities of target biomolecules are rapidly expanding fields of endeavour.

The past decade has brought impressive advances in surface and materials science and engineering, as well as in the development of new microelectronic components. These tools hold the promise of miniaturizing diagnostic devices, which could dramatically reduce costs and increase the throughput and sensitivity of a wide range of diagnostic tests for veterinary applications. They also raise the tantalizing possibility of so-called point-of-care diagnostics in veterinary medicine, in which an entire test is contained in a hand-held device. Most authors agree that microfluidics, nanotechnology, microarrays and other such devices have made steady progress and are now sufficiently advanced to act as individual components of an integrated device. It seems likely that the next generation of protein-based predictive assays will have to be rigorously quantitative, internally and externally standardized, and objectively reproducible.

Recent biotechnological developments, including micro- and nanotechnologies, have led to the proliferation of new, rapid diagnostic tests that hold promise for the improved management and control of infectious disease in animals. Clinical diagnostics applied to the diagnosis of diseases in animals is one of the most promising applications in veterinary medicine for such microfluidic lab-on-a-chip systems, especially in a point-of-care setting.

Microsystems and miniaturised assays are regarded as some of the key technologies for future progress in biochemistry, biotechnology and medicine. The advantages of miniaturisation are many and include lower reagent and energy consumption, less space requirement and lower manufacturing costs, which could lead to disposable units. Electronics and photo-electronics for detection can be integrated on-chip together with the analytical systems, which enable the development of in-field/in-situ/point-of-care analysers. Moreover, reduced molecular diffusion paths (i.e., faster kinetics) and more efficient patterns of reactions, both due to the small dimensions, may improve the analytical performance.

Microfluidics has slowly matured over the past twenty years, leading some to hope for a 'lab-on-a-chip' device capable of purifying, isolating and characterizing samples in one neat package. The devices promise greatly reduced sample and reagent volume. As with microfluidics, micro-electromechanical systems encompass the fabrication of microscopic electronic devices using techniques akin to those used in making silicon computer chips. Devices have been built with reservoirs, pumps, cantilevers, rotors, channels, valves, sensors and other structures from biocompatible materials.

The development of micro-analytical devices has also brought revolutionary changes to the area of deoxyribonucleic acid (DNA) assays. These micro-scale analyzers benefit not only from the reduction in sample/reagent volumes but also from enhanced analytical performance (e.g., speed and sensitivity). Most importantly, miniaturization offers the opportunity to integrate all the functional steps of the DNA analysis into a single microchip-based device. This integrated micro-total analytical system permits full automation, thereby minimizing sample contamination from manual operation.

Finally, microarrays are potential components in a wide variety of miniaturized diagnostics; they are the furthest along of the miniaturization technologies and have made inroads in diagnostics in human medicine, with interesting perspectives also in veterinary diagnostics.

## **Antimicrobial prescribing in veterinary practice is evidence-based: true or false?**

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Antimicrobial resistance (AMR) is currently a major public health risk. Inappropriate use of antimicrobials has been associated to AMR in both human and veterinary medicine. This has led to several initiatives and national and international level for the monitoring and surveillance of AMR and antimicrobial consumption in humans and animals. Guidelines promoting the responsible use in veterinary medicine are currently implemented in several countries and campaigns to raise awareness of the general public and the veterinary professionals for the risks of AMR are becoming more common.

Responsible use of antimicrobials should follow an evidence-based approach to minimise the risks of therapeutic failure and reduce the risk of occurrence of AMR. The application of evidence-based medicine involves the selection of antimicrobials based on clinical reasoning by the veterinary surgeon after evaluation of the evidence available collected through clinical examination, previous history of the animal and results diagnostic tests. The veterinary surgeon must also take into account current available scientific knowledge. Furthermore, the veterinary surgeon should follow guidelines and recommendations for responsible use of antimicrobials and follow the legislation implemented (i.e. Cascade) when prescribing antimicrobial therapy for their patients. However, there is currently scarce data on how what factors influence veterinarians when prescribing antimicrobials. Moreover, there is little evidence of the effectiveness and feasibility of current guidelines and recommendations in everyday practice. Empirical use of antimicrobials in veterinary practice is still common. Insufficient evidence may tamper the decision-making process if it compromises obtained a final diagnosis of the condition observed. This could lead to the selection of less efficacious or less adequate antimicrobials for animal therapy. The perceived expectations of animal owners, the risk of poor compliance, the animal characteristics, the cost of proper diagnostic procedures and the availability of drugs can influence the selection of antimicrobials by the veterinary surgeon. Additionally, lack of knowledge on available antimicrobials (i.e., pharmacodynamics, pharmacokinetics), therapeutic protocols and on the epidemiology of infectious diseases can also prevent the veterinarian from making an informed decision when prescribing antimicrobial therapy. This could result in the loss of antimicrobial efficacy if resistance emerged.

Evidence-based medicine should be taught and promoted to the veterinary profession. Guidelines and recommendations based on current available scientific evidence should be implemented in practice. Effectiveness studies of current guidelines should be implemented. The findings of these studies should be used to inform policy-makers working on strategies to tackle AMR in veterinary practice. Veterinarians need to be proactive in the acquisition of scientific knowledge and clinical skills for work in everyday practice that will help them improve their usage of antimicrobials. It is vital that veterinarians learn how to review critically published studies and assess validity of scientific findings in the literature available. It is important that veterinary surgeons evaluate the potential risks for resistance derived from antimicrobial usage in practice and take this into consideration when selecting antimicrobials, together with animal health and welfare issues.

## Case study: Guidelines for evidence-based responsible antimicrobial treatment of pigs in Denmark

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The guidelines for evidence-based responsible antimicrobial treatment of pigs in Denmark are based on available scientific evidence and for every combination of class of antimicrobials, swine disease and pathogen. An assessment of responsible use is provided by the Danish Veterinary and Food Administration (DVFA) in a simple spreadsheet on-line ([www.dvfa.dk](http://www.dvfa.dk)). The spreadsheet presents all currently registered veterinary antimicrobial products for the specific diseases in drop down lists with recommended dosages and treatment periods registered along with a colour coding – in red, yellow or green – indicating the most responsible treatments and giving the swine practitioners a simple choice.

**Background.** The new guidelines are the third step elaboration of responsible use treatment guidelines; the guidelines are part of the ongoing risk management strategy in Denmark for optimisation of antimicrobial consumption and reduction of antimicrobial resistance.

The guidelines result from a strong collaboration between all stakeholders in a task force hosted by the DVFA. Members of the task force are: the Danish Veterinary Association, the Danish Association of the Veterinary Pharmaceutical Industry, epidemiologists and risk assessors from the Danish Agriculture & Food Council and DVFA, researchers in pharmacology and swine diseases from the Faculty of Life Sciences, Copenhagen University as well as researchers and microbiologists from the National Food Institute (NFI) and the National Veterinary Institute (NVI).

**Objectives.** The guidelines are directed towards swine practitioners. In Denmark, all veterinary medicinal products are prescription only and this places the veterinary practitioners as key persons in responsible antimicrobial usage. The veterinary practitioners should use the guidelines as a working tool in their counselling of preventive veterinary strategies in herds, thereby optimizing antimicrobial usage with due consideration to both human and animal health. They can use the colours to choose a responsible treatment and the drop down lists to find products, dosage and treatment period. The evidence behind the scoring within the four criteria can be found in additional spreadsheets in the guidelines. Moreover, the DVFA control and supervision team, who visits all large animal practitioners biannually, uses the guidelines in their supervision of responsible use of antimicrobials.

**Methods.** Based on the available evidence, the different classes of antimicrobials are assessed by the following four criteria: (i) clinical documentation of efficacy; (ii) susceptibility based on national microbiological data; (iii) pharmacokinetics; and (iv) risk profiling of the human health concerns when the antimicrobial agents are used for veterinary antimicrobial treatment. The actual ranking is based on the scoring shown in Table 1, and the data evidence behind the ranking is given in separate spreadsheets in the guidelines.

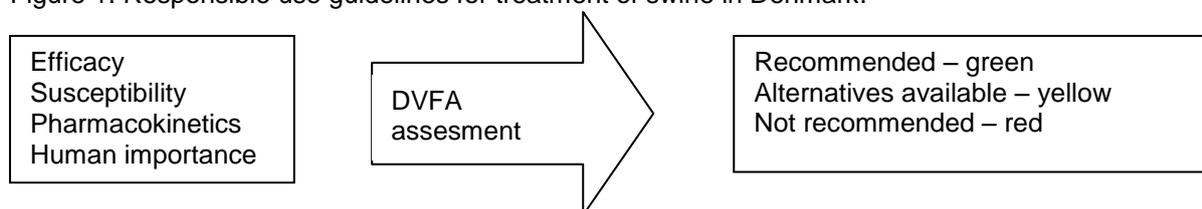
Table 1. Categorisation of antimicrobial agents in the guidelines.

Category	Scoring
Efficacy	1= Documented in SPC <sup>1)</sup> 2= Documented in peer-reviewed papers, EMA or FDA papers
Susceptibility	Percent susceptible among isolates sent to NFI and NVI
Pharmacokinetics	Based on ratio of MIC_Kill <sup>2)</sup> to MIC50: 1= 0-0,19; 2= 0,2-0,39; 3= 0,4-0,59;
Human importance	4= 0,6-0,79; 5= >0.8 Based on FDA and OIE guidelines: 1= very high; 2= high; 3=medium; 4= low; 5= very low

<sup>1)</sup> Summary of product characteristics (SPC). <sup>2)</sup> MIC\_Kill is the concentration at the site of infection at the time where 80% of the dosing interval has passed and where the animal has been administered the recommended dose.

The risk profiling is done according to the principles of FDA guidance 152 as well as the principles in OIE-guidelines. For every antimicrobial group, it is estimated whether the probability for selection of antimicrobial resistance, exposure of humans and human consequences are very high, high, medium, low or very low. Based on these estimates, a common estimate for the human health consequences of the use of this antimicrobial group for swine is estimated. The common estimate gives a qualitative ranking of the expected future human health consequences by antimicrobial usage for swine of the different antimicrobial groups (Table 1). Recommendations of usage of antimicrobial classes for the specific diseases (site of action) and specific pathogens are indicated by three different colours, green, yellow and red (Figure 1).

Figure 1. Responsible use guidelines for treatment of swine in Denmark.



Green indicates antimicrobials that are recommended to be used for that specific disease and pathogen combination. The green labelled antimicrobials will have susceptibility above 80%, good pharmacokinetics and a risk profiling of human health consequences assessed to have no or only low consequences and preferably also evidence based documentation of clinical efficacy. Yellow indicates antimicrobials that can be used, but where better alternatives are available. Red indicates antimicrobials not recommended due to an estimated high human health consequence or a very low susceptibility. Examples of red labelled antimicrobials are enrofloxacin, marbofloxacin and cephalosporins, but other antimicrobials with a low score on susceptibility for a specific pathogen are also labelled with a red colour.

## **Responsible use – contribution of the animal health industry**

### **Olivier Espeisse**

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IFAH (International for Federation Animal Health) is the organization representing manufacturers of veterinary medicines, vaccines and other animal health products in both developed and developing countries across five continents. The industry approach for use of antimicrobial agents relies on three fundamentals: (i) risk analysis; (ii) monitoring and surveillance; and (iii) responsible use.

Responsible use is encouraged and sponsored by many other organizations. Specifically, IFAH and its members are engaged in numerous activities regarding responsible use, at multinational, regional and national level, in collaboration with different stakeholders like government agencies, non-governmental organizations, veterinary and farmer organizations. The presentation will detail some examples of IFAH's activities around responsible use. In conclusion, responsible use is critical as, in spite of future innovations, use of antimicrobials for animals will continue to be necessary.

## **The responsible use of antimicrobials on a global level: the view of the profession**

**Tjeerd Jorna**

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Antimicrobial resistance is a problem that threatens both animal health and public health. Resistance to antimicrobials has the potential to take away this tool to protect animal health in two ways: (i) loss of effectiveness due to the development of resistance to antimicrobials by animal pathogens, and (ii) through the loss of approval to use important antimicrobials in animals in order to preserve their use in human medicine. Therefore, to protect the effectiveness of antimicrobials to treat animal and human diseases the World Veterinary Association (WVA) has developed responsible use guidelines for veterinarians. The WVA believes that the use of the guidelines will lessen the development and spread of antimicrobial resistance.

The guidelines recognize and acknowledge the fact that veterinarians must balance the sometimes competing needs of animal health and public health. Human medical providers are not challenged with achieving that balance. Instead they only need to concern themselves with protecting the health of humans. Decisions must be science-based and risk-based. Risk analysis needs to consider both the benefits and the risks to human health that are created through the use of antimicrobials to treat, control and prevent diseases in animals.

The use of the risk analysis process (risk assessment, risk communication, and risk management) can result in different risk management decisions in different countries or regions in the world because of differences in risk tolerances and in the respective importance given to human health over animal health. For example in the United States, the previously approved use of fluoroquinolones to treat colibacillosis in poultry has been withdrawn while other countries and regions continue to use fluoroquinolones. Similarly, Europe and other regions have banned the use of antimicrobials to promote feed efficiency and growth in animals while other countries and regions have not. These differences are due to a multitude of factors such as differing animal production systems, different patterns of antimicrobial use in animals and humans, differing acceptance of risk by different cultures, different values placed on the importance of animal health, different recognition of the benefits to human health from the use of antimicrobials in animals.

## Prudent use of antibiotics: an animal nutrition company's perspective

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The availability of antibiotics to prevent and treat infectious diseases has radically improved animal wellbeing and performance. Paradoxically, this very success threatens the future utility of antibiotics. Emerging antibiotic-resistant bacteria have become a major concern. As a result the development of alternative strategies is receiving high priority.

The main way forward to effectively establish prudent use of antibiotics is to implement prophylactic measures. Important advances have been realised in high health systems, vaccination strategies and livestock precision farming. Simultaneously, dietary strategies to improve animal health emerged. The feed supplier is an important stakeholder in the chain with co-responsibility to establish a prudent use of antibiotics.

The implementation of advanced HACCP based quality assurance systems in the animal feed industry is the basis. It will control the risks of presence of undesired substances in feed that may be harmful for animal health. Secondly, feeding strategies can be designed and applied that lower the risk of gastrointestinal disorders. Feeds with a higher digestibility of the ingredients, reduced protein content and with specific functional fibres or structure are known to lower the risk of diarrhoea in piglets and wet litter in broiler chickens. Special vitamin and trace element formulations can support the functioning of the immune system. Moreover, various feed additives have entered the market in recent years to support animal health, such as prebiotics, probiotics, bactericidal products and immune enhancers.

A challenge for the nutritionist is to implement strategies that have a good fit with each category of farm performance. Many feed companies have therefore economical least-cost feed programs for high-health farms besides more prophylactic programs for farms with suboptimal animal health.

Looking to the future, the impressive research developments in the 'omics' area will enable us to make fast scientific progress in the area of nutrition and health. Nutreco Research and Development recently adopted in its research program these technologies. This has led to new promising concepts for support of animal health.

## **Demonstrable controlled prudent use of antibiotics in the food chain**

**Bert Urlings**

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The use of antibiotics in animals that produce food needs to be controlled for various reasons, e.g., firstly individuals that work in the direct surrounding of the animals need to be protected from unnecessary contact with antibiotics, secondly the spread of residues in the environment needs to be prevented, thirdly the dispersion of resistant micro-organisms into the environment must be omitted, fourthly food items need to be free of residues of antibiotics and finally food items need to be controlled on the presence of food safety hazards.

Antibiotics are needed to treat animals in order to protect the welfare of the individual animal and unwanted spread of disease. The presence of ESBLs in men that originate from poultry meat shows that additional measures need to be taken to prevent the dispersion of this food safety hazard in the food supply chain. After the recognition that ESBLs are a food safety hazard a more stringent control of the use of antibiotics in the food supply chain is needed. HACCP rules prescribe that food business operators (FBOs), including farmers, need to show that they have controlled also ESBLs in the supply chain. This implies that all FBOs must demonstrate how they have controlled this risk. Several possibilities to comply with this legal requirement will be discussed.

# Antibiotics and animals – communicating societal uncertainties to an unknown public

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Controversial issues associated with the use of animal antibiotics may amplify consumer risk perceptions, in particular if such controversies are associated with disagreement between different scientific experts, or if associated risks are regulated differently in different regions of the world. Specific examples include the use of antibiotics as growth promoters in animal feed, where the use of sub-therapeutic doses of antibiotics in food-producing animals has been linked to antibiotic resistant infections in humans, although there is some uncertainty regarding this link, with differentiation in regulation regarding this practice in different parts of the world. Public negativity towards intensive animal husbandry systems may also reduce consumer acceptance of foods produced using non-therapeutic antibiotics, or even those which are applied as preventative treatments rather than in response to specific illnesses. Quality assurance certification may take non-therapeutic antibiotic use into account, suggesting that meat quality is dependent on the extent to which antibiotics are not routinely used in animal production systems. In addition, public concern may also be driven by other issues associated with antibiotic prescription to humans, for example advice from medical regulators to reduce prescriptions for some antibiotics in areas of the world where antibiotic resistant strains of bacteria causing human illness have evolved. Despite these controversies, there is little data available to allow systematic comparison of public attitudes and perceptions in different regions of the world. The available data suggest that levels of public awareness of the issue is low, but that, once consumers are aware of the issue, they tend to express negative attitudes. For example, Bostrom [1] notes that around 25% of American consumers are aware of the issue but, once they have been provided with information, 57% express a preference to avoid these products. From the few studies available, the data tend to suggest that generally consumer awareness is low, but become negative if information is provided or if consumers are 'prompted' by inclusion of a question about animal antibiotic use in a survey. It has also been suggested that other potentially controversial technologies, such as nanotechnology applied to animal production, may be more acceptable because of consumer negativity towards the use of antibiotics in animal husbandry [2], although empirical evidence to support this contention is required. Some scholars have argued that the economic benefit for consumers (lower meat prices) may potentially outweigh the perceived risks to human and animal health [3] although again empirical evidence to support this is scarce.

At this point, it is relevant to review the key determinants of consumer perceptions of risk, in particular in the context of the agrifood sector. From here it may be possible to extrapolate some policy implications regarding risk communication and management specifically relevant to antibiotic use. In general, potential hazards risks which are perceived to be unnatural, and to which people perceive that they are exposed on an involuntary basis, are more negatively viewed by the public than risks which are perceived to be natural, or to which people choose to expose themselves. Consumer decision making regarding consumption of foods associated with a specific type of risk depends on the characteristics of that risk- for example, "dietary" related hazards such as those associated with the negative health impacts of poor diet tend to be under rated by consumers, whereas the risks of food processing technologies are perceived to be more threatening, in part because they are associated with unnaturalness and involuntary consumer exposure. In the case of food

technologies in particular, the issue of distribution of equity of benefit associated with a specific technology application is highly relevant – for example, if the benefits are perceived to accrue to producers but the risks, even if they are (perceived to be) very small, are thought of as accruing to consumers, then consumer negativity will result. If the technology does not produce concrete and tangible consumer benefits, it is unlikely that the technology will be accepted by consumers (for review, see [4]). From this one might predict that consumer attitudes towards antibiotic use in meat production are more likely to be negative, unless lower prices are perceived to be a benefit which outweighs potential human and animal health risks, assuming these price reductions are passed on to consumers. It is also important to consider the issue of uncertainty associated with potential impacts of non-therapeutic use of animal antibiotics, either in terms of human and animal health or the potential impact on the environment.

Given that the topic of animal antibiotic use is controversial, and increasingly the focus of media debate, it is important that interested stakeholders, (for example, regulatory authorities or producers) instigate an appropriate communication strategy. This would need to address issues associated with both risks and benefits, including any uncertainties associated with these. In addition, trusted and transparent labelling practices may increase consumer confidence, (for example, linked to organic accreditation where non-therapeutic antibiotics are not used in production). However, if public concerns focus on generalised risk to human and animal health, and the environment, which cannot be mitigated by individual consumer choice, then research must be conducted to reduce uncertainties in the risk (benefit) assessments which inform the process of risk analysis and regulation associated with the use of antibiotics in livestock production. Communication with the public should focus not only on risk (benefit) assessment but also on management strategies adopted by regulators and other stakeholders.

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## Conclusions & path forward

### Brief presentations and panel discussion

#### Moderator

Prof.dr. Peter Silley MB Consult Limited and University of Bradford, UK

#### Panel members

Dr. Annette Cleveland Nielsen Danish Veterinary and Food Administration, Denmark

Dr. Christine Hoang American Veterinary Medical Association, USA

Peter J.G. Oostenbach, M.Sc. MSD Animal Health, the Netherlands

Dr. Stephen Page Advanced Veterinary Therapeutics, Australia

Prof.dr. Yong Ho Park Seoul National University, Korea

Dr. Thomas R. Shryock Elanco Animal Health, USA

During this three-day conference the present and future of the use antibiotics in animals have been discussed from different viewpoints. But has the path forward become clearer? What of the future?

During the first part of this final plenary meeting the panelists will give brief summaries tying the keynote talks to the content throughout the conference to the 'take-home' messages.

During the second part of the discussion questions from the participants will be answered.

## POSTERS

- P1 *PremiTest<sup>®</sup>, an efficient antibiotic screening assay*  
M. Mehl, T. Czymai and B. Reck  
R-Biopharm AG, Germany
- P2 *Modelling the effect of enforcement strategies to improve food safety – a case study on antibiotic use*  
E.D. van Asselt<sup>1</sup>, P. Sterrenburg<sup>1</sup> and S. Osinga<sup>2</sup>  
<sup>1</sup>RIKILT-Institute of Food Safety, Wageningen UR, the Netherlands and <sup>2</sup>Wageningen University, Logistics Decision and Information Sciences, the Netherlands
- P3 *Farm factors related to the use of antibiotics in pig farming in the Netherlands*  
H.J. van der Fels-Klerx, L.F. Puister-Jansen, E.D. van Asselt and S.L.G.E. Burgers  
RIKILT-Institute of Food Safety, Wageningen UR, the Netherlands
- P4 *Trends in antimicrobial resistance of indicator bacteria derived from foods*  
In-Sun Joo, Soon-Ho Lee and In-Gyun Hwang  
Food Microbiology Division, Food Safety Evaluation Department, National Institute of Food and Drug Safety Evaluation, Korea Food and Drug Administration, Korea
- P5 *Cordyceps militaris fermentatives as antibiotics alternative to enhance performance and modulate immune response of weaned piglets*  
Yeong-Hsiang Cheng, Chiu-Ming Wen, C.-M.J. Yang and Su-Der Chen  
Institute of Biotechnology, National I-Lan University, Taiwan and Department of Life Sciences, National University of Kaohsiung, Taiwan
- P6 *Consumption of antimicrobials in livestock in Germany – the pilot study ‘VetCab’*  
R. Merle<sup>1</sup>, P. Hajek<sup>2</sup>, A. Käsbohrer<sup>3</sup> and L. Kreienbrock<sup>1</sup>  
<sup>1</sup>WHO-Collaborating Centre Veterinary Public Health, Department of Biometry, Epidemiology and Information Processing, University of Veterinary Medicine, Germany, <sup>2</sup>Institute for Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, Universität Leipzig, Germany and <sup>3</sup>National Reference Laboratory for Antimicrobial Resistance, Department for Biological Safety, Federal Institute for Risk Assessment, Germany
- P7 *Emerging antimicrobial resistance against fluorochinolones and cephalosporins with public health relevance in commensal E. coli from animals and food in Germany*  
A. Käsbohrer, B.-A. Tenhagen, B. Guerra-Román, B. Appel and A. Schroeter  
National Reference Laboratory for Antimicrobial Resistance, Department for Biological Safety, Federal Institute for Risk Assessment, Germany
- P8 *Antimicrobial activity of crude extracts of Cassia surattensis Burm f.*  
K. Motina<sup>1</sup>, P. Wongwathanarat<sup>2</sup> and K. Charoenpornsook<sup>3</sup>  
<sup>1,2</sup>Department of Biotechnology, Faculty of Science and Technology, Thammasat University, Thailand and <sup>3</sup>Department of Food Technology, Faculty of Science and Technology, Thammasat University, Thailand
- P9 *Determination of antibiotic residues in food products in Thailand*  
K. Charoenpornsook<sup>1</sup> and P. Kavisarasai<sup>2</sup>  
<sup>1</sup>Department of Food Science and Technology, Faculty of Science and Technology, Thammasat University, Thailand and <sup>2</sup>Bureau of Quality Control of Livestock Product, Department of Livestock Development, Feed Quality Control Laboratories, Thailand

- P10 *Diversity of antimicrobial resistance profiles and PFGE patterns among vanA-type vancomycin-resistant Enterococcus faecium isolated from broilers, poultry slaughterers and hospitalized humans in Greece*  
I. Tzavaras<sup>1</sup>, V.I. Siarkou<sup>1</sup>, A. Zdragas<sup>2</sup>, C. Kotzamanidis<sup>3</sup>, G. Vafeas<sup>2</sup>, E. Chatzopoulou<sup>1</sup>, S. Pournaras<sup>4</sup> and D. Sofianou<sup>5</sup>  
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- P11 *Effect of a mixture of cinnamaldehyde, carvacrol and capsicum oleoresin on immunity against infection with Eimeria tenella in vaccinated broilers*  
H. Lillehoj<sup>1</sup>, S.H. Lee<sup>1</sup>, S.I. Jang<sup>1</sup>, K.W. Lee<sup>1</sup>, M.S. Park<sup>1</sup> and D. Bravo<sup>2</sup>  
<sup>1</sup>Agricultural Research Service, U.S. Department of Agriculture, USA and <sup>2</sup>Pancosma SA, Switzerland
- P12 *Effect of a mixture of capsicum and turmeric oleoresins on immunity against infection with Eimeria tenella in vaccinated broilers*  
H. Lillehoj<sup>1</sup>, S.H. Lee<sup>1</sup>, S.I. Jang<sup>1</sup>, K.W. Lee<sup>1</sup>, M.S. Park<sup>1</sup> and D. Bravo<sup>2</sup>  
<sup>1</sup>Agricultural Research Service, U.S. Department of Agriculture, USA and <sup>2</sup>Pancosma SA, Switzerland
- P13 *Effect of plant extracts on performance and immune status of weaned pigs experimentally infected with porcine reproductive and respiratory syndrome virus*  
Y. Liu<sup>1</sup>, M. Song<sup>1</sup>, T.M. Che<sup>1</sup>, J.A. Soares<sup>1</sup>, D. Bravo<sup>2</sup>, C.M. Maddox<sup>1</sup> and J.E. Pettigrew<sup>1</sup>  
<sup>1</sup>University of Illinois, USA and <sup>2</sup>Pancosma SA, Switzerland
- P14 *Effects of plant extracts on diarrhoea, gut morphology, immune and inflammatory status of weaned pigs experimentally infected with a pathogenic E. coli*  
Y. Liu<sup>1</sup>, M. Song<sup>1</sup>, T.M. Che<sup>1</sup>, J.A. Soares<sup>1</sup>, D. Bravo<sup>2</sup>, C.M. Maddox<sup>1</sup> and J.E. Pettigrew<sup>1</sup>  
<sup>1</sup>University of Illinois, USA and <sup>2</sup>Pancosma SA, Switzerland
- P15 *Contributing to the new concept of 'One World One Health'. Preliminary results of the study of methicillin-resistant Staphylococcus aureus (MRSA) – joining the veterinarian and medical efforts*  
C. Ortega<sup>1</sup>, M.C. Simón<sup>1</sup>, M. Zarazaga<sup>2</sup>, A. Rezusta<sup>3</sup>, E. Gomez-Sanz<sup>2</sup>, I. Ferrer<sup>3</sup>, M.J. Revillo<sup>3</sup>, P.-F. Nima<sup>3</sup> and C. Torres<sup>2</sup>  
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- P16 *SAPUVET and SAPUVETNET projects: a contribution to the understanding of the prudent use of antimicrobials*  
SAPUVETNET project, Universidade de Evora, Portugal

- P17 *Patterns of resistance to antibiotics in S. aureus and MRSA isolated in dairy herds (Aragón-Spain) – role in veterinary public health*  
C. Ortega, M. Espada and M.C. Simón  
Preventive Veterinary Medicine, Veterinary Faculty, University of Zaragoza, Spain
- P18 *Antimicrobial susceptibility of Escherichia coli isolated from acute bovine mastitis*  
L. Suojala<sup>1,2</sup>, H. Simojoki<sup>1</sup>, A.-L. Myllyniemi<sup>3</sup>, A. Pitkälä<sup>2</sup> and S. Pyörälä<sup>1</sup>  
<sup>1</sup>Faculty of Veterinary Medicine, University of Helsinki, Finland, <sup>2</sup>Central Union of Agricultural Producers and Forest Owners MTK, Finland and <sup>3</sup>Research Department of Finnish Food Safety Authority, Helsinki, Finland
- P19 *Evaluating in vitro susceptibility of bovine mastitis pathogens to a combination of kanamycin and cefalexin: recommendations for a disk diffusion test*  
C.M. Pillar<sup>1</sup>, L. Goby<sup>2</sup>, D. Draghi<sup>1</sup>, P. Grover<sup>1</sup> and C. Thornsberry<sup>1</sup>  
<sup>1</sup>Eurofins Medinet, USA and <sup>2</sup>Boehringer Ingelheim Animal Health, Germany
- P20 *In vitro activity profile of Ubrolexin® (kanamycin and cefalexin combination) against bovine coagulase-negative staphylococci and correlation between MIC and disk zone sizes*  
C.M. Pillar<sup>1</sup> and L. Goby<sup>2</sup>  
<sup>1</sup>Eurofins Medinet, USA and <sup>2</sup>Boehringer Ingelheim Animal Health, Germany
- P21 *Pan-European resistance monitoring programs of CEESA encompassing foodborne pathogens, commensal bacteria and target pathogens of food-producing and companion animals*  
V. Thomas, A. de Jong, U. Klein, H. Marion, S. Simjee and M. Vallé  
CEESA Study Groups, Belgium
- P22 *Analysis of antimicrobials prescribing patterns in dogs in small and mixed animal veterinary practices*  
L. Pokludová, J. Bureš and A. Hera  
Institute for State Control of Veterinary Biologicals and Medicines, Czech Republic
- P23 *Measuring the real carry-over of oxytetracycline in medicated feed production*  
V. Manti<sup>1</sup>, T. Zuidema<sup>1</sup>, H.J. van Egmond<sup>1</sup>, A.A. M. Stolker<sup>1</sup>, J. de Jong<sup>1</sup> and R. Herbes<sup>2</sup>  
<sup>1</sup>RIKILT-Institute of Food Safety, Wageningen UR, the Netherlands and <sup>2</sup>Food and Consumer Product Safety Authority, the Netherlands
- P24 *Microbial screening methods for antibiotic residues*  
M.G. Pikkemaat and H.J. van Egmond  
RIKILT-Institute of Food Safety, Wageningen UR, the Netherlands
- P25 *Extent and implications of carry-over of antibiotics in porcine feed production*  
T. Zuidema<sup>1</sup>, H.J. van Egmond<sup>1</sup>, J. Hooglugt<sup>2</sup>, P. Bikker<sup>1</sup>, E. Olde Heuvel<sup>2</sup> and L. Stolker<sup>1</sup>  
<sup>1</sup>RIKILT-Institute of Food Safety, Wageningen UR, the Netherlands and <sup>2</sup>Food and Consumer Product Safety Authority, the Netherlands
- P26 *A new breakthrough for poultry producers to manage coccidiosis using plant extracts*  
M. Forat<sup>1</sup>, B. Gozlan<sup>2</sup>, F. Clément<sup>2</sup> and F. Recoquillay<sup>2</sup>  
<sup>1</sup>IIIA, Mexico and <sup>2</sup>Phytosynthese, France

- P27 *Quantification of antimicrobial usage in farm animals: ABcheck, a free online web application*  
M. Postma<sup>1</sup>, D. Persoons<sup>1</sup>, D. Maes<sup>2</sup> and J. Dewulf<sup>1</sup>  
<sup>1</sup>Unit of Veterinary Epidemiology/<sup>2</sup>Unit Porcine Health Management, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Belgium
- P28 *Preliminary results on prophylactic and curative antimicrobial usage on 20 Flemish pig herds and advices on improvement*  
M. Postma<sup>1</sup>, D. Persoons<sup>1</sup>, D. Maes<sup>2</sup> and J. Dewulf<sup>1</sup>  
<sup>1</sup>Unit of Veterinary Epidemiology/<sup>2</sup>Unit Porcine Health Management, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Belgium
- P29 *Investigation of the relationship between biosecurity measures and production, health- and treatment-characteristics in pig herds*  
M. Laanen<sup>1</sup>, D. Persoons<sup>1</sup>, S. Ribbens<sup>2</sup>, E. de Jong<sup>1</sup>, B. Callens<sup>1</sup>, M. Strubbe<sup>2</sup>, D. Maes<sup>1</sup> and J. Dewulf<sup>1</sup>  
<sup>1</sup>Unit of Veterinary Epidemiology, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University Belgium and <sup>2</sup>Animal Health Care Flanders, Belgium
- P30 *Comparative study on group level antimicrobial use between 2003 and 2010 in Belgian pig herds*  
B. Callens<sup>1</sup>, D. Persoons<sup>1</sup>, D. Maes<sup>1</sup>, F. Boyen<sup>2</sup>, F. Haesebrouck<sup>2</sup>, P. Butaye<sup>3</sup> and J. Dewulf<sup>1</sup>  
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- P31 *HatchBrood: a tool to reduce antibiotic usage in poultry*  
R. Molenaar  
HatchTech B.V., the Netherlands

P1

## PremiTest<sup>®</sup>, an efficient antibiotic screening assay

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Due to increasing worldwide human population and decreasing space for farming and changing demands for human food consumption, more intensive animal farming will be necessary. This will require on one hand the application of increased antibiotic treatment. However, widespread use of antibiotics in animal farming may select more resistant bacteria or worse multiple resistant pathogens. Furthermore frequent uptake of antibiotics in food is discussed to cause negative effects in humans. Therefore demands for an easy to use, fast and sensitive screening test exists. Positive results of such a presumptive screening assay should be analysed with reference methods. A most easy to use screening system for laboratories as well as for food producers is the PremiTest<sup>®</sup> produced by DSM and distributed by R-Biopharm AG. This test is based on microbiological growth inhibition of spores of *Bacillus stearothermophilus* pre-filled in ampoules after treatment with the samples. Bacterial growth can be detected by colour change of the pH indicator bromocresol purple and the interpretation may be performed by eye or using flatbed scanners and specific software. The overall procedure time of the kit is approximately 4 hours and does not require sophisticated devices or intensive skills. The limit of detection (LOD) of most antibiotic parameters is below the maximum residue levels (MRL) of the Commission Regulation EU No. 37/2010. In contrast, the sensitivity for some parameters as triphenylmethane derivates as malachite green is lower (LOD>1 ppm) and this assay is not suitable for aquaculture where the use of malachite green is suspected. For other commodities as egg, meat, fish and poultry, the PremiTest<sup>®</sup> may serve as an efficient easy to use presumptive screening assay under current legislation.

P2

## **Modelling the effect of enforcement strategies to improve food safety – a case study on antibiotic use**

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According to the General Food Law, food producers are responsible for the production of safe products. Safe in this regard is often interpreted as compliant with EU food safety legislation. The level of compliance between companies differs and can be improved by measures such as education or sanctions. In order to determine the effectiveness of various enforcement strategies on the level of compliance we developed a simulation tool using Agent Based Modelling (ABM) as a method. This ABM tool allows to simulate with actions and reactions between autonomous agents, yielding an emerging overall effect. This emerging effect will give valuable insight in how the overall behaviour of the system and the individual behaviour of agents mutually depend on each other. As a case study, we focused on the use of antibiotics within primary pig production. The agents in this case were defined as individual farmers and food safety inspectors. The food safety inspectors could either educate or give sanctions. The ABM model showed that – given the assumptions – inspection frequency and sanctions had more effect on compliant behaviour than societal control or education. Furthermore, following a risk-based approach in inspections resulted in an overall increase in compliant behaviour. The model proved to be a powerful tool in exploring potential effects of different enforcement strategies on compliant behaviour.

P3

## **Farm factors related to the use of antibiotics in pig farming in the Netherlands**

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The aim of this study was to investigate farm-level economic and technical factors that are associated with the use of antibiotics on pig farms. Identification of such factors, like farm size and net farm result, may help to increase epidemiological knowledge and to specify farm advice and policy making to reduce the inappropriate use of antibiotics. The study used over 300 farm-year records collected in the period of 2004-2007 from pig farms in the Netherlands. Data included economic and technical factors as well as antibiotic administration. The data were statistically analyzed for factors associated with the use of antibiotics, both for fattening pig and sow farms (piglets only), separately. The response variable was the average number of daily dosages per average pig year. A set of in total 16 and 19 potential explanatory factors was analyzed for the fattening pig and sow farms, respectively. The results showed that, both on the fattening pig and sow farms, the average use of antibiotics increased over the years of 2004-2006, but decreased during 2007, but the effect of year was not significant ( $P>0.05$ ). The use of antibiotics varied highly between individual farms. A large farm repeatability for the use of antibiotics in the different years was found. Factors associated ( $P<0.05$ ) with the use of antibiotics included: farm system, number of pigs, and population density in the region of the farm (for sow farms only). As these factors are easy to collect and to register, they can be used to specify farm advice and investigation, as well as for policy making. The majority of the technical and economic factors were not significantly related to the on-farm use of antibiotics. Therefore, it is recommended to focus future research on the potential role of socioeconomic factors associated with antibiotic use on pig farms.

P4

## Trends in antimicrobial resistance of indicator bacteria derived from foods

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KFDA has monitored antimicrobial resistance of food poisoning bacteria from 2003 as part of a national surveillance called 'Korea National Antimicrobial Resistance Monitoring Programme'. Tetracycline antibiotics, in particular, are appointed as critical subject to control because they are the most used veterinary antibiotics in Korea and have high resistance rates among foodborne disease and indicator bacteria. Therefore the government implemented policies to reduce resistance such as forbidding addition of chlortetracycline into animal feed in 2005. As a result, usage of veterinary tetracycline antibiotics had decreased from 774 tons in 2002 to 284 tons in 2010. An investigation on *Escherichia coli*, *Enterococcus* and *Staphylococcus aureus* of antimicrobial resistance isolated from animal and marine products showed that resistance rate of *E. coli* had reduced from 80% in 2003 to 66% in 2010, of *Enterococcus* from 75% to 52%, and of *S. aureus* from 86% to 65%. However there was no change among other antibiotics.

P5

## ***Cordyceps militaris* fermentatives as antibiotics alternative to enhance performance and modulate immune response of weaned piglets**

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*Cordyceps militaris* is a fungus used in traditional Chinese medicine. *C. militaris* fermentatives (CMF) contain cordycepin which has many pharmacological properties including antiviral, antifungal and antitumor activities. In order to evaluate the effect of CMF on growth performance and immunocompetence of piglets, 144 weanling animals were allotted four different groups. They were feed for 4 weeks with diet supplemented with 0 (control group), 500, 1,000, or 1,500 µg CMF/kg feed. At 21 and 36 days of age the animals were immunized with a commercial hog cholera vaccine. At the end of the experiment, 6 to 12 randomly selected pigs in each treatment group were further analyzed for their biochemical and immune responses. CMF supplementation significantly increased growth performance in weaned piglets. Animals receiving the feed supplemented with 1,000 µg CMF/kg feed, had body weight gain, average daily gain and feed conversion rate increased by 11.5%, 14.9% and 6.1%, respectively when compared with animal receiving control feed. In addition, when added at 1,000 and 1,500 µg/kg, CMF decreased the serum concentration of aspartate aminotransferase, alanine aminotransferase, glucose, and triglycerides but did not modulate the concentration of creatinine and cholesterol. Analysis of the cytokines mRNA expression in the spleen indicated that CMF supplementation significantly increased the synthesis of Th1 cytokines, as indicated by the level of IL-2 and IFN-γ. By contrast, the CMF supplementation only had moderate effect on the mRNA expression level of the Th2-tested cytokines, IL-4 and IL-10. As expected from the increase expression of Th1 cytokines, the animal feed with the CMF supplement also displayed an increased cellular immune response. Indeed, alveolar macrophages isolated from piglets supplemented with 1000 and 1,500 µg CMF/kg feed had significantly higher chemotactic and phagocytotic indexes than those isolated from animal receiving control feed or feed supplemented with 500 µg CMF/kg. In relation with the absence of effect on Th2 cytokines, the CMF supplement had no effect on hog cholera antibody titer. In summary, feed supplementation with CMF improves growth performance, and enhances cell-mediated immunity. CMF supplementation may thus be useful at weaning to counteract the physiological and immunological stress during this period.

P6

## Consumption of antimicrobials in livestock in Germany – the pilot study ‘VetCAB’

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As in several other European countries, data on the consumption of antimicrobials in animal husbandry shall be continuously collected in Germany. To take into account national legislation, a national route for data collection needed to be developed. To achieve this, during the years 2007 and 2008 a feasibility study was conducted where different routes of data collection were evaluated. On the basis of these results, a pilot study was started with the aim to collect representative data. During 2011/2012 data are collected in both, animal holdings and veterinary surgeries on the antimicrobials used. A representative sample is collected in 8 provincial districts distributed over Germany and ensuring representativeness for the country. In this pilot study, fattening pigs, sows, piglets, dairy cattle, veal calves, beef cattle, laying hens, broilers and turkeys are covered and data are collected for each population separately. The data are collected retrospectively via the forms obligatory by German law concerning the treatment of animals and the delivery of animal drugs to the animal owners by the veterinarian. All information is entered into an online database and stored anonymously. To recruit a representative sample of farms and veterinarians, regional structures are analysed in detail beforehand. To achieve this, on the basis of statistical data for each provincial district, Germany is split into regions with similar density of animal husbandry (by each animal species). From each of these regions, 1 to 3 provincial districts are selected for the study. Participating farmers get access to the online-database via internet and are trained in data submission. This should enable them to complete the data collection sheets ensuring high data quality. Veterinarians participating in the study select suitable farms which are under their supervision. Data transfer is handled by specific interface between their computer software and the project database. In each region, about 3% of the animals by animal species are selected for data collection in the pilot study. This is achieved by selecting a sufficient number of medium and large sized holdings in each of the study regions. Farms are visited directly or contacted via the veterinarian. Data analysis will be focused on the amount of antimicrobials consumed, the number of applications as well as an estimate of the average number of applications for an animal in a holding or region. By fully taking into account experiences collected during the feasibility study, it is expected that representative data will be collected for each of the animal production groups targeted. Furthermore, experience from this study will be used to contribute to a harmonisation process for collection of data on consumption data on European level.

P7

## **Emerging antimicrobial resistance against fluoroquinolones and cephalosporins with public health relevance in commensal *E. coli* from animals and food in Germany**

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Resistance to antimicrobials in zoonotic bacteria and commensals is of special concern since this may compromise the effective treatment of infections in humans. To assess these risks and to identify factors of major impact, a systematic monitoring approach was established in Germany covering each of the major food production chains within a 3-year interval. All isolates were tested using the broth dilution method according to NCCLS/CLSI standards M31-A3 and minimum inhibitory concentrations were evaluated according to epidemiological cut-off values as published by EUCAST. Within the years 2009 and 2010, 3,802 *Escherichia coli* isolates could be collected, covering primary production, animals at slaughterhouses and animal derived food at retail level. Whereas the majority of isolates from laying hens and dairy cattle were susceptible to the fourteen antimicrobials tested, most isolates from broilers, turkeys, veal calves, chicken meat and turkey meat were resistant to at least one antimicrobial class. Most of these isolates were even resistant to several antimicrobial classes. Besides resistance to the commonly used antimicrobial classes, e.g., sulphonamides and tetracyclines, resistance to (fluoro)quinolones and cephalosporins was frequently observed. Resistance rates to ciprofloxacin, the fluoroquinolone tested, were highest in broilers and chicken meat, ranging between 43% and 54% resistant isolates. In turkeys and turkey meat, ciprofloxacin resistance was slightly lower, ranging between 30% and 34%. In the veal production chain, resistance to ciprofloxacin decreased markedly from 42% in veal calves tested at primary production, to 13% in calves tested at slaughterhouses and 4% in veal sampled at retail. In contrast, resistance rates to (fluoro)quinolones in *E. coli* isolates from laying hens, dairy cattle and pork were below 10%. Resistance to ceftazidime, a 3rd generation cephalosporins was observed in isolates from all production chains studied but till now in a low level. Highest rates could be observed in broilers, where an increase from 5.9 % to 13.5% was observed from 2009 to 2010 and chicken meat (6.2 %). The observed resistance levels to (fluoro)quinolones and cephalosporins in commensal *E. coli* isolates are of concern since these are critically important antimicrobials in human medicine. The emerging pattern warrants close monitoring and regular assessment. Together with continuous monitoring of the antimicrobial usage, this may allow for assessing and adapting management strategies continuously.

P8

## Antimicrobial activity of crude extracts of *Cassia surattensis* Burm f.

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*Cassia surattensis* Burm f. is a shrub plant which has been known for its diverse biological and pharmacological properties. Preliminary studies on crude extracts from various parts of *C. surattensis* Burm f. were examined for both antibacterial and fungal activity using agar well diffusion method. It was found that crude extracts from stem showed antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli* with the average of inhibition zone 25.2 and 25.1 mm, respectively. Minimum inhibitory concentration (MIC) of stem extract against both species was 1.57 mg/ml. Crude extracts from flower and leaf also showed antibacterial activity against *Klebsiella* sp. with the inhibition zones and MIC values ranging from 19.8-21 mm and 3.13-6.25 mg/ml, respectively, while crude extracts from root, stem, leaf and flower only showed antifungal activity against *Trichophyton mentagrophytes*. The average inhibition zone and MIC were found to be between 16-24 mm and 6.25-12.5 mg/ml, respectively.

## Determination of antibiotic residues in food products in Thailand

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Antibiotics are a group of important medicines in animal breeding farms. It is not only for curing animal diseases, but also for accelerating animal growth. Using antibiotics in inappropriate quantities and over prolonged time can lead to residues of antibiotics in the animals' tissue. The purpose of this study was to find out the occurrence of antibiotic residues in our main food (pork, poultry, egg, fish, shrimp and meat ball). Therefore, 621 samples were randomly chosen from fresh markets in Bangkok and the perimeter from 2004 to 2009. There were 121 samples of pork, 139 samples of fish, 142 samples of shrimp, 55 samples of chicken meat, 29 samples of chicken's liver, 92 samples of egg and 43 samples of meat ball. For antibiotic determination by ELISA the results showed that: (i)  $\beta$ -agonist was found in 100% of the pork samples (121/121, average 2.39 ppb); (ii) chloramphenicol was found only in 1% of both the pork and fish samples (0.2 ppb and 0.1 ppb); (iii) enrofloxacin was found in 41% of the fish samples (56/139, average 1.35 ppb), 65% of the shrimp samples (93/142, average 75.69 ppb), 38% of the chicken meat samples (21/55, average 5.02 ppb), 42% of the egg samples (39/92, average 16.57 ppb); (iv) sulfamethazine was found in 100% of both the pork (14/14, average 2.74 ppb) and chicken meat samples (28/28, average 6.89 ppb), 85% of the fish samples (80/94, average 4.51 ppb), 75% of the shrimp samples (68/91, average 15.21 ppb), 97% of the chickens' liver samples (28/29, average 13.64 ppb); (v) tetracycline was found in 41% of the fish samples (12/29, average 0.63 ppb), 96% of the shrimp samples (92/96, average 0.63 ppb); (vi) in meat ball only tetracycline was found (7% of the samples, 5/43). Overall, the antibiotic residues were lower than the maximum residue limits (MRLs).

P10

## Diversity of antimicrobial resistance profiles and PFGE patterns among *vanA*-type vancomycin-resistant *Enterococcus faecium* isolated from broilers, poultry slaughterers and hospitalized humans in Greece

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During the last two decades enterococci have emerged as important nosocomial pathogens mainly due to their resistance to glycopeptides vancomycin and teicoplanin. The use of glycopeptide avoparcin as growth promoter in farm animals and poultry resulted also in the emergence of vancomycin-resistant enterococci (VRE) in livestock, indicating that food-producing animals might be a potential reservoir for VRE. In Greece, there is no knowledge on VRE prevalence in broilers after the avoparcin ban. The present research was designed to investigate the prevalence of VRE in broiler production environment in Greece and their epidemiological relationship with human clinical VRE from the same geographical regions. Caecal content from broilers (n=500) from eight livestock farms and faecal samples from poultry slaughterers (n=50), all collected in two slaughterhouses, were analyzed for species and vancomycin-resistance gene identification using multiplex PCR. For the epidemiological analysis 63 human clinical vancomycin-resistant *Enterococcus faecium* (VREF) isolates were also examined. All VRE isolates were tested for their susceptibilities to 14 antimicrobial agents. The relationship of antimicrobial resistance profiles among broiler, poultry slaughterer and human clinical VREF isolates was determined using discriminant analysis (DA). Pulsed-field gel electrophoresis (PFGE) was conducted to study the genetic relatedness of the *vanA* gene-harboring VREF isolates. Multiplex PCR revealed the presence of 79 VREF *vanA* (14.4%) and 41 *Enterococcus gallinarum vanC* (8.2%) isolates with VREF isolates recovered from 5 out of 8 (62.5%) broiler farms. From the poultry slaughterer samples 10 VREF (20%) were recovered. Differences in resistance rates were revealed among VREF isolates from the three sources, with broiler isolates being constantly resistant to tetracycline and the vast majority of human clinical isolates being resistant to ampicillin. The human clinical VREF isolates showed clearly higher rates of multiresistance compared to broiler isolates. DA correctly classified 100% of broiler, 85.7% of human clinical but only 50% of poultry slaughterer VREF isolates, with 40% of the latter assigned closely to broiler source. PFGE analysis, however, revealed patterns clearly related to their source clustering broiler isolates distinctly from all human isolates. The results indicate a remarkable persistence of VREF even 8-11 years after the avoparcin ban. Human and broiler VREF isolates created clearly unrelated populations, with no sharing of any clones even in the case of close contact like that between broilers and poultry slaughterers despite the close association exhibited by DA. Analysis of 'broiler-specific' *vanA* elements will clarify their possible circulation among broiler and human environments and the mechanisms of VREF long-term persistence in Greece.

P11

## Effect of a mixture of cinnamaldehyde, carvacrol and capsicum oleoresin on immunity against infection with *Eimeria tenella* in vaccinated broilers

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Plant extracts are known to have a positive effect on the modulation of innate immunity in birds, while vaccines act as stimulating the development of acquired immunity. Therefore using plant extracts should enhance the immunity induced by vaccination in *Eimeria* infected birds. The objective of this trial was to evaluate the effects of an encapsulated blend of carvacrol, cinnamaldehyde, and capsicum oleoresin (XT, XTRACT® 6930, Pancosma) on local and systemic immune responses following immunization of chickens with an *Eimeria* recombinant protein. Chickens were fed from hatch with a non-supplemented diet (CT), or with diets supplemented with XT (XT), animals were immunized subcutaneously with profilin 7 days post-hatch, and orally challenged with virulent oocysts of *Eimeria tenella* 17 days post-hatch. Immunity against infection was evaluated by body weight gain, faecal oocyst shedding, anti-profilin serum antibody levels, splenic lymphocyte profilin recall responses, intestinal levels of cytokine gene transcripts, and peripheral blood lymphocyte subpopulations. Differences were considered as significant at  $P < 0.05$ . The infection reduced BW of birds, but vaccination maintained BW of challenged animals at the same level as unchallenged ones. Then, all vaccinated birds exhibited reduced oocysts excretion compared to non-vaccinated animals. Profilin-immunized and *Eimeria*-infected chickens fed XT had increased body weight gains compared with immunized and infected animals given CT. However, faecal oocyst shedding was similar among treated groups. Anti-profilin antibody levels, but not cell proliferation, were increased in birds given the XT-supplemented diet. Decreased levels of transcripts for IL-17F and TNFSF15 were observed with XT compared to non-supplemented controls but only in infected chickens. Transcripts levels of IFN- $\gamma$  and IL-6 were similar among CT and XT in vaccinated birds. Animals given the XT containing diet only exhibited an increase in K1+ macrophages. In conclusion, these results show that dietary supplementation of a mixture of capsicum oleoresin, cinnamaldehyde and carvacrol improves immune parameters following recombinant protein vaccination against avian coccidiosis.

P12

## Effect of a mixture of capsicum and turmeric oleoresins on immunity against infection with *Eimeria tenella* in vaccinated broilers

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Plant extracts are known to have a positive effect on the modulation of innate immunity in birds, while vaccines act as stimulating the development of acquired immunity. Therefore using plant extracts should enhance the immunity induced by vaccination in *Eimeria* infected birds. The objective of this trial was to evaluate the effects of a mixture of turmeric and capsicum oleoresins (XT, XTRACT® Nature, Pancosma) on local and systemic immune responses following immunization of chickens with an *Eimeria* recombinant protein. Chickens were fed from hatch with a non-supplemented diet (CT), or with diets supplemented with XT (XT), animals were immunized subcutaneously with profilin 7 days post-hatch, and orally challenged with virulent oocysts of *Eimeria tenella* 17 days post-hatch. Immunity against infection was evaluated by body weight gain, faecal oocyst shedding, anti-profilin serum antibody levels, splenic lymphocyte profilin recall responses, intestinal levels of cytokine gene transcripts, and peripheral blood lymphocyte subpopulations. Differences were considered as significant at  $P < 0.05$ . The infection reduced BW of birds, but vaccination maintained BW of challenged animals at the same level as unchallenged ones. Then, all vaccinated birds exhibited reduced oocysts excretion compared to non-vaccinated animals. Profilin-immunized and *Eimeria*-infected chickens fed XT-supplemented diets had increased body weight gains compared with immunized and infected animals given the non-supplemented diet. However, faecal oocyst shedding was not affected in the experimental vs. control groups. Immunized chickens given XT-supplemented diet displayed increased anti-profilin antibody levels and greater profilin-induced lymphocyte proliferation compared with non-supplemented controls. Prior to *Eimeria* infection, immunized chickens fed XT had reduced levels of IFN- $\gamma$  and IL-6 mRNAs, and increased expression of TNFSF15, compared to non-supplemented controls. Interestingly, post-infection levels of IFN- $\gamma$  and IL-6 were increased, while IL-17F transcripts were decreased, with XT supplementation. Finally, immunized chickens fed XT exhibited increased percentages of MHC class II+, CD4+, CD8+, TCR1+, and TCR2+ lymphocytes compared to non-supplemented controls. In conclusion, these results show that dietary supplementation of a mixture of capsicum and turmeric oleoresin improves immune parameters following recombinant protein vaccination against avian coccidiosis.

P13

## Effect of plant extracts on performance and immune status of weaned pigs experimentally infected with porcine reproductive and respiratory syndrome virus

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Recent research has shown that dietary modulation could affect the immune response of non-gut tissues. Plant extracts have demonstrated potent effects on gut function and immunity. Therefore the objective of this work was to evaluate the effects of 3 plant extract on performance and immune response of weaned pigs experimentally infected with porcine reproductive and respiratory syndrome virus (PPRSV). 64 Weaned pigs (7.8 kg bodyweight, 21 days old) were housed in disease containment chambers for 28 days and used in a 2\*4 factorial design. First factor was with or without PRRSV challenge after 14 days (0 day post infection, DPI). Second factor was the diet type: control diet (CON), 10 ppm *Capsicum* oleoresin (CAP), turmeric oleoresin (TUR) or garlic (GAR). Performance parameters were determined at -14, 0, 7 and 14 DPI. Rectal temperature was measured every 3 to 4 DPI. Blood samples were collected 0, 7 and 14 DPI for determination of serum viral load (SVL), PRRSV antibody titers (AT), white blood cells (WBC), serum TNF- $\alpha$ , C-reactive protein (CRP) and haptoglobin. In the absence of infection, no animal was PRRSV positive. GAR increased CRP and monocytes at 7 DPI and decreased monocytes and the ratio neutrophils: lymphocyte. CAP increased lymphocyte at 7 DPI and improved final BW and ADFI for 0 to 14 DPI. In challenged animals, infection reduced ( $P<0.01$ ) performance, WBC, lymphocytes at 7 DPI and neutrophils:lymphocyte ratio at 7 DPI. Rectal temperature, SVL, AT at 14 DPI, TNF- $\alpha$ , CRP, haptoglobin, lymphocytes at 14 DPI and the neutrophils:lymphocyte ratio at 14 DPI were increased ( $P<0.05$ ). Compared to unsupplemented group, CAP reduced ( $P<0.05$ ) rectal temperature (-0.5 °C) at 4 DPI and TNF- $\alpha$  (-18.6%), CRP (-32.0%), SVL (-14.7%) at 7 DPI and increased haptoglobins at 14 DPI. GAR enhanced ( $P<0.05$ ) BWG from 0 to 7 DPI (328 vs. 236 g/d) and haptoglobins at 14 DPI (+66.9%) and decreased ( $P<0.05$ ) rectal temperature at 14 DPI (-0.5 °C). TUR improved ( $P<0.05$ ) BWG from 7 to 14 DPI (469 vs. 333 g/d) and G:F from 0 to 14 DPI (0.58 vs. 0.42) and AT (+23.7% S/P ratio) and reduced SVL (-14.9%) and TNF- $\alpha$  (-22.3%) at 7 DPI. This trial shows that dietary supplementation of these plant extracts affected performance and immune response of pigs challenged with PRRSV. The immunomodulatory effects of CAP and TUR can be profitable to the pigs.

P14

## Effects of plant extracts on diarrhoea, gut morphology, immune and inflammatory status of weaned pigs experimentally infected with a pathogenic *E. coli*

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Plant extracts are known to positively affect gut function and immune modulation of healthy animals. So the objective of this trial was to evaluate if these beneficial effects were also observed in weaned piglets infected with a pathogenic F-18 *E. coli*. 64 Weaned pigs (6.3 kg bodyweight, 21 days old) were housed in disease containment chambers for 11 days and used in a 2\*4 factorial design. First factor was with or without an F-18 *Escherichia coli* challenge (toxins LT, STb, SLT-2) with 10<sup>10</sup> cfu/3 ml daily oral dose for 3 days from day 0. Second factor was the diet type: control diet (CON), 10 ppm *Capsicum* oleoresin (CAP), turmeric oleoresin (TUR) or garlic (GAR). Performance parameters were measured at day 0, 5 and 11. On day 5 and day 11, half of the pigs were euthanized to collect intestine to measure villi height (VH), crypt depth (CD), and their ratio (VH:CD). Diarrhoea (DS) was daily scored individually (1, normal, to 5, watery diarrhoea). Frequency of diarrhoea (FD) was the percentage of pig days with DS ≥ 3. Blood was collected on day 0, 5, and 11 to measure white blood cell (WBC) counts, serum TNF-α and haptoglobin. The infection reduced overall performance and VH and increased DS and FD as expected. It increased (*P*<0.05) lymphocytes, TNF-α and haptoglobin on d5, and WBC, neutrophils, lymphocytes, monocytes, and haptoglobin on day 11. In uninfected pigs, plant extracts improved (*P*<0.05) ADG from day 0 to 5 and reduced average DS from day 3 to 5 and FD. On day 5 CAP decreased (*P*<0.05) haptoglobin. GAR increased (*P*<0.05) monocytes and decreased (*P*<0.05) haptoglobin. In challenged groups, the supplementation reduced (*P*<0.05) DS from day 0 to 2 (-1.2 points) and day 6 to 11 (-2.0 points), and overall FD (20 vs. 40%). It increased ileal VH on day 5 (+20.2%, *P*<0.05), jejunum VH (+17.0%, *P*≤0.1) and VH:CD (+23.5%, *P*≤0.1), without affecting growth performance. CAP decreased (*P*<0.05) TNF-α (-22.8%) and haptoglobin (-41.2%) on day 5, and WBC (-32.9%) and neutrophils (-39.4%) on day 11. GAR decreased (*P*<0.05) lymphocytes (-35.2%) and haptoglobin (-36.6%) on day 5, and WBC (-28.6%), lymphocytes (-43.4%), and haptoglobin on day 11. TUR decreased (*P*<0.05) TNF-α (-20.7%) on day 5 and neutrophils (-40%) on day 11. In conclusion, these three plant extracts affected performance, gut health and humoral and cellular immune responses of pigs infected with *E. coli*.

P15

## **Contributing to the new concept of ‘One World One Health’. Preliminary results of the study of methicillin-resistant *Staphylococcus aureus* (MRSA) – joining the veterinarian and medical efforts**

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Along the last decades of the 20th century the World Health Organization (WHO) was concerned about the emergency of new and old infectious and parasitic diseases around the world. Entering the 21st century the WHO established a series of important decisions to control diseases diffusion defining the new concept of ‘Global Health Security’. It was elaborated into a list of the most important health threats highly recommending the collaboration between the different health statements concerned, clamping the idea of ‘One World One Health’. Antimicrobial resistance was considered one of the most important threats and the emerging MRSA in the veterinary and medical fields is on top of this ranking. In 2004, the Infectious Diseases Diagnostic Laboratory of the Veterinary Faculty of Zaragoza (Spain), started the collection of some of the bacterial strains defined as health threats by the WHO (*Staphylococcus aureus*, *Staphylococcus intermedius/pseudintermedius*, coagulase-negative staphylococci, *Escherichia coli* and *Pseudomonas aeruginosa*). Later, the study of MRSA centred on the main objective of the study. More recently, the medical and veterinary efforts has been joined to contribute to the knowledge of these important resistant bacteria. This communication presents the preliminary results on antibiotic resistance in MRSA and *S. pseudintermedius* after the medical and veterinary collaboration. This study started with 28 *Staphylococcus* spp. strains recovered from 21 dogs, 5 horses, one cat and one rabbit. All animals but two dogs showed any kind of pathology. Isolates were identified by biochemical (Microscan®-Post Combo Panel, PC31 and Brilliance MRSA agar 2, Oxoid) and molecular methods (PCR and PCR-RFLP). The susceptibility to antibiotics was tested by disc diffusion and the minimal inhibitory concentration test was carried out in parallel. The presence of *mecA* gene was tested by PCR. The following staphylococcal species were detected in our collection of isolates: 8 *S. aureus* (2 dogs, 5 horses and one rabbit), 18 *S. pseudintermedius* (17 dogs and one cat) and 2 *S. intermedius* (both dog origin). All *S. aureus*, except one recovered from rabbit, showed methicillin resistance and harboured the *mecA* gene. In addition, all *S. pseudintermedius* recovered from dogs and a cat showed methicillin resistance and harboured the *mecA* gene. Nevertheless, the two *S. intermedius* strains of dog origin were negative for *mecA* gene detection. Different phenotypes of antibiotic resistance were identified among staphylococcal isolates of this study.

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## **SAPUVET and SAPUVETNET projects: a contribution to the understanding of the prudent use of antimicrobials**

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SAPUVET and SAPUVETNET III (n. DCI-LA/2008/75) are a series of projects, co-financed under the EU ALFA programme during the last 8 years, aimed to support an educational Veterinary Public Health (VPH) network. Latin-american and European Veterinary schools and some international organizations are contributing to the project ([www.sapuvetnet.org](http://www.sapuvetnet.org)).

The objectives of the projects are the development of strategies to improve learning and teaching VPH. The main results of the project can be summarized in the proposal of a common VPH curriculum, to design teaching material as case studies or a manual on VPH, the co-ordination of some e-conferences and a journal 'One Health'. All the materials can be freely distributed or used for distance learning by the web site. Some activities and materials are related to the increase of antimicrobial-resistant microorganisms and their role in public health. These activities are: (1) Development of the chapter 'Bacterial resistance; control strategies, good practices and prudent use of antimicrobials' in the manual of Veterinary Public Health-One Health; (2) Organization of two open electronic conferences to discuss the problem of the use of antimicrobials in veterinary medicine, the associated risks to their use in public health, and the alternatives to apply based on the prudent use of antimicrobials. The e-conference 'Local practices in production and animal health, with special reference to the use of medicines and the resistance to antibiotics: consequences in veterinary public health' took place in March 2007, and the e-conference 'Prudent use of antimicrobials in animals: solution or utopia?' took place in September 2010. Both these e-conferences used the moodle platform and involved students, lecturers and professionals from Canada, Latin America, Africa and Europe. In the last e-conference, the general opinion agrees on the use of antimicrobials for therapeutic and prophylactic purposes. However, their use as additives was not consensual, both among group members and other participants. Some opinions defended the view that the use of some specific antimicrobials as additives could make sense in some circumstances, as long as under serious and strict control. However, all professionals agree that veterinaries should work based on sanitary planning and preventive medicine to avoid the use of antimicrobials, except when their use is clearly necessary, and that multicenter research is absolutely needed to take more informed decisions. It also introduced the concern of the inappropriate use of other chemical products and its relation with antimicrobial resistance. Related with this all members of the group agree with the most correct use of the term 'antimicrobials' to describe any substance that kill microorganisms including natural compounds, produced or not by microorganisms, and synthetic antimicrobials compounds, instead of the term 'antibiotic', more restricted, although previously used by the group. (3) SAPUVETNET members have also published a manuscript in the first volume of the journal 'One Health' called 'The problem of antibiotics resistance in public health'. (4) A group of case studies concerning to health problems linked to the use of antimicrobials have been included in the learning materials 'Multiresistant *Salmonella enterica thyphimurium* in human and animal populations', 'Multiresistance to antibiotics of porcine origin', 'Relapsed multiresistant infection in a Golden Retriever dog' and 'Residues of antimicrobial substances in bovine meat and viscera'. These materials are tested actually by the lecturers of the universities involved in the SAPUVETNET project.

P17

## Patterns of resistance to antibiotics in *S. aureus* and MRSA isolated in dairy herds (Aragón-Spain) – role in veterinary public health

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In the last century the World Health Organization (WHO) alerted of the increase in the isolation of human *Staphylococcus aureus* in hospitals and other health environments as one of the important nosocomial infections. In association to the nosocomial characteristics an increase in the resistance to antibiotics in strains of *S. aureus* has been observed, especially to  $\beta$ -lactams and vancomycin. Consequently, the European Union has designed the surveillance program, EARS-Net, in which the study of *S. aureus* resistant to methicillin (MRSA) is one of the objectives. In animals *S. aureus* is also observed as a nosocomial infection being occasionally responsible of diseases in both farm and companion animals, and MRSA is also increasing in both groups of populations. In the case of swine and horse, some studies have demonstrated a direct relationship between animal and human isolates. *S. aureus* is usually isolated from the udder of cows suffering from mastitis. Antibiotics are applied to solve mastitis as a therapeutic or prophylactic treatment, which could be a risk for public health: *S. aureus* can develop resistance to antibiotics and could be transmitted by means of the final product (milk) or by direct contact with the farmer. The European Food Safety Authority (EFSA) has suggested that more information on *S. aureus* and MRSA in cows is necessary. A study on 28 dairy farms was developed to know their prevalence and resistance patterns in the udder of animals, the environment of the farm and the farmers and to analyze the role of the interchange of *S. aureus* strains and their resistance characteristics between these (animals, environment and humans). *S. aureus* was detected in 4% of the samples of milk tanks and in 0.9% of the animals sampled in the udder, but none of the isolates was MRSA (methicillin, oxacillin) nor vancomycin-resistant. Resistance to penicillin and ampicillin (65.7%) was the highest, while resistance to amoxicillin (1.39%) was the lowest. No *S. aureus* was isolated in the environment, however, two farmers working in positive herds carried the bacteria on their hands. These isolates were not MRSA nor vancomycin-resistant, but they were resistant to amoxicillin in opposite to the isolates of the animals in the same farms that were sensitive to this antibiotic. According to the resistance patterns in these populations (human and animals) we concluded that human and animal strains are different, suggesting that no interchange happened in these cases. Genetic comparison of these strains is being carried out.

P18

## Antimicrobial susceptibility of *Escherichia coli* isolated from acute bovine mastitis

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The aim of this study was to determine the *in vitro* antimicrobial susceptibility of *Escherichia coli* isolated from acute clinical mastitis in Finland. Use of antimicrobials for food animals is generally strictly controlled in Finland. Clinical mastitis suspected to be caused by Gram-negative bacteria is often treated with broad-spectrum antimicrobials such as enrofloxacin or trimethoprim-sulfonamides. It is known that the use of antimicrobials causes selection pressure resulting in antimicrobial resistance. Minimal inhibition concentration (MIC) values of 140 *Escherichia coli* isolates from acute bovine mastitis were determined for ampicillin, gentamicin, tetracycline, cloramphenicol, sulfametoxazol, ceftiofur, streptomycin, trimethoprim and ciprofloxacin by the VetMIC™ microdilution method (SVA, Uppsala, Sweden) in Research Department of Finnish Food Safety Authority EVIRA, Helsinki, Finland. Isolates were from samples collected during years 2003-2006 from acute clinical mastitis from 64 different farms in Southern Finland. A total of 27.9% isolates showed resistance to one or more antimicrobials tested. Among them, 18.6% was resistant to ampicillin, 16.4% to streptomycin, 15.7% to tetracycline, 13.6% to sulfametoxazol and 10.7% to trimethoprim. No resistance was found for gentamicin, ceftiofur and ciprofloxacin. Antimicrobial resistance of *E. coli* isolated from mastitis in the present study is at the same level than found in the national resistance surveillance (FINRES-Vet 2005-2006) and markedly lower than reported in most other countries. In conclusion, antimicrobial resistance appears to be no problem among mastitis caused by *E. coli* in Finland. However, strict antimicrobial policy should be continued in therapy of food animals to retain this good situation.

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## Evaluating *in vitro* susceptibility of bovine mastitis pathogens to a combination of kanamycin and cefalexin: recommendations for a disk diffusion test

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In conjunction with clinical experience, antimicrobial susceptibility testing of the causal pathogen helps in selecting the most appropriate antibiotics for therapy of mastitis treatment. A combination of kanamycin and cefalexin (1:1.5 w/w) has recently been licensed in Europe for the treatment of bovine clinical mastitis (Ubrolexin<sup>®</sup>, Boehringer Ingelheim). However, there exists little information about how to appropriately test for *in vitro* susceptibility of the target organisms to these agents in combination. The goal of this study was to determine the appropriate broth microdilution testing criteria for the combination, to develop preliminary interpretive criteria, and to evaluate the feasibility of a disk test. The current activity profile of kanamycin and cefalexin, alone and in combination (at a pharmacokinetically relevant ratio of 10:1 kanamycin:cefalexin) was evaluated against 268 random bovine isolates (93% clinical mastitis) from various European countries. An additional 39 isolates with varied susceptibility to either kanamycin or cefalexin and resistance to both were chosen to further evaluate the ability of the selected testing conditions to detect such isolates. In all, a total of 307 isolates (100 *Escherichia coli*, 104 *Staphylococcus aureus*, and 103 *Streptococcus* spp. were evaluated. The minimum inhibitory concentrations (MICs) of those isolates were determined by broth microdilution in accordance with the Clinical Laboratory Standards Institute (CLSI) M31-A2 Standard, and by disk diffusion. Based on achievable concentrations in milk and the resulting MIC distributions, preliminary broth breakpoints for kanamycin:cefalexin (10:1 fixed ratio) of < 8/0.8 µg/ml susceptible, 16/1.6 µg/ml intermediate, and > 32/3.2 µg/ml resistant were applied to evaluated staphylococci, streptococci and *E. coli*. Parallel testing by disk diffusion and resulting error-rate bounded analysis utilizing a combined disk concentration of 30 µg kanamycin and 15 µg cefalexin resulted in the establishment of preliminary disk interpretive breakpoints of ≥ 20 mm susceptible, 18 to 19 mm intermediate, and ≤ 17 mm resistant for staphylococci, streptococci (*S. uberis* and *S. dysgalactiae* only) and *E. coli*.

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## ***In vitro* activity profile of Ubrolexin® (kanamycin and cefalexin combination) against bovine coagulase-negative staphylococci and correlation between MIC and disk zone sizes**

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Ubrolexin® is a combination of kanamycin and cefalexin used to treat clinical bovine mastitis. The feasibility of using a disk containing both kanamycin and cefalexin for *in vitro* susceptibility testing of the combination against major mastitis pathogens (*Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae* and *Escherichia coli*) has been previously established (Journal of Dairy Science, 2009) and preliminary interpretive criteria for the susceptibility testing of the latter pathogens by broth microdilution (10:1 w/w kanamycin:cefalexin) and disk diffusion have been suggested. However, it is unknown how well the disk result correlates with broth MIC when testing coagulase-negative staphylococci (CNS). This study evaluated the overall activity profile of the combination by broth microdilution and disk diffusion against 148 recent isolates of CNS (from bovine mastitis field cases). The overall correlation of broth MIC to disk zone size was determined against these isolates by testing by broth microdilution and disk diffusion in parallel per CLSI guidelines. In addition, 8 isolates known to be resistant to kanamycin and/or cefalexin were included to challenge the performance of the disk test. The proposed interpretive criteria previously described by Pillar *et al.* (<8/0.8 mg/l, > 20 mm = susceptible; 16/1.6 mg/l, 18-19 mm = intermediate; > 32/3.2 mg/l, < 17 mm = resistant) were subjected to error rate bounding per CLSI to determine whether these criteria are suitable for use when testing CNS. Bovine mastitis isolates of CNS show a high degree of susceptibility to the kanamycin/cefalexin combination, with minimal resistance to either agent alone. It was concluded that the combined disk concentration of 30 µg kanamycin and 15 µg cefalexin currently used for testing the susceptibility of bovine mastitis isolates of *S. aureus*, *S. uberis*, *S. dysgalactiae* and *E. coli*, is also reliable for use in the testing of CNS, as disk results correlated with broth MICs. Furthermore, the interpretive criteria published for interpreting broth and disk results for this combination also can be applied when testing CNS, as there were minimal error rates detected when using these criteria.

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## **Pan-European resistance monitoring programs of CEESA encompassing foodborne pathogens, commensal bacteria and target pathogens of food-producing and companion animals**

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Antimicrobial resistance is a concern for animal and human health. Veterinary programs to monitor resistance of animal pathogens and zoonotic pathogens are therefore essential. Various European countries have implemented national programmes, particularly for zoonotic and commensal bacteria, to assess susceptibility to antibiotics. However, harmonization is identified as a current weakness but as an important need in order to compare data across countries. Such comparisons of resistance monitoring data between European national resistance monitoring programs are hampered by virtue of differences between programs such as sampling and testing methodology, interpretive criteria, e.g., CLSI vs. EUCAST and the use of different epidemiological cut-off values. Moreover, only very few valid data are currently available regarding target pathogens. The European Animal Health Study Center (CEESA) attempts to fill these gaps. The resistance monitoring programs of CEESA have been a collaboration of 8 veterinary pharmaceutical companies for more than a decade and include two different European projects: the European Antimicrobial Susceptibility Surveillance in Animals (EASSA) program which collects foodborne pathogens and commensal bacteria at slaughter from healthy animals and the pathogen programs which collect first intention target pathogens from acute diseased animals. The latter exist of three different subprograms: VetPath, MycoPath and ComPath. These two CEESA projects include uniform sample collection and bacterial identification to species level in various EU Member States. A central laboratory conducts quantitative susceptibility testing (Minimum Inhibitory Concentrations: MICs) to a range of antimicrobial agents either important in human medicine (EASSA) or used in veterinary medicine (VetPath, MycoPath, ComPath). Conducting MIC determination in a single central laboratory has a number of advantages including eliminating any inter-laboratory variation and interpretation of results using identical interpretive criteria. Additionally, the three pathogen programs are the only longstanding pan-EU programs to conduct resistance monitoring of a large variety of target pathogens. VetPath is an ongoing pan-European resistance monitoring program in 11 countries for veterinary pathogens from respiratory tract infections, mastitis or PPDS (MMA) and digestive diseases isolated from diseased antimicrobial-naive cattle, pigs and poultry. MycoPath exclusively addresses the recovery of mycoplasma organisms from cattle, pigs and poultry in five EU countries. ComPath collects isolates from untreated dogs and cats in 11 EU countries from skin/wound/ears, urinary tract and respiratory tract infections. The 'harmonisation' of methodology and interpretation of data of these four unique CEESA programs allow for easy comparisons to be made between bacterial isolates recovered from different EU Member States and thus make these longstanding veterinary pharmaceutical industry sponsored resistance monitoring programs robust and valuable to address food safety and efficacy of antibiotics.

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## **Analysis of antimicrobials prescribing patterns in dogs in small and mixed animal veterinary practices**

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In this paper data from questionnaires completed by veterinary practitioners dealing with small animals in small and mixed animal practices were collected. The information in the questionnaire forms was recorded during the visits to these veterinary practices by person (national medicines authority representative) not involved in the analysis of the datasets to ensure anonymity of the respondents. Over the five month period, data from total 25 practices, 12 small animal practices and 13 mixed practices (with also food producing animals) were collected. The most frequently treated animals were dogs and cats, only in rare cases other companion animals as ornamental birds, rabbits, reptiles and small rodents were taken care about. Antibacterials were prescribed for the treatment of gastroenteritis in 28.4%, dermatitis/skin disorders 21.6%, respiratory diseases 16.2%, otitis 13.5%, urogenitary tract infections 10.8%, other (injuries, gingivitis, stomatitis, neutropenia) 9.5%. Antibacterials according the frequency of use: amoxicillin/clavulanic acid 22.1%, cephalosporines total 17.3% (approx. equal 1st and 2nd cephs vs. 3rd and 4th cephs), penicillins 16.4% (mostly amoxicillin), fluoroquinolones 11.5%, aminoglycosides 6.7%, metronidazole 6.7%, polymyxin B 5.8%, spiramycin 3.8%, clindamycin 3.8% , sulphonamides 2.9% and other (lincomycin, rifaximin, colistin) 2.9%. Veterinarians also responded to questions on factors (except professional knowledge) influencing antimicrobials prescription, on stratification of knowledge used in decisions, performance of susceptibility testing, use of the results from susceptibility testing, availability of laboratories, etc. As the questionnaire was performed by the national competent authority (NCA) the questions related to the certain requirements for improvement related to the antimicrobials were identified too: improvement of summary of product characteristics – deeper information on combinations and interactions, adverse reactions, recommended web sites on resistance, possibilities of dose regimen changes in the cases of the treatment failure, updated SPCs freely available at the NCA website.

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## Measuring the real carry-over of oxytetracycline in medicated feed production

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Medicated feed is still the most common way of oral administration of antimicrobials in animal husbandry in the EU. The major disadvantage of this practice is the unavoidable carry-over of the active substances on the production lines. The cross-contamination of the feed by the previous batch results in consumption of antimicrobials by non-targeted animals and thus in uncontrolled use of antibiotics at sub-therapeutic levels, a well-known cause of antimicrobial resistance. Acknowledging the importance of the control on antibiotics the Dutch Office of Risk Assessment issued a recommendation to set a limit of 2.5% on the carry-over of medicated feed in the production lines. According to the Good Manufacturing Practice scheme of feed producers in the Netherlands, the carry-over of all production lines has to be periodically determined using accepted markers. There are indications though that this method does not give the same results with the carry-over of antibiotics. Oxytetracycline (OTC) belongs to the antibiotic class of tetracyclines. Within the Netherlands the use of tetracyclines for veterinary purposes is several tons a year. The determination of the real carry-over of OTC in four different Dutch feed producers is the objective of our case study. Two producers were selected to have a low carry-over percentage and the two others a high one, based on the tracers and proteins estimation of carry-over. Four lines with different production rates were sampled in close time intervals during the flushing, thus twenty samples were collected on every one of the four presses, following the production of medicated feed of 400 and 500 mg/kg OTC. All the samples were analysed with an in-house developed, validated and accredited analytical method based on LC-MS detection. The limit of determination of the method for OTC in feed is 1mg/kg. Our results show a considerable inhomogeneity in OTC content of the four flushing batches. This should be considered when sampling. In all four lines the mean carry-over was smaller, than the estimate based on the tracers and smaller than 3% of the level of OTC in the medicated batch. However the carry-over was much higher than 3% (max of 9.5%) in the first 1,000 kg of feed produced after a medicated batch. Details of the results will be presented and discussed.

**P24**

## **Microbial screening methods for antibiotic residues**

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Monitoring of food products from animal origin for the presence of antimicrobial residues is preferably done using microbial screening methods, because of their high cost-effectiveness. Microbial inhibition methods rely on the inhibitory action of antibiotics against bacteria: the presence of antimicrobial residues is manifested as absence of growth of the test organism. These effect-based bioassays are extremely low cost and high throughput and they do not require sophisticated equipment or specialized technicians, which makes them very attractive for large scale monitoring. An efficient screening method should be able to effectively identify potential noncompliant samples from a large set of negative samples, while maintaining an acceptable low percentage of false positive results. Traditionally applied methods, like the EU-four plate method, fail to detect the maximum residue limits (MRLs) which were established when Council Regulation (EEC) No 2377/90 came into effect. In order to comply with EU legislation, RIKILT -Institute of food safety, developed a series of improved multi-plate tests, dedicated to antibiotic residue screening in typical animal tissue matrices. The Nouws Antibiotic Test (NAT) is an integrated two step screening system for slaughter animals, involving analysis of renal pelvis fluid, and subsequent analysis of muscle and/or kidney samples of a suspect animal. Additionally several other matrix specific, so called SCAN tests (SCReening Antibiotic residues) were derived from this test, among others for screening of eggs, poultry, urine and fish. The NAT and SCAN test methods are multi-plate tests. A sample is applied on five individual test plates, each comprising a balanced combination of test-organism, growth medium and synergistic compounds, yielding them preferably sensitive to either  $\beta$ -lactam and macrolides, aminoglycosides, tetracyclines, quinolones or sulfonamides/trimethoprim. After overnight incubation the test is either negative (no growth inhibition > compliant) or suspect (growth inhibition, potentially non-compliant). The test plate showing the largest inhibition reveals the group specific identity of the residue. Group specific identification significantly reduces confirmatory efforts and costs. All methods have been validated according to Commission Decision 2002/657/EC and several methods are currently used by the Food and Consumer Product Safety Authority in national monitoring programs. Additionally, they are used in private monitoring programmes and disseminated to a number of European and non-European countries. RIKILT offers support on implementation and quality assurance of this new generation of microbial inhibition tests.

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## **Extent and implications of carry-over of antibiotics in porcine feed production**

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A broad range of feeds and different types of products may have to be manufactured after each other in the same production line. Because of this it may happen that unavoidable carry-over may occur when antibiotics are used for the production of medicated feed. To avoid the carry-over from feed to food-producing animals GMP<sup>+</sup>-criteria are set for the production of feed for critical species (lactating cows, laying hens, etc) in The Netherlands. To apply to these criteria at least one (rinsing) feed for non-critical species has to be produced after the production of a medicated feed. Most of the times porcine feed is being used for that purpose. To determine the extent of carry-over from medicated feed in The Netherlands a survey has been performed. In this survey 21 feed mills were selected and in total 169 (porcine) rinsing feeds (140 'first', 29 'second' and 'third' rinsing feeds) were sampled. The samples were analyzed for antibiotics. Furthermore detailed information about the production process and the samples was collected. Results show that 87% of the 140 'first' rinsing feed samples and 76% of the 'second' and 'third' rinsing feed samples contain antibiotics. More results, observations and the implications will be presented.

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## A new breakthrough for poultry producers to manage coccidiosis using plant extracts

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The objective of this study was to compare treatment with standard coccidiostats with programmes using plant extracts (PE) only or plant extracts combined with a coccidiostat. 1,680 Male chickens Ross 308 were reared in pens, divided among 6 treatments, from day 1 to day 49. The animals received the same feed programme; only the coccidiostatic supplementation varied among the groups. At day 12 all the chickens were orally inoculated with 1 ml of a suspension containing 220,000 oocysts: 10,000 *Eimeria tenella*, 200,000 *E. acervulina* and 10,000 *E. maxima*. The control R1 did not receive any treatment; R2, narasin + nicarbazine (100 ppm) from day 1 to day 25; R3, salinomycine (60 ppm) from day 1 to day 25; R4, robenidine (33 ppm) from day 1 to day 25; R5, PE (35 ppm diallylsulfures + 4 ppm diterpenes lactones) from day 1 to day 25; R6, robenidine (33 ppm) from day 1 to day 25 + ½ amount of PE from day 25 to day 49. The chickens' weight was recorded at day 1, 11, 25, 42 and 49. Feed intake was monitored at day 11, 25, 42 and 49. Samples of faeces were collected to determine oocysts' excretion as well as the viability and sporulation rate of oocysts at day 11, 25 and 35. The lesion scores were performed on chickens at day 11 and 25. From day 1 to 11 the chickens' feed intake was the same for R1 to R5; R6 presented a lower feed intake resulting in a lowered feed conversion rate (FCR). From day 12 to 25 R2 to R6 showed a high significantly improved average daily gain ( $P < 0.00003$ ) as compared to R1. The FCR showed the same results: a highly significant difference between R1 and R2 to R6 ( $P < 0.00006$ ). At day 42, the body weights of R2, R3 and R6 were significantly increased as compared to R1. Overall, R2 to R6 had a final weight higher than R1, the weight of R6

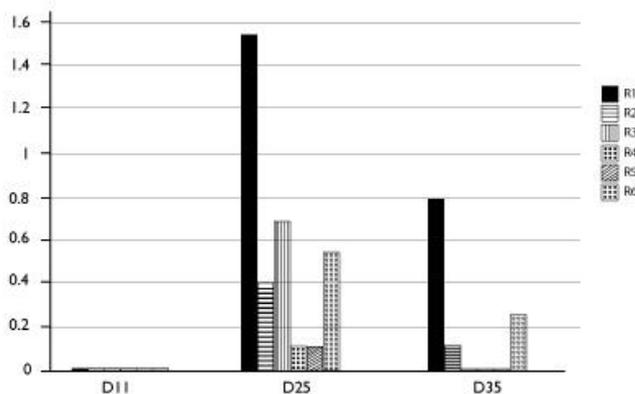


Figure 1. Lesion scores at day 11, 25 and 35.

a lower infectious capacity. Lesion scores were significantly reduced at day 25 and 35 for R2 to R6 as compared to R1 (Figure 1). In conclusion, it has been shown that a formulation of plant extracts can give results comparable to standard coccidiostats. The results obtained in this study open a research path to plant extracts alone and in combination with coccidiostats, and on the possibilities of rotation programmes.

being improved 4.81% as compared to R1 ( $P < 0.09$ ). The FCR of R6 was also significantly improved as compared to the control ( $P < 0.03$ ). At day 11 the oocysts' excretion was zero for all groups. 14 Days after inoculation the oocysts' excretion of R2 to R6 was very significantly reduced ( $P < 0.001$ ). The reduction associated with the plant extract treatment was comparable to that of robenidine. R2 to R6 showed sporulation rates lower than R1, resulting in a very significant reduction of oocysts' excretion with

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## Quantification of antimicrobial usage in farm animals: ABcheck, a free online web application

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Knowing the magnitude of antimicrobial usage in a herd is of great importance to implement changes and focus on prudent use. In order to quantify the antimicrobial usage on pig, poultry and turkey farms an 'antibiotic check' scoring system has been developed by the Veterinary Epidemiology Unit of the faculty of Veterinary Medicine, Ghent University. From June 2011 onwards this system is freely available online ([www.ABcheck.ugent.be](http://www.ABcheck.ugent.be)). The Dutch and English version are already operational, the French version is expected soon as well as a module for ruminants. The ABcheck gives farmers, veterinarians and herd advisors the opportunity to calculate treatment incidences (TI) on a farm, using herd specific data. Results are subdivided into treatment incidences per animal category and can be recalculated to other usage quantification systems such as the Dutch daily dosage. Additional information is provided, for example if the antimicrobial is critically important according to the lists of the World Health Organization (WHO) or the World Organisation for Animal Health (OIE). The system works totally anonymous, no login is required and the only information that needs to be provided by the farmer is the number of animals per production round, the type of product and the amount used (ml, l, g, kg) and duration of treatment. Herd specifics like production round duration and weight of the animals are prefilled, but can be adjusted. The ABcheck can be used for prophylactic and curative treatments. If wanted, scores can be saved and reclaimed after registration. The farmer can see on a graph the placing of his herd versus other farms in the database. The database behind the calculator consists of all antimicrobials registered in Belgium for the specific animal categories. For each antimicrobial, the animal daily dose (ADD) was established by taking the average dosage prescriptions from the product leaflets. Factors for long acting antimicrobials were estimated from available scientific research papers. All used ADD's can be found on the website. At the moment data from 13 reproduction, 67 slaughter pig, 47 broiler and one turkey herds are in the database. The database is dynamic and newly saved data are added directly to the graphs. The graphs show a wide variety in antimicrobial usage amongst pig and poultry herds in Belgium, with minimal treatment incidences from zero till 800 for production, zero to almost 600 for reproduction pig herds and around 50 till 500 for broiler herds.

## Preliminary results on prophylactic and curative antimicrobial usage on 20 Flemish pig herds and advices on improvement

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Between January and August 2011 data on antimicrobial usage and herd management characteristics were collected retrospectively on fifteen closed or semi closed, four finisher and one multiplying herd. The aim of the research was to promote prudent antimicrobial usage and optimize herd management. Antimicrobial usage was calculated with the ABcheck ([www.ABcheck.ugent.be](http://www.ABcheck.ugent.be)), using treatment incidences (TI) as the quantifying value. Average total  $TI_{ADDpig}$  for reproduction animals was 63.8 (min. 0.2, max. 279.5), of which 41.70% was prophylactic, 58.30% curative. For suckling piglets and weaners 237.1 (min. 1.6, max. 770.9, 68.21% prophylactic, 31.79% curative) and for finisher pigs 118.9 (min. 0, max. 648.9, 28.20% prophylactic). Thirteen out of 20 farmers have used prophylactic treatments for several years. For reproduction animals the most frequently administered prophylactic antimicrobials were amoxicillin and lincomycin-spectinomycin (both 23.08%). Based on  $TI_{ADD}$  trimethoprim-sulphonamide ( $TI_{ADDpig}$  237.1) is the primarily used antimicrobial. In suckling piglets only three antimicrobial classes were used, all parentally administered; macrolides (tulathromycin 12.50%,  $TI_{ADDpig}$  80.9), aminopenicillin (amoxicillin 31.25%,  $TI_{ADDpig}$  173.0) and cephalosporins (ceftiofur 56.25%,  $TI_{ADDpig}$  513.6). In weaners and finishers all used antimicrobials were administered orally, with amoxicillin (30.78%,  $TI_{ADDpig}$  637.1) and colistin (34.33%,  $TI_{ADDpig}$  710.6) for the weaners and doxycycline (66.67%,  $TI_{ADDpig}$  232.5), lincomycin-spectinomycin (16.67%,  $TI_{ADDpig}$  135.9) and trimethoprim-sulphonamide (16.67%,  $TI_{ADDpig}$  70.6) for finishers as main antimicrobials. Parenterally administered prophylactic treatments were overdosed in 15.38% and 62.50% of the treatments in reproduction animals and in suckling piglets, respectively. Oral treatments were underdosed for weaners (43.33%) and finishers (50.00%). Curative treatments in reproduction animals mainly consisted of fluoroquinolones (32.86%) and cephalosporins (17.14%). In piglets, mainly aminopenicillins (amoxicillin 25.00%) and polymyxines (colistin 22.06%) were used, and in finisher pigs tetracyclines (36.51%). Reliable slaughterhouse findings were only available from 11 farms, with on average 6.64% of the lungs affected, 2.82% showed pneumonia/fissures, 2.63% pleuritis and 5.71% of the livers showed white spots lesions. Most advices were given on 'general' biosecurity and management measures such as: washing of sows, cleaning and disinfection, hygiene lock and personal hygiene. Also supplemental vaccinations or adjustments to the existing vaccination scheme and frequent follow up were advised. Concerning antimicrobial usage mainly a higher awareness on the necessity of prudent antimicrobial usage was advised. A switch from prophylactic to curative treatments was strived for as well as less use of, according to the World Health Organization (WHO), critically important antimicrobials. For the necessary treatments, choice of antimicrobial, dosage and duration of the therapy were optimized. In the follow up of this study, the effect of given advise, relations between production parameters and antimicrobial usage will be studied.

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## Investigation of the relationship between biosecurity measures and production, health- and treatment-characteristics in pig herds

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It is generally believed that biosecurity measures in pig production improve performance and health status of the pigs, and may thus decrease the need for antimicrobial treatments. However, limited quantitative data is available to support this hypothesis. In the present study, 95 randomly selected Belgian closed or semi-closed pig herds were visited to quantify the biosecurity status of the herd by means of a risk-based weighted biosecurity scoring system (Biocheck). This score ranges from 0 (= total absence of biosecurity measures) to 100 (= perfect biosecurity). During the same visit, data concerning the herd, farmer and production characteristics and the use of antimicrobials (quantified as treatment incidences) were collected. The external biosecurity score (measures preventing infectious agents from entering the herd) ranged between 45 and 89/100 with an average of 65/100, whereas the internal biosecurity score (measures reducing within herd spread of infectious agents) was on average 52/100 (min 18; max 87). The herd size was positively associated with the external biosecurity score and a negative association was observed between the internal biosecurity score and the age of the buildings as well as the experience of the farmer. These results indicate that biosecurity is generally better implemented in larger herds, in more modern facilities and by younger farmers. A higher overall, external and internal biosecurity score had a significantly positive influence on daily weight gain of fattening pigs ( $P < 0.01$ ) and the external biosecurity score was negatively associated with the feed conversion ( $P < 0.05$ ). Whereas the internal biosecurity was negatively associated with treatment incidence ( $P < 0.05$ ), indicating an improved biosecurity is associated with a reduction of antimicrobial drug use. No significant associations were found between the mortality rate of fattening pigs or the seroprevalence of *Salmonella* and the respective biosecurity scores. In conclusion, this study demonstrated clear associations between several aspects of the biosecurity and both production and treatment characteristics in pig production.

## Comparative study on group level antimicrobial use between 2003 and 2010 in Belgian pig herds

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Between January and October 2010 data on prophylactic and metaphylactic antimicrobial use, from birth until slaughter, were collected retrospectively on 50 closed or semi-closed pig herds. Treatment incidences (TI) based on the used daily dose pig (UDD<sub>pig</sub>) and the animal daily dose pig (ADD<sub>pig</sub>) (number of pigs treated with one UDD<sub>pig</sub> or ADD<sub>pig</sub>/1000 pigs at risk/day, respectively) were calculated to quantify the prophylactic and metaphylactic use in group. The proportional TI<sub>ADDpig</sub> and TI<sub>UDDpig</sub> for each individual antimicrobial drug was calculated by dividing the TI<sub>ADDpig</sub> and TI<sub>UDDpig</sub> of each individual antimicrobial by the total TI<sub>ADDpig</sub> and TI<sub>UDDpig</sub> for injectable and oral administrations, respectively. In 2003-2004 a similar study on the antimicrobial group level use in pig production in Belgium was performed (Timmerman et al., 2006). Based on these results, changes in the antimicrobial drug consumption between 2003 and 2010 can be assessed. The average TI<sub>ADDpig</sub> and TI<sub>UDDpig</sub> in 2010 (235.7 and 200.7 respectively) were higher than those in 2003 (178.1 and 170.3 respectively). The increase is the result of a higher number of prophylactic group treatments (93%) whereas a drastic decrease of the portion of metaphylactic group treatments (7%) is observed since 2003 (44% metaphylactic and 56% prophylactic). Results for 2010 showed that the penicillins were the most frequently used antimicrobial class (proportional TI<sub>UDDpig</sub> equals 27.6%), mainly due to the frequent use of amoxicillin both as injectable and oral administration, together with the less frequently used injectable penicillin and ampicillin. Polymyxins follow very close with a frequency of 27.0%. Antimicrobial classes with a moderate relative importance are the macrolides/lincosamides (17.8%), the trimethoprim-sulfonamides (11.5%) and tetracyclines (10.0%). Cephalosporins represent 5.3% of the total use and aminoglycosides (0.7%), phenicols (<0.1%) and quinolones (<0.1%) are less frequently used. In 2010, doses used during group treatments with injectable products were generally overdosed, as 79.5% of the administered UDD<sub>pig</sub> was higher than the ADD<sub>pig</sub>. Oral treatments were generally underdosed (47.3%). This trend was already seen in 2003. Most of the antimicrobials used in 2003, are currently still in use. Although, a shift between 2003 and 2010 on antimicrobial group level use was marked by a partial yet substantial replacement of older, orally administered compounds (e.g., doxycycline and trimethoprim-sulphonamides) by new injectable long acting products (e.g., cephalosporins, macrolides). This evolution warrants an assessment of antimicrobial resistance trends in commensal and pathogenic bacteria.

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## **HatchBrood: a tool to reduce antibiotic usage in poultry**

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Antibiotic usage is high in the poultry industry in the Netherlands and needs to be reduced by 50% in 2013 to prevent the growing resistance to antibiotics of disease-causing bacteria in humans. To reduce antibiotic usage, optimal management of the environment of chickens is important, especially directly after hatch. Although the chicken is anatomically complete at hatch, maturation of different regulatory systems occurs during the first four days of the chicken's life (the brooding period). During this period, chickens are unable to regulate their own body temperature. Their body temperature depends on the environment but is optimal between 40.0-40.6°C and this is often difficult to achieve at a broiler farm. Low body temperatures caused by a cold floor are often found in practice and negatively affect the development of young chickens. A suboptimal brooding environment results in delayed chick development such as the intestinal and immune function, a lower uniformity in body weight, and a higher mortality rate. Furthermore, antibiotic usage can be increased as well. HatchBrood is a system that is designed to control the environment of the first days of a chicken's life. After hatching, day-old chickens are placed in the HatchBrood unit. Inside this unit, factors such as air temperature, air velocity, relative humidity, and CO<sub>2</sub> are constantly monitored and adjusted to the requirements of the chickens to ensure an optimum environment and body temperature. All chickens have easy access to fresh air, water and feed. After 4 days, the chickens are transported from the hatchery to the farm. HatchBrood optimises the development of the thermoregulatory, intestinal and immune system of the birds and can be a tool to reduce antibiotic usage. Different aspects from the HatchBrood system achieve this: (i) Chickens are uniformly brooded at the right body temperature (40.0-40.6°C) and with feed and water; there is no cold floor or other stressors to impair the development and maturation of the thermal, digestive, and immune system of the chicken. (ii) The environment can be cleaned and disinfected to eliminate disease challenges; the immune system develops before the chickens are exposed to the field disease challenges. In a recent interview in *Nieuwe Oogst* (Vol. 3, 2011), a Dutch poultry farmer, Mr. Kees Nuijten, shared his experience with HatchBrood chickens. He has been able to grow four batches of HatchBrood chickens completely free of antibiotics and he was able to maintain the total mortality below 3% throughout the four cycles. Optimal brooding in HatchBrood may enable chickens to respond better to field disease challenges. With enhanced natural disease resistance and optimal development of the bird, antibiotic usage can be reduced in the future.