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  • abstracts of lectures and posters are grouped separately
  • lectures are grouped according to the daily programme
  • posters are grouped in an alphabetical order according to the corresponding author

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WELCOME

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.

The international conference series on Responsible Use of Antibiotics in Animals is an initiative independent from commercial companies, official organisations or authorities. The objectives of the initiative are:

- to give an overview of the ongoing activities with respect to the issue of antibiotic use in animals and antimicrobial resistance in animals and humans
- to learn about the expectations of different parties involved
- to focus on innovative approaches to disease prevention, rapid diagnostics and alternatives to antibiotics
- to identify the areas which need further research and action with respect to the current scientific knowledge and political expectations

In 2005, the series started with the international debate conference ‘Antimicrobial Growth Promoters: Worldwide Ban on the Horizon?’ reflecting the state of AGPs and alternatives at that time. The second conference took place in 2011 and focused on exchanging views on the path forward. Key focuses of the third conference in 2014 were current insights, sustainable initiatives and transparency. Key themes of the 4th International Conference on Responsible Use of Antibiotics in Animals include:

- integration of animal, human and environmental health
- approaches at global and national levels
- engagement of stakeholders
- innovations in disease prevention and detection, including gut health, alternatives to antibiotics, rapid diagnostics, etc.
- new areas to explore

The conference is designed for the animal health industry and the medical community; all users of antibiotics in animals, such as veterinarians, animal feed producers, livestock and aquaculture producers, and nutritionists; food processors and manufacturers, and retailers; policy makers and regulatory agencies; researchers in universities and research institutes; and others with an interest in resistance and in the sustainability of antibiotics, such as educators, agricultural extension staff, consultants, and consumer organisations.

The members of the Advisory Board of the 4th International Conference on Responsible Use of Antibiotics in Animals are looking forward to meeting you and assure that your participation will be fruitful and productive!

ADVISORY BOARD

Prof.dr. Peter Borriello
Dr. Delia Grace
Prof.dr. Samuel M. Kariuki
Dr. Arie K. Kies
Prof.dr. Jan Kluytmans
Dr. Cristina Muñoz Madero
Peter J.G. Oostenbach, M.Sc.
Dr. Stephen Page
Prof.dr. Mark G. Papich
Dr. Thomas R. Shryock
Prof.dr. Peter Silley
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Veterinary Medicines Directorate, UK
International Livestock Research Institute, Kenya
Kenya Medical Research Institute, Kenya
DSM Nutritional Products and Wageningen University & Research, the Netherlands
University Medical Center Utrecht and Amphia Hospital, the Netherlands
Spanish Agency of Medicines and Medical Devices, Spain
MSD Animal Health, the Netherlands
Advanced Veterinary Therapeutics, Australia
North Carolina State University, USA
Antimicrobial Consultants, USA
MB Consult Limited and School of Life Sciences, University of Bradford, UK
Federal Rural University of Rio de Janeiro, Brazil
# PROGRAMME AT A GLANCE

## MONDAY 26 SEPTEMBER 2016

<table>
<thead>
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<th>Time</th>
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<tbody>
<tr>
<td>10:00 – 10:15</td>
<td>Conference opening</td>
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<tr>
<td>10:15 – 10:45</td>
<td>Keynote lecture</td>
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<tr>
<td>10:45 – 12:30</td>
<td>Plenary meeting&lt;br&gt;Antibiotic use and resistance: cross-cutting themes</td>
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<tr>
<td>12:30 – 13:30</td>
<td>Lunch break &amp; poster viewing</td>
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<td>13:30 – 16:00</td>
<td>Plenary meeting&lt;br&gt;Global approaches to responsible use of antibiotics</td>
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<td>16:00 – 16:30</td>
<td>Networking break &amp; poster viewing</td>
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<tr>
<td>16:30 – 18:30</td>
<td>Plenary meeting&lt;br&gt;Antibiotic use and resistance – case studies from developing countries</td>
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<td>18:30 – 19:30</td>
<td>Lounge party</td>
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## TUESDAY 27 SEPTEMBER 2016

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<tr>
<td>08:30 – 10:45</td>
<td>Parallel session 1&lt;br&gt;National approaches to antibiotic use and resistance – focus on EU and USA</td>
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<td>Parallel session 2&lt;br&gt;Importance and relevance of animal intestinal and respiratory health</td>
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<td>10:45 – 11:15</td>
<td>Networking break &amp; poster viewing</td>
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<td>11:15 – 13:00</td>
<td>Parallel session 1 (cont’d)</td>
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<td>13:00 – 14:00</td>
<td>Lunch break &amp; poster viewing</td>
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<tr>
<td>14:00 – 16:00</td>
<td>Parallel session 3&lt;br&gt;The stakeholders’ view on antibiotic use and resistance</td>
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<td>Parallel session 4&lt;br&gt;Ongoing research to control antibiotic use and resistance</td>
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<td>Networking break &amp; poster viewing</td>
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<td>16:30 – 18:15</td>
<td>Parallel session 3 (cont’d)</td>
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<td>18:15 – 19:00</td>
<td>Poster viewing &amp; drinks</td>
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<td>20:00 – 22:15</td>
<td>Conference dinner</td>
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## WEDNESDAY 28 SEPTEMBER 2016

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<tr>
<td>08:45 – 11:00</td>
<td>Plenary meeting&lt;br&gt;Towards a global approach to veterinary antimicrobial stewardship</td>
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<td>11:00 – 11:30</td>
<td>Networking break &amp; poster viewing</td>
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<tr>
<td>11:30 – 13:00</td>
<td>Final plenary meeting&lt;br&gt;Where do we go from here?</td>
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<tr>
<td>13:00</td>
<td>Conference closing</td>
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CONFERENCE PROGRAMME

MONDAY 26 SEPTEMBER 2016

10:00  Conference opening

10:15  Keynote lecture
Superbugs attack! How the world can win the war on antimicrobial resistance
Dr. Steven J. Hoffman, Director, Global Strategy Lab at the University of Ottawa, Canada

PLENARY MEETING: ANTIBIOTIC USE AND RESISTANCE: CROSS-CUTTING THEMES

Chairs: Prof. dr. Peter Borriello, Veterinary Medicines Directorate, UK
Dr. Timothy Robinson, International Livestock Research Institute, Kenya

10:45  Modelling the distribution of intensifying livestock production systems
Dr. Marius Gilbert, Spatial Epidemiology Lab, Université libre de Bruxelles, Belgium

11:10  Mapping the global use of antimicrobials in food animals
Dr. Thomas P. Van Boeckel, Department of Environmental Systems Science, ETH Zurich, Switzerland

11:35  Moving beyond the classic foodborne pathogens to quantify the total public health burden of using antibiotics in food animals
Prof. dr. Lance B. Price, Milken Institute School of Public Health, The George Washington University, USA

12:00  Resistance from food to humans: a multidimensional problem
Prof. dr. Jan Kluytmans, University Medical Center Utrecht and Amphia Hospital, the Netherlands

12:30  Lunch break & poster viewing
MONDAY 26 SEPTEMBER 2016

PLENARY MEETING: GLOBAL APPROACHES TO RESPONSIBLE USE OF ANTIBIOTICS

Chairs: Prof.dr. Peter Borriello, Veterinary Medicines Directorate, UK
       Prof.dr. Jan Kluytmans, University Medical Center Utrecht, the Netherlands

13:30 Collection of data on the use of antimicrobial in animals: an important tool to develop a policy for responsible use
Dr. Jean-Pierre Orand, Director, The French Agency for Veterinary Medicinal Products/The French Agency for Food, Environmental and Occupational Health & Safety, France

13:55 Unpacking factors influencing antimicrobial use in global aquaculture
Dr. Patrik J.G. Henriksson, Stockholm Resilience Centre, Sweden and WorldFish, Malaysia

14:20 Antimicrobial resistance: exploring the human animal link! Perspectives from the Latin American Caribbean region
Prof.dr. H. Morgan Scott, Department of Veterinary Pathobiology, Texas A&M University, on behalf of PAHO Foundation, USA

14:45 Working together: Australia’s response to antimicrobial resistance
Dr. Mark Schipp, Chief Veterinary Officer, Department of Agriculture and Water Resources, Australia

15:10 Antimicrobial resistance transmission between animal and human; what we can do from now?
Prof.dr. Yong Ho Park, College of Veterinary Medicine, Seoul National University, Korea

15:35 Transatlantic Taskforce on Antimicrobial Resistance (TATFAR), collaboration in veterinary medicine
Jordi Torren Edo, Veterinary Medicines Department, European Medicines Agency, UK

16:00 Networking break & poster viewing

PLENARY MEETING: ANTIBIOTIC USE AND RESISTANCE – CASE STUDIES FROM DEVELOPING COUNTRIES

Chair: Dr. Timothy Robinson, International Livestock Research Institute, Kenya
       Dr. Delia Grace, International Livestock Research Institute, Kenya

16:30 Chair’s introduction

16:40 Development and migration of antimicrobial resistance in livestock in China
Dr. Dengpan Bu, Institute of Animal Science, Chinese Academy of Agricultural Sciences, China

17:00 Antibiotic use in small holder peri-urban dairy farms in India: sowing ignorance, reaping resistance
Dr. Manish Kakkar, Public Health Foundation of India, India

17:20 A veterinary intervention to reduce antimicrobial usage in chicken production in Vietnam
Dr. Juan J. Carrique-Mas, Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Vietnam

17:40 Multiresistant E. coli in small vs. large scale pig farms in North Eastern Thailand
Dr. Jatesada Jiwakanon, Faculty of Medicine, Khon Kaen University, Thailand

18:00 Antimicrobial resistance along the meat value chain in Kenya
Prof.dr. Samuel M. Kariuki, Kenya Medical Research Institute, Kenya

18:20 Chair’s summary

18:30 – 19:30 Lounge party
TUESDAY 27 SEPTEMBER 2016

PARALLEL SESSION 1: NATIONAL APPROACHES TO ANTIBIOTIC USE AND RESISTANCE – FOCUS ON EU AND USA

Chairs: Dr. Cristina Muñoz Madero, Spanish Agency of Medicines and Medical Devices, Spain  Dr. Rick Sibbel, Merck Animal Health, USA

08:30 Update on the U.S. National Action Plan to Combat Antibiotic-Resistant Bacteria
Dr. Thomas R. Shryock, Antimicrobial Consultants, USA

08:50 Implementation of the Veterinary Feed Directive in the USA: issues and impacts
Dr. Jeffrey L. Watts, Zoetis, USA

09:10 The CVMP Strategy on Antimicrobials 2016 to 2020 – an EU regulatory perspective
Dr. Helen Jukes, Veterinary Medicines Directorate, UK

09:30 Organic farming and antimicrobial resistance in Germany – the example of E. coli in dairy herds
Dr. Bernd-Alois Tenhagen, Department Biological Safety, Federal Institute for Risk Assessment, Germany

Case studies from the USA

09:50 Strategies in U.S. veterinary medicine for antibiotic use and surveillance of antibiotic resistance
Dr. Rick Sibbel, Merck Animal Health, USA

10:10 Incentivising R&D for new antimicrobial drugs and non-antibiotic alternatives
Dr. Stacy Sneeringer, Structure, Technology, and Productivity Branch, Economic Research Service, U.S. Department of Agriculture, USA

10:30 Environmental, economic, and animal health implications from antibiotic-free production of broilers in the U.S.
Dr. Matthew J. Salois, Elanco Animal Health, USA

10:50 Networking break & poster viewing

Measures taken in the EU to reduce the need for and the use of antibiotics in animal husbandry

11:15 Actions promoting prudent use of antibiotics in Spain: One Health approach
Dr. Antonio Lopez, Spanish Agency of Medicines and Medical Devices (AEMPS), Spain

11:35 The French action plan to reduce the risk of antimicrobial resistance in veterinary medicine: a global collective and voluntary approach
Dr. Jean-Pierre Orand, The French Agency for Veterinary Medicinal Products/The French Agency for Food, Environmental and Occupational Health & Safety (ANMV/ANSES), France

11:55 Evolution of antimicrobial use and resistance in animals in Belgium
Dr. Bénédicte Callens & Wannes Vanderhaeghen, Center of Expertise on Antimicrobial Consumption and Resistance in Animals (AMCRA), Belgium

12:15 The effect of reduced use of antibiotics on resistance levels and next steps in achieving responsible use of antibiotics in livestock
Dr. Hetty van Beers-Schreurs, The Netherlands Veterinary Medicines Authority (SDa), the Netherlands

12:35 Antimicrobial resistance and use, the Danish experience
Dr. Elisabeth Okholm Nielsen, Animal Welfare and Veterinary Medicine Division, Danish Veterinary and Food Administration, Denmark

13:00 Lunch break & poster viewing
TUESDAY 27 SEPTEMBER 2016

PARALLEL SESSION 2: IMPORTANCE AND RELEVANCE OF ANIMAL INTESTINAL AND RESPIRATORY HEALTH

Chairs: Dr. Arie K. Kies, DSM Nutritional Products and Wageningen University & Research, the Netherlands
Dr. C. Stephen Roney, College of Veterinary Medicine, The University of Georgia, USA

08:30 Chair’s introduction

08:40 Microbial colonisation is essential for the development of the gut immune system in chickens
Prof. dr. Bernd Kaspers, Department of Veterinary Sciences, LMU Munich, Germany

09:05 Antibiotic usage during early life perturbs gut development in pigs
Dr. Dirkjan Schokker, Animal Sciences Group, Wageningen University & Research, the Netherlands

09:30 Antibiotic therapy in poultry: friend or foe to the gastrointestinal tract?
Dr. C. Stephen Roney, College of Veterinary Medicine, The University of Georgia, USA

09:55 Modulating the gut flora to reduce antibiotic usage in livestock
Prof. dr. Roberto Marcello La Ragione, Veterinary Pathology Centre and Department of Pathology and Infectious Diseases, University of Surrey, UK

10:20 Factors affecting the gastro-intestinal microbial balance and the impact on the health status of pigs
Dr. Paolo Trevisi, Department of Agricultural and Food Sciences, University of Bologna, Italy

10:45 Networking break & poster viewing

11:15 Proteolytic fermentation and the effects on intestinal health in broilers
Francois J. Nell, M.Sc., Animal Nutrition and Health, DSM Nutritional Products and Department of Animal Sciences, Wageningen University & Research, the Netherlands

11:40 ResPig: impact of a structural respiratory health approach on antibiotic use
Victor Geurts, M.Sc., MSD-AH Intervet, the Netherlands

12:05 The nutritional costs of the immune response in pigs
Dr. Elodie Merlot, Physiology of Adaptation, Animal Nutrition and Health, INRA/Agrocampus Ouest (PEGASE), France

12:30 Costs of broiler and fattening pig diseases, with reference to antimicrobials
Dr. Helmut Saatkamp, Business Economics Group, Department of Social Sciences, Wageningen University & Research, the Netherlands

12:55 Chair’s summary

13:00 Lunch break & poster viewing
TUESDAY 27 SEPTEMBER 2016

PARALLEL SESSION 3: THE STAKEHOLDERS’ VIEW ON ANTIBIOTIC USE AND RESISTANCE

Chairs: Peter J.G. Oostenbach, M.Sc., MSD Animal Health, the Netherlands
Dr. Stephen Page, Advanced Veterinary Therapeutics, Australia

14:00 Veterinary antibiotics: a perspective on usage, innovation incentives and effective policies
Carel du Marchie Sarvaas, Executive Director, HealthforAnimals (Global Animal Medicines Association)

14:20 Stewardship through sound science – the role of the veterinarian
John M. Blackwell, Past president British Veterinary Association, on behalf of the World Veterinary Association (WVA)

14:40 Possible measures to reduce antimicrobial use in livestock and fish; a veterinary perspective
Nancy De Briyne, Deputy Executive Director, Federation of Veterinarians of Europe (FVE)

15:00 Reducing the need for antibiotics through animal nutrition
Predrag Peršak, Chairman of the Animal Nutrition Committee, European Feed Manufacturers’ Federation (FEFAC)

15:20 The links between animal health, welfare and antibiotics use from a public health perspective
Sascha Marschang, Policy Manager for Health Systems, European Public Health Alliance (EPHA)

15:40 Public health points of view regarding antibiotic resistance
Dr. Aura Timen, President of the Section Infectious Disease Control, European Public Health Association (EUPHA)

16:00 Networking break & poster viewing

16:30 The future of broiler breeding in an ever-changing world
Dr. Mitchell Abrahamsen, Senior Vice President R&D, Cobb-Vantress, USA

16:50 Interventional genomics for the prevention of antibiotic resistance
Prof. dr. Alex Friedrich, Medical Microbiology and Infection Prevention, University Medical Center Groningen, the Netherlands

17:10 Animal production without any antimicrobials: potential scenario or license for euthanasia?
Prof. dr. Jaap Wagenaar, Department of Infectious Diseases and Immunology, Utrecht University, the Netherlands

17:30 Panel discussion

18:15 – 19:00 Poster viewing & drinks

20:00 -22:15 Conference dinner (for detailed information, see page 11)
TUESDAY 27 SEPTEMBER 2016

PARALLEL SESSION 4: ONGOING RESEARCH TO CONTROL ANTIBIOTIC USE AND RESISTANCE

Chairs: Dr. Thomas R. Shryock, Antimicrobial Consultants, USA
Prof.dr. Peter Silley, MB Consult Limited and University of Bradford, UK

14:00 A rapid procedure of PCR and a microarray lateral flow test for detection of antibiotic resistance genes
Dr. Aart van Amerongen, BioSensing & Diagnostics, Wageningen University & Research, the Netherlands

14:20 Host defence peptides: natural anti-infectives?
Prof.dr. Henk Haagsman, Department of Infectious Diseases and Immunology, Utrecht University, the Netherlands

14:40 Unlocking the medieval medicine cabinet to help fight antimicrobial resistance
Dr. Stephen Diggle, Centre for Biomolecular Sciences, University of Nottingham, UK

15:00 Disarming pathogens: an evolution-proof approach to manage infections?
Dr. Rolf Kümmerli, Department of Plant and Microbial Biology, University of Zürich, Switzerland

15:20 Human contact with dogs and the risk of carriage of antimicrobial resistant E. coli
Emma Ormandy, Institute of Infection and Global Health, University of Liverpool, UK

15:35 The influence of farm-specific characteristics and production management on antibiotic usage in conventional broilers
Tommy Van Limbergen, Department of Obstetrics, reproduction and herd health, Ghent University, Belgium

15:50 Impact of facilitated structural animal health management on antimicrobial use and animal health parameters in Dutch dairy farming.
David Speksnijder, Department of Infectious Diseases and Immunology, Utrecht University and Veterinary Clinic Tweestromeland, the Netherlands

16:05 Networking break & poster viewing

Focus on the environment

16:30 Antibiotic resistance in aquatic environments: from the evidences to the mitigation measures
Dr. Célia Manaia, Centre for Biotechnology and Fine Chemistry, Catholic University of Portugal, Portugal

16:55 Does application of antibiotic molecules to farm soil significantly increase resistance genes abundance?
Dr. Pascal Simonet, Environmental Microbial Genomics Group, École Centrale de Lyon, France

17:20 Antibiotics in aquaculture: facts and alternatives
Dr. Igor Hernández, Food Research Division, AZTI-Tecnalia, Spain

17:45 Selection for, dissemination of and exposure to antibiotic resistant bacteria in the natural environment
Dr. William H. Gaze, European Centre for Environment and Human Health, University of Exeter Medical School, University of Exeter, UK

18:15 – 19:00 Poster viewing & drinks

20:00 – 22:15 Conference dinner (for detailed information, see page 11)
WEDNESDAY 28 SEPTEMBER 2016

PLENARY MEETING: TOWARDS A GLOBAL APPROACH TO VETERINARY ANTIMICROBIAL STEWARDSHIP

Chairs: Prof.dr. John F. Prescott, University of Guelph, Canada  
Dr. Stephen Page, Advanced Veterinary Therapeutics, Australia

08:45 Introduction to veterinary antimicrobial stewardship  
Prof.dr. John F. Prescott, Department of Pathobiology, University of Guelph, Canada

09:00 Antimicrobial stewardship in healthcare in Scotland – key success factors  
Dr. Jacqueline Sneddon, Healthcare Improvement Scotland, UK

09:25 Antimicrobial stewardship in livestock: principles and practice  
Prof.dr. Guy H. Loneragan, Department of Animal and Food Sciences, Texas Tech University, USA

09:50 Antimicrobial stewardship in livestock: experiences in dairy practice  
Dr. Andy Millar, Purata Farming Ltd., New Zealand

10:15 Antimicrobial stewardship in companion animal practice: a pilot programme in Canberra, Australia  
Dr. Alison Taylor, Kippax Veterinary Hospital, Australia

10:40 Developing a global veterinary antimicrobial stewardship support programme  
Dr. Stephen Page, Advanced Veterinary Therapeutics, Australia

11:00 Networking break & poster viewing

FINAL PLENARY MEETING: WHERE DO WE GO FROM HERE?

Chairs: Prof.dr. Peter Borriello, Veterinary Medicines Directorate, UK  
Prof.dr. Jan Kluytmans, University Medical Center Utrecht, the Netherlands

11:30 The global response to antimicrobial resistance  
Prof.dr. Mark Woolhouse, Centre for Immunity, Infection & Evolution and Usher Institute of Population Health Sciences & Informatics, University of Edinburgh, UK

11:50 One Health; bridging the gap between surveillance and stewardship  
Dr. Luke Moore, Department of Medicine, Imperial College London, UK

12:15 Panel discussion: Conclusions and path forward

During this three-day conference the present and future of antibiotic use and resistance have been discussed from different viewpoints. Has the path forward become clearer? What are the 'take-home' messages? After a brief introduction by the moderators, questions from the participants will be answered.

Moderators:
Prof.dr. Peter Borriello, Veterinary Medicines Directorate, UK  
Prof.dr. Jan Kluytmans, University Medical Center Utrecht, the Netherlands

Panel members:
Dr. Arie K. Kies, DSM Nutritional Products and Wageningen University & Research, the Netherlands  
Dr. Delia Grace, International Livestock Research Institute, Kenya  
Dr. Cristina Muñoz Madero, Spanish Agency of Medicines and Medical Devices, Spain  
Peter J.G. Oostenbach, M.Sc., MSD Animal Health, the Netherlands  
Dr. Stephen Page, Advanced Veterinary Therapeutics, Australia  
Dr. Thomas R. Shryock, Antimicrobial Consultants, USA  
Prof.dr. Peter Silley, MB Consult Limited and University of Bradford, UK

13:00 Conference closing
MINIATURE THEME PARK ‘MADURODAM’
CONFERENCE DINNER

TUESDAY 27 SEPTEMBER 2016

20:00 – 22:15  Conference dinner

Buses will leave from The Hague Marriott Hotel to Madurodam at 19:30, and back from Madurodam to The Hague Marriott Hotel at 22:15.

IMPORTANT NOTES

- THE CONFERENCE DINNER IS ONLY OPEN TO PARTICIPANTS WHO REGISTERED IN ADVANCE. YOU WILL FIND YOUR TICKET FOR THE CONFERENCE DINNER AT THE BACK OF YOUR NAME BADGE.

- PARTICIPANTS WHO HAVE REGISTERED FOR THE CONFERENCE DINNER MUST WEAR AND SHOW THEIR NAME BADGE WITH THE TICKET FOR ENTRANCE TO MADURODAM.

Where:
Madurodam
2584 RZ  George Maduroplein 1
The Hague

As a special end to the 2nd day of the 4th International Conference on Responsible Use of Antibiotics in Animals there will be a unique dinner at Madurodam, with views all over the Netherlands!

The conference dinner will start at 20:00. After dinner, you will have ample time to visit the miniature theme park Madurodam. The interactive miniature park is the ultimate place to discover what makes Holland so unique within a short period of time. Madurodam presents you all the relevant Dutch stories behind the miniature buildings through multimedia and numerous interactive installations. Canal houses, tulip fields, cheese market, a wooden shoes factory, windmills, the Peace Palace, the Delta Works: you will find them all in Madurodam!
LECTURES

MONDAY, 26 SEPTEMBER 2016

KEYNOTE LECTURE

Superbugs attack! How the world can win the war on antimicrobial resistance

Steven J. Hoffman
Director, Global Strategy Lab at the University of Ottawa, Canada
steven.hoffman@globalstrategylab.org

Antimicrobial resistance (AMR) is a transnational and multi-sectoral issue that requires global collective action, since individual states cannot fully address the threat that migrating 'superbugs' pose to their citizens. Understanding the underlying global market and governance failures that prevent collective action makes it clear that an international legal response is needed to tackle this challenge. An international AMR treaty could promote access, conservation and innovation for antimicrobials by designating universally appropriate use standards, mobilising financial resources, co-ordinating national responses, and setting surveillance expectations. This presentation will review how an AMR treaty could work as a mechanism for co-ordinating states to safeguard sustainable access to effective antimicrobials. Specifically, this presentation will identify the functions needed to address AMR globally which individual countries cannot achieve on their own; the policies that would most benefit from international legalization as opposed to other approaches; as well as a roadmap to transform these functions and their implementation mechanisms into components of a treaty.
Modelling the distribution of intensifying livestock production systems

*Spatial Epidemiology Lab (SpELL), Université Libre de Bruxelles, Belgium
marius.gilbert@ulb.ac.be

Preventive and curative drugs, including antibiotics, are typically part of the inputs used in intensive livestock production systems, alongside others such as specialised breed, improved feed or infrastructure. In high-income countries, much of the intensification of livestock production systems took place in the 20th century, but the production has now mostly stabilised, or keeps increasing at a moderate rate. In contrast, in low and middle-income countries (LMICs), the combination of increasing populations and consumption per capita linked to better income, translates into a fast increase of the demand for animal source food (ASF). This has resulted in fast increases of the livestock sector, which will keep intensifying in the next few decades in response to these changing demands. A better understanding of these trends and of how these will translate into the spatial and temporal distribution of intensive production is important to understanding the present and future drivers for antibiotics consumption patterns in food animals. Here, we present a recently developed method to map the global distribution of intensively raised chickens and pigs, based on a relationship that links the national proportion of extensively raised animals to the gross domestic product (GDP) per capita (in purchasing power parity). This relationship is modelled and used together with the global distribution of rural population to disaggregate existing 10 km resolution global maps of chicken and pig distributions into extensive and intensive systems. The approach is also used to project how extensive and intensive chicken and pig production systems will develop in the future. Our results highlight countries and regions where extensive and intensive chicken and pig production systems are most important, now and into the future. We discuss the sources of uncertainties, the modelling assumptions and ways in which this approach could be extended in the future to account for different possible development scenarios.

Mapping the global use of antimicrobials in food animals

Thomas P. Van Boeckel
Department of Environmental Systems Science, ETH Zurich, Switzerland
thomas.vanboeckel@env.ethz.ch

Demand for animal protein for human consumption is rising globally at an unprecedented rate. Modern animal production practices are associated with regular use of antimicrobials, potentially increasing selection pressure on bacteria to become resistant. Despite the significant potential consequences for antimicrobial resistance, there has been no quantitative measurement of global antimicrobial consumption by livestock. We address this gap by employing Bayesian statistical models combining maps of livestock densities, economic projections of demand for meat products and current estimates of antimicrobial consumption in high-income countries to map antimicrobial use in food animals for 2010 and 2030. We estimate that the global average annual consumption of antimicrobials per kg of animal produced was 45, 148 and 172 mg/kg for cattle, chicken and pigs, respectively. Starting from this baseline, we estimate that between 2010 and 2030, the global consumption of antimicrobials will increase by 67%. Up to a third of the increase in consumption in livestock between 2010 and 2030 will be imputable to shifting production practices in middle-income countries where extensive farming systems currently dominates. For Brazil, Russia, India, China and South Africa, the increase in antimicrobial consumption will be 99%, up to seven times the projected population growth in this group of countries. The rise in antimicrobial consumption in food animals is likely to be driven by the growth in consumer demand for livestock products in middle-income countries and a shift to large-scale farms where antimicrobials are used routinely. Better understanding of the consequences of the uninhibited growth in veterinary antimicrobial consumption is needed to assess its potential effects on animal and human health. Our findings call for initiatives to preserve antibiotic effectiveness while simultaneously ensuring food security in low- and lower-middle income countries.
Moving beyond the classic foodborne pathogens to quantify the total public health burden of using antibiotics in food animals

Lance B. Price
Milken Institute School of Public Health, The George Washington University, USA
lprice@gwu.edu

It is well established that antibiotic use in food-animal production selects for antibiotic-resistant *Salmonella* and *Campylobacter* that make their way through the food supply to infect people. However, the human health risks of agricultural antibiotic use extend well beyond these classic foodborne pathogens and likely includes a number of colonising opportunistic pathogens (COPs), including extra-intestinal pathogenic *Escherichia coli*, *Klebsiella pneumoniae*, *Clostridium difficile*, and *Staphylococcus aureus*. Quantifying the incidence of foodborne COP infections is particularly challenging because these species have broad host ranges leading to multiple reservoirs and bidirectional transmission between livestock and humans. Furthermore, colonisation is typically asymptomatic and the period between exposure and infection is indefinite. The rapidly decreasing cost of genome sequencing is revolutionising infectious disease epidemiology and providing new insights into the transmission of antibiotic-resistant COPs from livestock to people. However, our surveillance systems have not kept up with technological advancements, and most countries do not have integrated surveillance systems that actively monitor COPs from livestock, meat, healthcare and the community. I will review some of the latest research on zoonotic COP infections and discuss how to move towards truly integrated systems that would enable the public health community to accurately quantify the resistance spillover from livestock to people.

Resistance in food and humans: a multidimensional problem

Jan Kluytmans
University Medical Centre Utrecht and Amphia Hospital, the Netherlands
jankluytmans@gmail.com

With the increase of highly resistant micro-organisms (HRMO) all over the world we are approaching a post-antibiotic era. The reservoir and transmission routes of HRMO are manifold and therefore control is difficult. Many studies have shown that HRMO are present in the food chain and pose a potential threat for humans. However, it is difficult to determine the contribution of HRMO in the food chain to the disease burden caused by HRMO in humans. The reasons are the low risk per exposure for acquisition, the enormous amount of exposures every day and the various level of transmission of the resistance genes. The genes may spread by transmission of strains, plasmids or other mobile genetic elements. With the high background of resistance in the population it is almost impossible to quantify the amount of resistance that is coming from the food chain.

In the Netherlands, the levels of resistance in the population are still relatively low and there is very intensive farming with high use of antimicrobials agents. This provides a unique setting to determine the role of the food chain. Examples on MRSA and ESBL will be presented, as well as the effect of a strong reduction of antimicrobial use in animals.
MONDAY 26 SEPTEMBER 2016

PLENARY MEETING
GLOBAL APPROACHES TO RESPONSIBLE USE OF ANTIBIOTICS

Collection of data on the use of antimicrobials in animals: an important tool to develop a policy for responsible use

Jean-Pierre Orand
The French Agency for Veterinary Medicinal Products/The French Agency for Food, Environmental and Occupational Health & Safety (ANMV/ANSES), France
jean-pierre.orand@anses.fr

Surveillance of data on the use of antimicrobial is considered together with collecting information on bacterial resistance as essential elements of information needed to the fight against antimicrobial resistance. Data on antimicrobial use are useful for monitoring trends in the use of antimicrobial agents in animals over time and potential associations with antimicrobial resistance in animals. This information may also assist in risk management to evaluate the effectiveness of efforts to ensure responsible and prudent use and mitigation strategies (for example, by identifying changes in veterinary prescribing practices) and to indicate where change of antimicrobial usage practices might be appropriate.

In France, such a system was established in 1999 and has evolved to enable to follow antimicrobial sale per animal species but also to evaluate exposure of animals to antibiotics. At the EU level, the ESVAC project was initiated in 2008 and currently covers data from 26 European countries. However, the level of detail provided is quite low, only sales data are available without any detail by animal species. Obtaining more precise data on antimicrobial used in animals by species and production category is recognised as a priority in France, in Europe and it is also recommended by OIE at international level. Regulatory changes to improve data collection have been adopted in France and are discussed at the EU level. Tools to collect more precise data on antimicrobials used in animals are currently under development.

At the international Level, in the framework of the Global Action Plan on Antimicrobial Resistance, developed by WHO with the support of the OIE in the spirit of the ‘One Health’, the OIE is tasked with, the construction and maintenance of a global database on the use of antimicrobial agents in animals. In the end of 2015, the OIE launched an annual collection of data on the use of antimicrobial agents in animals in OIE member countries. The first annual collection on sales of antimicrobial agents in animals had a successful participation from the member countries (72% (130/180) of OIE member countries submitted the completed template to the OIE Headquarters). Among the 130 responses, 89 countries were able to provide quantitative data. This survey shows that a number of countries have started to collect data on antimicrobials. It also shows the need to provide additional support to countries to improve their national data collection systems.

Unpacking factors influencing antimicrobial use in global aquaculture and their implication for management

1Stockholm Resilience Centre, Sweden; 2WorldFish, Malaysia; 3IMDEA Water Institute, Spain; 4Princeton University, USA; 5Beijer Institute, Sweden; 6Centro i-mar and CeBiB, Universidad de Los Lagos, Chile; 7British Columbia Centre for Aquatic Health Sciences, Canada; 8Shanghai Ocean University, China
patrik.henriksson@beijer.kva.se

Aquaculture is currently the fastest growing animal food production sector worldwide, driven by increasing demands for seafood and overexploited capture fisheries. Increases in production are to a large extent being achieved through intensification of existing farming systems, resulting in a higher risk of disease outbreaks. The initial response to disease has been increased use of antimicrobials (AMs), which in some places has resulted in antimicrobial resistance. This has raised criticism towards aquaculture products impacting negatively on human health. Some countries have responded to this
criticism and restricted their AM use, others have not. In order to better understand AM use within the aquaculture industry, the present study sets out to identify the underlying main drivers.

AM use in aquaculture differs from that in livestock farming due to more diverse species and farming systems, alternative ways of administration and less consolidated farming practices in many regions. This together with less research on AM use in aquaculture in general suggests that large data gaps persist with regards to overall use and breakdowns by species and system. However, available data do allow for identification of main mechanisms behind application rates. Type of species, strain and farming environment are obvious factors, but also underlying factors determining farmed species vulnerability to bacterial diseases are key. These were in their turn influenced by farm management, where less educated farmers with physical and monetary access to AMs often tends to over use, unless regulations that limit AM use exist and are enforced. Consolidation, strong extension services, and institutional capacity can also help to reduce AM use. If all abovementioned factors fail to limit AM use, market food safety controls and certification schemes have proven efficient measures, given that most seafood is traded on international markets.

From the set of identified mechanisms, it can be concluded local governments can reduce AM use through farmer training, spatial planning, assistance with disease identification and stricter regulations. Developing countries and international organisations could, in turn, assist with the development of more disease resistant strains, vaccines and enforce rigid testing of AM residues. The problem of AM use and resistance should be tackled internationally adopting one common approach, as the spread and transfer of antibacterial resistance to humans is likely to happen simultaneously in different parts of the globe.

Antimicrobial resistance: exploring the human animal link! Perspectives from the Latin American Caribbean region

H. Morgan Scott
Department of Veterinary Pathobiology, Texas A&M University, on behalf of PAHO Foundation, USA
hmscott@cvm.tamu.edu

The PAHO Foundation is an independent non-governmental organisation whose mission is to develop programmes, share insight, and collaborate with experts and partners to have a profound, positive impact on seemingly intractable public health problems that threaten the health and prosperity of people in Latin America and the Caribbean (LAC). One of our strategic priority areas is mobilising resources to support LAC countries’ capacity to address the threat and implications of antimicrobial resistance (AMR).

The rapid increase in AMR is a threat to global health and the global economy given the challenges of poor antimicrobial stewardship, misuse of antibiotics, and weak surveillance systems in human health sectors and the growing linkages to inappropriate use of antibiotics within the agriculture and aquaculture communities. Yet, while guidance towards solutions is emerging globally, there remains a dearth of information to inform effective and sustainable solutions with LAC.

While data from the LAC are limited, it has been estimated that in some countries, as much as three-quarters of antimicrobials are used in the production of food animals, including aquaculture [1]. Throughout the region, antimicrobials are used routinely and extensively to prevent disease and to promote growth in food animals, including via mass drug administration to healthy animals. Such practices provide favourable conditions for the emergence, spread, and increase of AMR resistance. This increase of bacterial pathogens exhibiting multiple resistance to current treatments has narrowed the potential use of antimicrobial drugs for treatment of infections in both humans and animals. Such conditions have brought serious consequences to the linkages between human health and agricultural practices as LAC has among the highest extended beta-lactamase (ESBL) bacterial prevalence among the world regions [2].

The World Health Organization has called for the development of National AMR plans (NAPs) by May 2017, with the Pan American Health Organization (PAHO) leading the LAC response by providing the technical support to member countries in the development of their NAPs. At the regional level, recent recommendations have been endorsed by the regional ministries at the Inter-American Ministerial Meeting on Health and Agriculture (RIMSA 17). These include: to adopt a ‘One Health’ approach for
issues related to the interface between human health and animal health, including limiting the impact of AMR; to adopt standards for antimicrobial use that put human health at the center; to develop, fund and implement NAPs based on multi-sectoral interventions, including an integrated AMR surveillance; and to promote the appropriate use of antimicrobials in health and agriculture, using a One Health approach [2]. While these recommendations have been endorsed, there are many challenges to the implementation phase as there exists great variability in terms of health and economic development and resources among LAC countries and even within each country.

This presentation will include discussion of the current issues and the pressing case for action for AMR in the LAC region. Examples will be provided on the current strategies and systems that are in place, efforts to build on these current capacities, and plans to combat AMR using the One Health approach from the country and regional level. The work of the PAHO Foundation in engaging with non-traditional partners to bring together non-governmental organisations, institutional partners, private sector, health associations and other interested stakeholders to improve collaboration and capacities for AMR, prevention, control and surveillance will be also outlined.

References

Working together: Australia’s response to antimicrobial resistance

Mark Schipp
Australian Chief Veterinary Officer – Australian Government Department of Agriculture and Water Resources, Australia
mark.schipp@agriculture.gov.au

Antimicrobial resistance (AMR) is a global issue gaining increasing momentum due to the serious consequences for human and animal health if it is not addressed. A whole of ecosystem approach that crosses traditional boundaries of government, research and industry will be necessary at national, regional and international levels. In recognition of this problem, Australia established a collaborative One Health partnership between the human and animal sectors in 2012. Subsequently, Australia’s AMR framework was setup with the Australian Antimicrobial Resistance Prevention and Containment Steering Group (AMRPCSG) and the Australian Strategic and Technical Advisory Group on antimicrobial resistance (ASTAG). These groups are providing oversight and strategic, technical, scientific and clinical advice to inform the development and implementation of Australia’s approach to the issue. A series of stakeholder engagement events, which occurred throughout this period, discussed AMR itself, the issues and what government action was required. Australia’s first National Antimicrobial Resistance (AMR) Strategy (the Strategy) was released in June 2015. The Strategy aligns with the requirements of the World Health Organization’s Global Action Plan on AMR for national action plans.

The Strategy is intentionally high level, supports a One Health approach and identifies broad areas where action can be taken to address seven objectives covering: appropriate use of antibiotics; surveillance; infection prevention and control; communication and education; research and development; international engagement; and governance. Priority areas for action are identified for each objective in the Strategy. The majority of the proposed action areas apply to both human health and animal health/agriculture, and are intended to outline the broad scope of activity that may be undertaken over the life of the Strategy.

To translate the Strategy into action, a National AMR Stakeholder Forum was held in November 2015 to consult and inform the development of the Strategy’s Implementation Plan. The Implementation Plan identifies which immediate actions need to be undertaken – the high level priority areas for action – this is where the greatest contribution in assisting the achievement of the seven objectives identified in the Strategy will occur. The Implementation Plan also incorporates activities being undertaken across various sectors to encourage and enable collaboration and information sharing.

For animal health, proof-of-concept model projects for AMR surveillance are being undertaken in several food animal sectors. These surveillance projects will provide comparable data on current resistance levels in key bacteria within the various food animal industries. This work is being overseen by an AMR
Surveillance Task Group, demonstrating further collaboration within the animal sector of animal health industry bodies, experts, and governments.

For many years, Australia has adopted a conservative approach towards the use of antimicrobials for animal health. For example, fluoroquinolones have not been permitted for use in food producing animals. The outcome of this conservativeness has been reflected in the Review on Antimicrobial Resistance’s published report ‘Antimicrobials in agriculture and the environment: reducing unnecessary use and waste’, which shows Australia is in a good position as a low user of antibiotics in agriculture. Antimicrobial stewardship and awareness are also critical for the maintenance of this result, which can only be done as a collaborative One Health partnership between governments and human, animal and environmental sectors.

In conclusion, Australia, to date, has a good track record with low AMR in the animal sector. However, there is no room for complacency. Continual progress is critical for retaining our human and animal health, and associated treatment options. The development of the National AMR Strategy’s Implementation Plan and further surveillance projects will allow us to maintain momentum in combatting AMR in Australia. We hope that this approach is of interest to other countries. Given the complexity of AMR and to ensure the sustainable and judicious use of antibiotics in both human and animal medicine, close co-operation of countries at regional and international levels is also essential.

**Antimicrobial resistance transmission between animal and human; what we can do from now?**

Yong Ho Park  
College of Veterinary Medicine, Seoul National University, Korea  
yhp@snu.ac.kr

Antimicrobials have played an important role in maintaining the animal health and in producing the high quality food. The concern that the use of antimicrobials in food animal production can increase the risk of selection of antimicrobial resistant bacteria that may cause failure of treatment has led to international expert meeting and reports. we summarised the current status of antimicrobial resistance in animal and some important activities or recommendations for effective implementation of policies and regulations to combat antimicrobial resistance. In particular, companion animal owners and their families are likely in close contact with their animals daily, which provides the opportunity for transfer of bacteria that encoding resistant gene between them. Therefore, the animal resistant profile should be screened not only to food animals but also to companion animals at science-based approaches. Although the prevalence of zoonotic antimicrobial resistant bacteria in food animals or companion animals is maintained still low, however, resistant genotypes similar to or identical with those of the human isolates were also found in non-human sources. Therefore, the risk management interventions should be urgently implemented. Furthermore, a ‘One Health’ approach to antimicrobial use and resistance is essential to minimise the antimicrobial resistance in humans and animals, because these are the responsibility of all three health communities: human health, animal health, and environmental health-communities.

*Methicillin Resistant Staphylococcus aureus (MRSA)*. Recent reports have documented MRSA detection in animals, foods and animal workers. Now it is considered as one of the most important zoonotic pathogens. In Korea, MRSA has been isolated from various non-human sources such as pet, live pigs, raw meat, and bovine milk. In a recent study, two different lineages of MRSA were identified namely human associated (HA) type (ST5, ST59, ST72) and livestock associated (LA) type (ST398, ST541, ST692) in non-human sources and slaughterhouse workers. Although the prevalence of MRSA in food animal products in Korea is still maintained at the low level compared to many other European countries, occurrence and increase in multiple resistant LA MRSA lineage and virulent HA MRSA lineage can be potential threat to public as animal related job workers and consumers are constantly exposed to these MRSA lineages.

*CTX-M producing Enterobacteriaceae*. Extended-spectrum β-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, CTX-M producing *E. coli* and *Salmonella* were detected in animals, raw meat,
farm environment, and farm workers. Although a variety of CTX-M types was involved in the resistance against 3rd generation cephalosporins, blaCTX-M-14 and blaCTX-M-15 were the most prevalent in non-human sources. Furthermore, identical PFGE patterns and conjugative IncFIIIs and IncI1-ly plasmids were detected in non-typhoidal Salmonella (NTS) from both human and animal sources. These results suggest that a combination of clonal and horizontal transmission is spreading of CTX-M resistant NTS between animal and human sources.

Policies and regulations to control antimicrobial resistance.

- Strengthening the monitoring systems on antimicrobial usage and resistance. Surveillance of antimicrobial usage and resistance provides important data for the identification of resistance problems and contributing factors for the development and spread of resistance at a national and local level. Antimicrobial resistance trends should be consistently monitored over time and across geographical areas and should be shared at the regional and global levels. Furthermore, harmonisation and standardisation are needed to compare situations at the national and international levels.
- Regulatory decisions based on risk assessment. The purpose of risk assessment is to supply the proper information to risk managers, policy makers, and other stakeholders. It is based on Codex and OIE frameworks of risk assessment with the incorporation of several adaptations and alternative approaches in response to important data gaps and the needs of risk manager. As there are increasing needs for evidence-based and transparent decision-making in public health, much works including development of further methodology and training of risk analysts are needed.
- Strict enforcement of prudential use guidelines for veterinarians and producers. International organisations have emphasised the importance of prudential and rational use of antimicrobials in animals in order to minimise the possible impact of animal antimicrobial usage on public and animal health. A guiding principle with respect to antimicrobial usage should be ‘as little as possible, as much as necessary’ since we owe it to both present and future generations to use these agents with care and discrimination. It is essential that all parties work together to ensure safe use and to minimise the development of resistance.
- Reduction of antimicrobial use in animal husbandry. Prevention and control of infections in food animals is essential in fighting antimicrobial resistance. Thus, to minimise infections in food animal production and decrease the volume of antimicrobials used, efforts should aim to improve animal health. This can be achieved by improving hygiene, biosecurity, and health management on farms and preventing disease through the use of vaccine and other alternative measures such as probiotics, prebiotics, or competitive exclusion products.
- Communication and education. The purpose of communication on the antimicrobial resistance is to raise awareness of the importance of antimicrobials in treating bacterial infections and public health challenges of antimicrobial resistance. Education strategies that emphasise the importance and benefits of the prudent use of antimicrobials should be developed and implemented to provide relevant information about resistance to farmers, veterinarians, and the public.

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Transatlantic Taskforce on Antimicrobial Resistance (TATFAR), collaboration in veterinary medicine

Jordi Torren-Edo, Z. Kunsagi and D.K.J. Mackay
Veterinary Medicines Division, European Medicines Agency, UK.
jordi.torren@ema.europa.eu

The Transatlantic Taskforce on Antimicrobial Resistance (TATFAR) was created in 2009 with the goal of improving co-operation between the EU and the USA in three key areas: appropriate therapeutic use of antimicrobial drugs in the medical and veterinary communities, prevention of both healthcare- and community-associated drug-resistant infections, and strategies for improving the pipeline of new antimicrobial drugs in human medicine. TATFAR is composed of US and EU institutions. Canada and Norway have recently joined the task force.

TATFAR initially identified and adopted 17 Recommendations for future collaborations between the EU and the USA. These recommendations have been implemented through increased communication (including regular meetings and workshops) and sharing of information (e.g. on approaches to common problems, best practices and methods) [1]. These recommendations are divided in 3 key subjects according to the mandate. Only those relevant for veterinary medicine are listed here:

- Collaborate in collection of data on sales and use of veterinary antimicrobials in food-producing animals.
- Collaborate on implementation of the Guidelines for Risk Analysis of Foodborne Antimicrobial Resistance prepared by Codex Alimentarius.
- Enhance information sharing on approaches to promoting appropriate use in veterinary communities.
- Encourage efforts to harmonise, to the extent possible, epidemiological interpretive criteria for susceptibility reporting of bacterial isolates across surveillance programmes in the US and EU.

A new Recommendation 18 was introduced in May 2014 [2]. This recommendation focused on the need to identify and address gaps in our knowledge of the emergence and transmission of antimicrobial resistance in bacteria related to veterinary uses of antimicrobial drugs and development of effective interventions. A report was published on the TATFAR website [3] identifying the key knowledge gaps and highlighting those that would be suitable for action within the Task Force.

Experience from the European Medicines Agency (EMA) on working with TATFAR shows that it is a good tool to reduce duplication of tasks, to gain access to experts in each area, to avoid diversion in regulatory approach, and to gain benefits of scale and additional ‘critical mass’ that allow the complex issues related to antimicrobial resistance (AMR) to be tackled more effectively. TATFAR is also a practical implementation of the One Health approach bringing together expertise from both human and veterinary medicine to address common issues. TATFAR does not initiate work on topics already under development by other international organisations including OIE and FAO in areas such as the surveillance of sales of antimicrobials in veterinary medicine. Rather TATFAR focusses on providing a forum for increased communication and exchange of information between Task Force members on priority topics which in turn enhances the contribution that members can then make to these international activities.

In October 2015, TATFAR extended the collaboration for an additional five years (2016-2020).

References
Development and migration of antimicrobial resistance in livestock in China

Dengpan Bu¹, L. Ma¹, J. Zhang¹, A. Ostermann², J. Xu³ and T. Robinson⁴
¹State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, China; ²World Agroforestry Centre East and Central Asia Regional Office, China; ³Kunming Institute of Botany, Chinese Academy of Sciences, China; ⁴International Livestock Research Institute (ILRI), Kenya
budengpan@126.com

Antibiotic pollution has become a public health issue in China. With the growing Chinese livestock industry (swine, poultry and dairy), consumption of antimicrobials is projected to further increase. Based on the annual report on development of veterinary medicine in China (2014), the total annual production of antimicrobial drugs was 53,000 ton. By category from sales, the major antimicrobial drugs were florfenicol, doxycycline hydrochloride, colistin sulfate, and enrofloxacin. It was estimated that 52% of the antibiotic substances consumed in China was applied in livestock industry and the total amount of antibiotics used was estimated to 162,000 tons in 2013. Irrational application and overdosing of antibiotics in livestock will increase the burden of antibiotic resistance in exposed environment and can pose a potentially serious threat to the health and well-being of both humans and animals. Several studies have collected data on which antibiotic substances were found in different environmental compartments, such as animal manure, agricultural fields, rivers and lakes, vegetables, wastewater and surface water, even residents’ tap water, royal jelly, and marine aquaculture in China, which gives an indirect idea that the abundance, types of antibiotic resistance, and distribution of resistance in populations of symbiotic and pathogenic bacteria vary enormously. Recently, a China national wide picture of bacterial resistance was reported through the CHINET surveillance system between 2005 and 2014. Furthermore, a few comprehensive analyses on spreading and distribution characteristic of antibiotic resistance genes (ARGs) conferring resistance to tetracyclines, sulfonamides, quinolones, aminoglycosides, and macrolides in livestock production related systems. These studies showed that ARGs distribution in soils or water depended on the animal type of the adjacent livestock farms. Few studies also showed that manure management like composting provides an opportunity to contain and/or destroy antibiotic resistance. However, research addressing the distribution and migration of antibiotics and ARGs in livestock of China is still scarce.

Antibiotic use in small holder periurban dairy farms in India: sowing ignorance, reaping resistance

Manish Kakkar
Public Health Foundation of India, India
manish.kakkar@phfi.org

Antibiotic use in the veterinary sector has been identified as a contributory factor in the global antimicrobial resistance crisis. In India, periurban ecosystems have emerged secondary to rapid urbanisation. Owing to poor regulatory and quality support infrastructure, farmers in these areas, who are mostly small holders, practice intensive industry-style livestock rearing, indulging in harmful practices to boost productivity. Such practices then lead to a greater incidence of infections and consequent higher veterinary use of antibiotics. A large and comprehensive study was undertaken recently to identify the drivers, determinants and extent of irrational use of antibiotics in smallholder dairy farms of periurban India in five cities.

Farm level antibiotic use is driven by a complex interplay of market, farm and systems-level factors. These factors, either directly (proximal drivers) or indirectly (distal factors), and by operating at different levels (micro, meso and macro) increase antibiotic consumption, worsening risks of developing resistance in the community. The study revealed several important drivers that operate in the periurban milieu in India, causing antibiotic overuse in farm animals. The lack of knowledge about antibiotics in the farmers was seen to be a factor promoting antibiotic overuse. This was triangulated by a systematic
stakeholder analysis that also revealed similar trends; at the micro (local) level, there was a lack of knowledge, empowerment and resources, whilst at the macro (national) and meso (state) levels, there was a crisis of leadership that was found facilitating ignorance. The absence of comprehensive animal healthcare services and penetrative outreach programmes further contributed to the problem. This led to increased interactions with non-formal caregivers, who were more likely to indulge in uninformed choices thus promoting liberal antibiotic use by farmers. Over-the-counter access to cheap antibiotics led farmers to self-administer drugs for common and recurrent ailments in the animals, increasing antibiotic consumption at the farm level.

The findings thus suggest that until comprehensive healthcare services are made available to the farmers through accredited health workers or licensed veterinarians, the extent of non-prescription use of antibiotics is unlikely to come down. And even if knowledge and awareness among stakeholders improve, gaps in leadership, power and resources at different levels can lead to local contexts that are conducive to irrational veterinary use of antibiotics.

With an annual output of 146.3 million tons, India is the top milk producer in the world, accounting for 18.5% of the global output. However, India has a large bovine population, resulting in relatively low output per head of cattle. India also happens to be one of the top agricultural consumers of antibiotics, accounting for 3% of the global consumption. Thus, it is critical to identify sustainable solutions that can impact antibiotic use without significantly reducing productivity. To this end, a holistic approach, that transcends the traditional biomedical approaches, needs to be adopted to combat overuse in order to effectively contain emergence of antibiotic resistance.

A veterinary intervention to reduce antimicrobial usage in chicken production in Vietnam (ViParc programme)

Juan J. Carrique-Mas
Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Vietnam
jcarrique-mas@oucru.org

The ViParc Programme aims at developing a support system for farmers in the Mekong Delta of Vietnam to help them raise meat chickens using lower amounts of antimicrobials. We will formally address this by conducting a farm-based randomised before-and-after controlled ‘trial’, that will be conducted over about three years. The trial will consist of a ‘baseline period’ to be followed by an ‘intervention period’ on a randomly subset of farms, the other group remaining as control farms. The final result from the trial (i.e. the impact of the intervention) will be fully evaluated at the end of the intervention period. Farmers assigned to the ‘intervention group’ will be provided with free veterinary support (administered through a Farm Health Plan) during the intervention phase of the trial. This support will help farmers improve the productivity of their farms whilst reducing disease, therefore reducing also their reliance on antimicrobials. In addition, half of the farmers in the intervention group will be asked not to use antimicrobials in feed. To these farmers we will provide advice on alternatives to control and treat disease as they appear in the flocks as a result of changing the feed.

Throughout ViParc we will collect samples (chicken faecal samples using cloacal/gauze swabs, and other environmental samples) that will be investigated in the laboratory for AMR in enteric bacteria present in farms. We will also investigate chickens at the end of production for the presence of antimicrobial residues in meat. For all participating farms we will conduct data collection along the production cycle of farms to help us understand: (i) antimicrobial usage; (ii) disease and productivity in farms; and (iii) levels of antimicrobial resistance in commensal enteric bacteria (these are bacteria that do not cause any disease problems in chickens). In addition to evaluating the sustainability and cost-effectiveness of the intervention, the ViParc Programme will aim to answer the following questions:

- How do farmers use antimicrobial medicines for their chickens (including in feed) and what is their knowledge about these medicines?
- How do chicken enteric bacteria become antimicrobial resistant after having used antimicrobials? Is this process reversible?
- What is the contribution of hatcheries and inadequate cleaning and disinfection on antimicrobial resistance?
- Does meat from chicken farms contain antimicrobial residues?
The ViParc Programme is led by Dr. Juan Carrique-Mas, Oxford University Clinical Research Unit (OUCRU) in Ho Chi Minh City, and is funded by the Wellcome Trust Major Overseas Programme (UK). The project will be implemented in Dong Thap province (Mekong Delta) by the Sub-Department of Animal of Dong Thap province. The project has Vietnamese and overseas collaborators: Prof. Viet Thu Ho Thi (University of Can Tho), Prof. Jonathan Rushton (Royal Veterinary College, UK), and Prof. Hafez M. Hafez (Institute of Poultry Diseases, Free University of Berlin, Germany).

Multiresistant *E. coli* in small vs. large-scale pig farms in North Eastern Thailand

Jatesade Jiwakanon1, M. Halje2, D. Karlsson2, G. Ström2, M. Pringle3 and U. Magnusson1
1Research Group for Preventive Technology in Livestock, Faculty of Veterinary Medicine, Khon Kaen University, Thailand; 2Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), Sweden; 3National Veterinary Institute (SVA), Sweden
jatgiw@kku.ac.th

Antimicrobial resistance (AMR) is regarded to be more frequent in intensive livestock farming systems than in small-holdings due to a more extensive use of antibiotics in the former systems. To investigate if this is true in settings where the livestock sector has been in a rapid transition from small-scale family farming to larger intensive farming, we compared AMR in small scales pig farms with that in in large scale farms in North-Eastern Thailand. Faecal samples were collected from three clinically healthy sows at each farm at 25 small-scale (< 20 sows; median 5 sows) and 27 large-scale (100-500 sows; median 210 sows) farms. *E. coli* was cultured and isolated from the samples and one confirmed *E coli* isolate from each sample was subjected to antimicrobial susceptibility testing. Microdilution susceptibility panels (VetMIC GN-mo, National Veterinary Institute, Uppsala, Sweden) were used to determine susceptibility to 13 antimicrobials. The minimum inhibitory concentration for each antimicrobial was visually determined. Epidemiological cut-off values, defined by the European Committee on Antimicrobial Susceptibility Testing, were used to differentiate between susceptible and resistant strains of the bacteria isolates. A semi-structured questionnaire revealed that the large-scale farms were company-owned and that the small-scale ones were family run and owned. In all large scale farms antibiotics were added in the feed, whereas in the small scale only 4% did this. In the large-scale farms there was always a veterinarian who decided when to treat an animal with antibiotics, whereas in 64% of the small-scale farms it was the farmers themselves who decided this. Multidrug-resistance (resistance to three or more antibiotics) was found in 68% of isolates from small-scale and in 89% of isolates large-scale farms ($P=0.003$). Isolates from large-scale farms were more likely to be resistant to streptomycin ($P<0.001$), sulfamethoxazole ($P=0.006$), trimethoprim ($P<0.001$) and chloramphenicol ($P=0.023$), compared to isolates from small-scale farms.

This study shows that the frequency of AMR is indeed higher in larger intensive pig farms than in small-scale farms, even if both farm-types were located in a geographically limited area. This difference might be due to a more extensive use of antibiotics in the large-scale farms.

Antimicrobial resistance along the meat value chain in Kenya

Samuel Kariuki, R. Onsare and J. Mwituria
Kenya Medical Research Institute, Kenya
samkariuki2@gmail.com

In Kenya, as the human population grows, we will see increased use of antimicrobials to enhance food production. We have witnessed commensurate increase in resistance to commonly used antimicrobials, a scenario that does not bode well for treatment and management of infections in both humans and animals. In our sentinel surveillance sites, we have observed that in some regions of the country there was evident overuse and abuse of antimicrobials. Paradoxically, some communities were unable to access these vital lifesaving medicines. The main objective of the study was to determine bacterial contamination and antimicrobial resistance along the meat value chain in Kenya.
A total of 1,200 *E. coli* isolates, 110 *Salmonella* spp. and 500 *Campylobacter* isolates were assessed for antimicrobial susceptibility. Among *E. coli* isolates from beef carcasses at three abattoir locations, bacteria were most frequently resistant to tetracycline, ampicillin and co-trimoxazole with 33% resistant to two or three antibiotics. In the poultry supply chain, *E. coli* isolates were more frequently resistant to a broader range of antibiotics, including streptomycin, quinolones and third generation cephalosporins at varying frequencies. Prevalence of resistance in *E. coli* isolates was higher in samples from a commercial abattoir sourcing chicken from medium and large scale commercial farms, as compared with samples from small scale poultry farms; in the case of ampicillin (44 vs. 18%), chloramphenicol (28 vs. 5%), and ceftazidime (14 vs. 2%); but similar in the case of co-trimoxazole (60 vs. 45%), nalidixic acid (20 vs. 24%), and fluoroquinolones (2 vs. 5%); and lower in the case of tetracycline (45 vs. 65%). A larger proportion of isolates from commercial operations were multi-drug resistant. The high prevalence of resistance in *E. coli* isolates from retail chicken meat in Nairobi outlets was consistent with those from chickens on small scale farms in peri-urban small holder producers. *Salmonella* isolates from beef carcasses were mainly resistant to tetracycline (42%), co-trimoxazole (38%), and chloramphenicol (35%); *Campylobacter* isolates from rectal swabs of broilers and neck rinsates from carcasses were resistant to ciprofloxacin (5%), gentamicin (12%), tetracycline (65%), and sensitive to erythromycin (MIC <0.04 μg/ml). Resistance rates among *E. coli* and *Salmonella* spp. from pig carcasses were higher than for beef but lower than for chickens; mainly tetracycline (39%), ampicillin (32%), streptomycin (28%), and also nalidixic acid (3%) and ceftazidime (6%), a third were resistant to at least the three most common antibiotics. Oxytetracycline was the most commonly used antibiotic among small scale poultry farmers. Others were fluoroquinolones (norfloxacin and enrofloxacin), erythromycin, sulphonamides and co-trimoxazole. Antimicrobials and other products were easily available and mostly purchased from shops (62%), or from animal health assistants.

In conclusion, antimicrobial resistance in livestock in Kenya has been rising over the recent past. It will be important to monitor antimicrobial use in livestock in order to determine factors driving overuse and misuse and put in place mitigating measures to address increasing resistance to commonly available antimicrobials.
Update on the U.S. National Action Plan to Combat Antibiotic-Resistant Bacteria

Thomas R. Shryock
Antimicrobial Consultants, LLC, USA
trshryockact2@gmail.com

In September, 2014 the President’s Council of Advisors on Science and Technology (PCAST) issued a comprehensive report on antimicrobial resistance (AMR) and how it could be addressed. An Executive Order was subsequently issued by President Obama that directed federal agencies to implement the recommendations from the PCAST report, which included creation of a federal interagency Task Force on Combating Antibiotic-Resistant Bacteria (CARB), a national strategy and action plan (NAP), as well as directing the Secretary of the U.S. Department of Health and Human Services (HHS), in consultation with the Secretaries of the Departments of Defense (DoD) and Agriculture (USDA), to establish a President’s Advisory Council on Combating Antibiotic Resistant Bacteria (PACCARB). The mission of the PACCARB is to provide advice, information, and recommendations to the HHS Secretary regarding programmes and policies intended to support and evaluate the implementation of the NAP, which will then be transmitted by the HHS Secretary to the President.

The five Goals of the NAP, whose implementation began in March, 2015, are:

- Slow the emergence of resistant bacteria and prevent the spread of resistant infections.
- Strengthen national one-health surveillance efforts to combat resistance.
- Advance development and use of rapid and innovative diagnostic tests for identification and characterisation of resistant bacteria.
- Accelerate basic and applied research and development for new antibiotics, other therapeutics, and vaccines.
- Improve international collaboration and capacities for antibiotic-resistance prevention, surveillance, control and antibiotic research and development.

The NAP divides the work under each goal into objectives, sub-objectives and Year 1, Year 3, Year 5 milestones.

In March, 2016, PACCARB identified six overarching themes of the initial NAP implementation that will require further attention by the U.S. government in order to have the strongest impact in combating antibiotic-resistant bacteria:

- Fully embracing a One Health approach
- A lead federal champion of the CARB initiative
- Co-ordination of the federal response
- Resource allocation
- Development of critical partnerships
- Economic incentives for developing and deploying new diagnostic, preventive, and therapeutic tools

Full length documents are located at:
http://www.hhs.gov/ash/advisory-committees/paccarb/resources/index.html#

Implementation of the Veterinary Feed Directive in the USA: issues and impacts

Jeffrey L. Watts, A.L. Schroeder and J.W. Hallberg
Zoetis, Inc., USA
jeffrey.l.watts@zoetis.com

In 2012, The United States Food and Drug Administration Center for Veterinary Medicine (FDA-CVM) announced the issuance of Guidance for Industry (GFI) 209 which focused on the responsible use of antimicrobials in food animals. This GFI indicated the intent of FDA-CVM to limit the use of medically-important antimicrobials in food-producing animals as well as moving all such agents to veterinary
oversight. Subsequent to the issuance of GFI 209, the agency issues two additional GFIs in December of 2013: (i) GFI 213 which provided guidance to sponsors for the removal of the growth promotion or performance claims for all medically important antimicrobials in food-producing animals; and (ii) the Veterinary Feed Directive (VFD) that would move all medically important antimicrobial agents administered through feed or water under the direct supervision of a veterinarian with a documented veterinarian-client-patient relationship (VCPR). GFI 213 used a collaborative approach with the animal health industry to both phase out the growth promotion claims through a transitional process that would end on 31 December 2016 as well as provide a path for sponsors to include prevention indications as appropriate to some products.

Since the initiation of GFI 213 and VFD process in 2013, much progress has been made. Of the 26 animal health companies with products with performance claims, all 26 have agreed to collaborate with FDA in this transition. Moreover, the process of removing the growth promotion claims from the labels and updating labelling of almost 300 New Animal Drug Applications (NADA) products or approvals is on target for completion by mid-December of 2016. Once this process is completed, any additional label changes will require a full regulatory review with supportive data packages. The implementation of the VFD has required development of both the traditional paper form of documentation and created the opportunity for the development of electronic systems to provided added support to: veterinarians, feed companies, and producers when documenting the oversight and use of feed and water products. Additionally, extensive training has occurred over the past year and will continue for the next year or two to veterinary and producer groups on the new regulatory requirements. Zoetis has conducted and presented GFI 213/VFD information in over 600 veterinary, producer and industry meetings since early 2015. Overall, the process used by the FDA-CVM to remove the growth promotion claims from medically important agents used in food-producing animals has been a successful partnership between the agency, the animal health companies, veterinarians, feed companies, nutritionists and producers. The importance of this type of public-private collaborative effort coupled with a transition period to allow for development of systems to support veterinarians and producers in this change cannot be over-emphasised.

The CVMP’s Strategy on Antimicrobials 2014 to 2020 – an EU regulatory perspective

Helen Jukes
Veterinary Medicines Directorate, UK
h.jukes@vmd.defra.gsi.gov.uk

The European Medicines Agency’s Committee for Medicinal Products for Veterinary Use (CVMP) is responsible for preparing opinions on questions concerning the use of veterinary medicines in the EU. The CVMP’s Strategy on Antimicrobials is being finalised for the period from 2016 to 2020 against the background of the proposed EU Regulation on veterinary medicinal products (VMPs) which is currently undergoing legislative process. The strategy has six aims which include: providing opinions on marketing authorisations for antimicrobial VMPs, addressing the public health risks arising from the use of antimicrobials in animals, maintaining the effectiveness of authorised veterinary antimicrobial VMPs and supporting the development of new veterinary antimicrobials and their alternatives. The presentation will highlight some of the actions the CVMP intends to take to address the challenge of AMR in relation to VMPs. The CVMP is increasingly collaborating with colleagues from the human medicine arena to provide recommendations on the use of critically important antimicrobials, such as colistin, in animals. The CVMP will continue to provide scientific review of the marketing authorisations of existing antimicrobial VMPs under referral procedures where an AMR risk is identified, and is preparing for the harmonisation of the SPCs foreseen under the new Regulation. The development of new antimicrobials and alternatives, especially vaccines, will be encouraged through provision of regulatory guidance and scientific advice. Finally, the CVMP is supporting the responsible use of antimicrobials in food-producing animals by providing, jointly with the European Food Safety Authority (EFSA), a scientific opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the EU.
Organic farming and antimicrobial resistance in Germany – the example of *E. coli* in dairy herds

Bernd-Alois Tenhagen, M. Grobbel, B. Guerra, K. Alt and A. Käsbohrer
Department Biological Safety, Federal Institute for Risk Assessment, Germany
bernd-alois.tenhagen@bfr.bund.de

Public perception relates AMR rather to conventional than organic farming. It was the purpose of this study to compare AMR in *E. coli* harvested from bulk tank milk (BTM) in organic and conventional dairy herds in Germany.

Sampling was carried out in 2014. Sampling was distributed across the country based on the regional distribution of conventional and organic dairy cows in Germany within a national monitoring framework. BTM samples (25 ml) were analysed by regional laboratories for *E. coli* and isolates were sent to the National Reference Laboratory for Antimicrobial Resistance at the Federal Institute for Risk Assessment (BfR). Resistance to 14 antimicrobials was tested using the broth microdilution method according to the prescriptions of Commission Decision 2013/652/EC on the harmonised monitoring of AMR in the food chain in the EU. Minimum inhibitory concentrations were evaluated according to EUCAST epidemiological cut off (ECOFF) values. Testing included (ECOFFS in mg/l in brackets): gentamicin, colistin, trimethoprim (>2), chloramphenicol, nalidixic acid, azithromycin (>16), ciprofloxacin (>0.06), ampicillin, tetracycline (>8), sulfamethoxazole, meropenem (>0.125), tigecycline (>1), cefotaxime (>0.25), ceftazidime (>0.5). For azithromycin, no ECOFF was available. We therefore used the value recommended by the European Food Safety Authority (EFSA) and the European Union Reference Laboratory for antimicrobial resistance. Regional laboratories sent 74 and 122 isolates of *E. coli* from organic and conventional dairy farms, respectively. The vast majority of the isolates was susceptible to all 14 test substances. Of the 3 isolates from organic herds (4.1 %) that were resistant to at least one substance, all were resistant to tetracycline, one additionally to ampicillin and another one to tetracycline and sulfamethoxazole. Among the 13 resistant isolates from conventional herds (10.7 %) resistance to ampicillin (7 isolates), sulfamethoxazole (7) and tetracycline (6) was also most prevalent. However, resistance to ciprofloxacin, a fluoroquinolone (2), colistin (1) and ceftazidime, a 3rd generation cephalosporin, (1) was also observed.

The results underline that overall, AMR is not a major issue in dairy herds. However, the proportion of resistant *E. coli* was even lower in organic dairy herds and resistance was only observed to comparatively old antimicrobials with widespread resistance in other food animal populations. In contrast, isolates from conventional farms showed some diversity in resistance which might reflect differences in selective pressure in the herds.

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Strategies in U.S. veterinary medicine for antibiotic use and surveillance of antibiotic resistance

R.L. ‘Rick’ Sibbel
Merck Animal Health, USA
rick_sibbel@merck.com

The presentation will give an overview of the past and current activities currently being deployed and proposed in the United States in food animal agriculture in the strategy of antibiotic stewardship. It will give an overview of antibiotic use information and soon to be adopted regulation in antibiotic feed and water use for the food animal industries. It will also discuss proposed policy and legislation in additional strategies being considered in antibiotic use in animal agriculture in the United States.
Incentivising R&D for new antimicrobial drugs and non-antibiotic alternatives

Stacy Sneeringer
Structure, Technology, and Productivity Branch, Economic Research Service, U.S. Department of Agriculture, USA
ssneeringer@ers.usda.gov

Antibiotic resistance has the potential to lower efficacy of antimicrobials in humans and animals, yielding higher morbidity and mortality. While research is done to better understand resistance and proliferation, a generally agreed-on route of action is to encourage development of new antibiotics. While much literature explores such programmes in human pharma, nothing yet examines similar programmes for the veterinary sector. We examine incentive programmes for new products that can reduce the use of medically important antibiotics in animal agriculture. To do this we address three overarching questions. First, what are the relevant characteristics of the veterinary product development market, and how is it related or different from the human pharmaceutical market? Second, can programmes to incentivise new human antibiotic development be leveraged for development of new animal antibiotics? Third, would programmes like those proposed or used in the human pharmaceutical market work similarly in animal pharma?

Environmental, economic and animal health implications from antibiotic-free production of broilers in the USA

Matthew J. Salois
Elanco Animal Health, USA
msalois@elanco.com

Rising consumer interest in how food is produced coupled with global conversations over the use of antibiotics in the raising of farm animals has resulted in the growth of antibiotic-free (ABF) meat production and consumption. The antibiotic-free classification is most prominent in the poultry market as several producers and retail-chain companies have moved their marketing in that direction. Related to the antibiotic-free movement is the dual challenge of meeting global demand for affordable food, especially for affordable animal proteins, in a sustainable manner. Given the role that antibiotics play in supporting animal welfare and production efficiency, it is important to understand the impacts of eliminating the use of antibiotics in poultry production.

This research examines the potential environmental, economic, and animal health impacts of withdrawing antibiotics (including animal-only ionophores) from U.S. broiler production using a simulation approach. In addition, this research also examines the impact of antibiotic-free production on key health conditions that are indicative of broiler welfare using data collected by Elanco Health Tracking System (HTS). Specifically, risk and severity of eye lesions, footpad lesions and airsacculitis are assessed. These conditions disrupt biologic function and are painful for the broiler. Findings show that ABF production reduces the quantity of meat produced at the house-level and requires additional birds to be placed to keep supply constant. A simulated hypothetical 100% ABF market requires between 680-880 million more birds to maintain supply and results in between 95-230 million more bird deaths than a conventional system. In terms of the impact to resource utilisation, a 100% ABF market requires between 5.4-7.6 million tons of additional feed which requires between 2.5-3.3 million more acres of land to grow. The additional birds required for 100% ABF require between 1.9-3 billion gallons of water and produces between 4.6-6.1 million tons of additional manure than a conventional system. A 100% ABF system at the market-level results in an additional production cost of between $3.0–3.8 billion dollars. Moreover, findings reveal a detrimental impact from ABF production on broiler health and welfare. First, estimated odds ratios from the analysis demonstrate that, antibiotic free birds are 3.1 times more likely to have eye lesions compared to broilers that have been given antibiotics. There is also an increased likelihood of footpad lesions by 25% for antibiotic free broilers compared to birds produced in a conventional system. Similarly, there is a 58% increase in risk for an antibiotic-free bird having airsacculitis compared to a bird that was raised conventionally.

Overall, findings suggest that eliminating the use of antibiotics in the raising of broilers is associated with an increased risk of certain disease states leading to an overall reduction in animal welfare. Moreover, the antibiotic-free production may have a negative effect on the conservation of natural
resources as well as a negative economic effect via increased prices to the consumer. Taken together, our study suggests the need to communicate to consumers the supportive role that prudent, responsible use of antibiotics for animal disease treatment, control, and prevention plays in the sustainable production of chicken.

References

Actions promoting prudent use of antibiotics in Spain: One Health approach

Antonio Lopez
Spanish Agency of Medicines and Medical Devices (AEMPS), Spain
alopezn@aemps.es

Spain has performed a National Plan to Combat Antibiotic Resistance (PRAN) that includes six strategies with the objective to combat the resistance and to ensure, for as long as possible, continuity of successful treatment of bacterial disease with effective and safe medicines, used in a responsible way, and accessible to all who need them. The plan has a holistic, multisectorial approach, involving many different sectors (human medicine, veterinary medicine, research, animal husbandry, education and communication) because it is only by taking action in all of these areas simultaneously that further spread of resistance will be limited and antibiotic kept effective. Since its adoption in 2014, we have been working on the development of specific actions to combat this serious public health problem. Currently, a number of documents linked to each of the measures and strategic lines of the PRAN have been developed. More than 260 experts, 70 scientific societies, representatives from 6 ministries (health, agriculture, education, economy, interior, and defence), and all the autonomous regions are involved in the development and implementation of the national plan.

In consensus with representatives of the autonomous communities and scientific societies, we are now prioritising specific measures whose implementation will have a strong impact on improving the use of antibiotics and therefore in controlling resistance. Priority measures include:

• The implementation of antibiotic stewardship programmes in hospitals and primary care.
• The development of an epidemiological map by livestock regions, allowing us to address the real problems in a specific way.
• On-line guideline where the practitioner can find all the information that he needs to make a proper prescription (epidemiological situation in your area, treatment recommendations for the method of administration, etc.) and of course, to provide greater security than currently exists when making an exceptional prescription.
• The development, promotion and implementation of recommendations and programmes of prevention of health care related infection.
• The creation of a national network of sentinel laboratories or reference for monitoring antibiotic resistance with the support of reference centres in each region.
• The improvement the system of collection and use of data consumption of antibiotics in primary care and in hospitals.
• The development and implementation of electronic prescriptions and computerised control systems of related treatments in the veterinary sector, so as to know the consumption at farm level.
• In animal health, the improvement of the vigilance systems of antibiotic sales, including data at a distribution level.
• The training of health professionals at all stages of their training (university, training for specialisation and continuous training).
• Raising awareness of the population on the prudent use of antibiotics and the problem of resistance to them.
• Ensuring the exploitation and analysis of data at a local, regional and national level and information feedback.
• Monitoring programme target pathogens in food-producing animals in the EU.
Standardising antimicrobial susceptibility testing for the characterisation of resistant bacteria and promoting the use of rapid diagnostic tests to improve medical prescriptions

We can say that the development of resistance to antibiotic is one of the most serious problems we have to face now. The causes are multiple and therefore isolated and uncoordinated measures are doomed to failure. Any plan or strategy for the fight against antibiotic resistance should include all those involved, mobilising them for the same purpose, to prevent post-antibiotic time, with the serious consequences associated lead. We all need to educate us that this struggle depends on each of us and no longer have excuses for inaction.

The French action plan to reduce the risk of antimicrobial resistance in veterinary medicine: a global, collective and voluntary approach

Jean-Pierre Orand
The French Agency for Veterinary Medicinal Products/The French Agency for Food, Environmental and Occupational Health & Safety (ANMV/ANSES), France
jean-pierre.orand@anses.fr

The national action plan for the reduction of antibiotic-resistance risks in veterinary medicine, called ‘Ecoantibio2017’ was launched in November 2011 by the French ministry of agriculture. This action plan sets specific targets: a quantitative objective to reduce antibiotic use in veterinary medicine by 25% within 5 years and a qualitative one focus on the reduction of the use of critically important antibiotics (particularly fluoroquinolones and third and fourth-generation cephalosporins). The action plan has two objectives: firstly, to reduce the contribution to bacterial resistance made by the antibiotics used in veterinary medicine and its consequences for public health, and secondly, to preserve the therapeutic arsenal on a sustainable basis, especially given that the prospects for development of new antibiotics are limited in veterinary medicine.

This action plan is organised into five priorities:
- promote best practices and raise awareness among the stakeholders involved to the risks arising from antibiotic resistance and the need to preserve the effectiveness of antibiotics;
- develop alternatives to antibiotic use;
- reinforce the regulation of commercial practices and prescribing rules;
- improve the system for monitoring antibiotic use and antibiotic resistance; and
- promote the same approach on the European and international scale.

The main key points for success consisted in:
- mobilising all stakeholders;
- improving livestock farming practices;
- reinforcing partnerships between prescribers and animal owners; and
- promoting prevention and alternatives.

These priorities were organised in 40 measures and have been implemented. In the framework of this plan, both legislative and voluntary measures have been taken. After 4 years, this has led to encouraging results. For example, a voluntary restriction of the use of cephalosporins in pigs led to a decrease by 82% in the use of cephalosporins and a decrease of 20% of the use of all antimicrobials in veterinary medicine was registered.
Evolution of antimicrobial use and resistance in animals in Belgium

Bénédicte Callens1,2, Wannes Vanderhaeghen1, V. Piessens1, F. Dal Pozzo1, S. Lagast3, P. Wattiau2 and J. Dewulf1,4
1Center of Expertise on Antimicrobial Consumption and Resistance in Animals (AMCRA), Belgium; 2Veterinary and Agrochemical Research Centre (CODA-CERVA), Belgium; 3Belpork vzw, Belgium; 4Faculty of Veterinary Medicine, Ghent University, Belgium
benedicte.callens@amcra.be; wannes.vanderhaeghen@amcra.be

Antimicrobial resistance (AMR) is a worldwide concern in both human and veterinary medicine. The main factor in the development, selection and spread of AMR is antimicrobial use (AMU). This presentation summarises efforts made and results established towards reducing AMU and AMR levels in animals in Belgium in recent years.

Veterinary AMU is monitored at national level through the collection of sales data of all pharmaceutical formulations containing antibacterials registered on the Belgian market. These data, presented in the yearly BelVetSAC reports, have shown a consecutive decrease of AMU in 2012 and 2013 followed by a slight increase in 2014. In 2015, however, AMU decreased again, reaching 129.4 mg/kg biomass and resulting in a cumulative reduction between 2011-2015 of 15.9%. For pigs, a sector-driven, herd-level AMU monitoring system called ‘AB Register’ has been set up in 2014 by Belpork, owner of the pork-quality system Certus. AMU is calculated as the number of treatment days (TD) per 100 animal-days. Results of 2014-2015 show AMU is highest in weaners (mean TD: 30.33) and lowest in sows/boars (mean TD: 0.39). Also in this system a slight decrease was measured in all weight categories between 2014 and 2015. Extended-spectrum penicillins, tetracyclines and trim-sulfa antimicrobials together accounted for approx. 60% of TD. In the future, AB Register AMU monitoring will be applied in the poultry and dairy cattle sector. In addition, a national data collection system called ‘Sanitel-Med’ has been established in June 2016, for the collection of herd-level AMU data in pig, broiler, layer and veal calf farms, based on compulsory national legislation. The first results are expected in 2017.

Since 2011, the national annual monitoring programme for AMR in commensal bacteria collects data on the prevalence of antibiotic resistant Escherichia coli strains in Belgian livestock, under the supervision of the Federal Agency for the Safety of the Food Chain. Based on the data from 2011 to 2015, a trend analysis of the prevalence of AMR in commensal E. coli, considered as representative of Gram-negative bacterial species of the commensal flora from livestock, has been performed by the CODA-CERVA (the National Reference Laboratory for AMR). The analysis checked for statistical significance of the observed trends (increase or decrease). Also the level and trend of multi-resistance over the same period was calculated for each animal category. The results indicated an overall decreasing trend of E. coli AMR in Belgian production animals. However, this decrease appeared to slow down in 2015, especially for veal calves and broilers. In pigs, 2015 was the first year AMR was lower than 50% for all tested substances. Worryingly, resistance to critically important fluoroquinolones remained at very high levels in broilers (64.5%) and high levels (24.0%) in veal calves. A significantly decreasing trend of multi-resistant strains of E. coli was observed from 2012 onwards. Yet, the proportions of multi-resistant strains remained high during the five consecutive years, especially in chickens (>60%) and veal calves (>50%). Moreover, in chickens, an increase was seen again in 2015, resulting in a mere borderline significant decreasing trend for the five consecutive years. E. coli strains from broiler chickens were also resistant to the highest number of antibiotics (highest entropy), whereas E. coli from pigs had the lowest entropy. Again, multi-resistance and entropy revealed broiler chicken production as the animal production sector where additional measures should be taken in terms of rationally reducing AMU.

The effect of reduced use of antibiotics on resistance levels and next steps in achieving responsible use of antibiotics in livestock

Hetty van Beers-Schreurs
The Netherlands Veterinary Medicines Authority (Sda), the Netherlands
vanbeers@autoriteitsdiergeneesmiddelen.nl

In order to reduce further increase of the antibacterial resistance (ABR) the government of the Netherlands together with the livestock sectors (pigs, poultry, veal and dairy) and veterinarians developed an action plan to reduce the use of antibiotics (Abs), in the assumption that reduction in use
is associated with reduction in resistance. Use of ABs reduced since 2009 with 58.4%, therefore, research of existing data was performed to check whether the assumption is correct for commensal *E. coli*. Furthermore, the policy to reduce the use of ABs at farms that are high users for consecutive years will be presented.

**Effect of reduced use of antibiotics on ABR.** Data of the use of ABs were obtained from the Agricultural Economic Institute (2004 to 2012) and from the Netherlands Veterinary Medicines Authority (2011-2014) and expressed as defined daily dosage per animal per year (DDDA/Y). Data of ABR were obtained from Wageningen Bioveterinary Research as published in MARAN and presented in percentages based on MICs interpreted using epidemiological cut-off values. AB use increased from 2004 till 2009 and decreased thereafter with 58.1%. ABR decreased in all animal sectors, but levels of reduction differ in animal species and specific type of resistance. ABR reductions were most prominent in pigs and veal: pigs, 54% reduction in AB use and 22% reduction in ABR; and veal, 28% and 26%, respectively. Reductions in ABR in poultry were less distinct (57% and 8%, respectively). AB use in dairy is very low, ABR reduction is very high (79%). So the decrease in AB use is associated with a decrease in ABR. Reduction in use has proven an efficient way to reduce ABR in farm animals. Further reduction of AB use is therefore the target for farms with average and high AB use as these livestock farms are particularly at risk for development of antibiotic resistance and spread of resistant bacteria.

**Next steps to responsible use of antimicrobials.** The factors contributing to the high usage levels at these livestock farms are largely unknown. Identification of contributing factors should help distinguish between avoidable and unavoidable use of ABs at livestock farms in the signalling and action zones. By having livestock farms in the signalling and action zones implement improvement measures targeted specifically at these contributing factors, they will be able to achieve further reductions, leading to more narrow and uniform usage level distributions. In response to the lower usage levels and less pronounced between-farm variations in usage levels, the benchmarking method will be reviewed and updated accordingly. If trend analysis indicates the emergence of low and uniform usage levels within a particular livestock sector or subsector, the two benchmark thresholds may be replaced by a single and potentially final benchmark threshold representing prudent, uniform usage of antibiotics. In such cases, the sector or subsector concerned has managed to limit its antibiotic use to the minimum level achievable when keeping livestock. New benchmark values will be available in 2018.

**Antimicrobial resistance and use, the Danish experience**

Elisabeth Okholm Nielsen
Danish Veterinary and Food Administration, Denmark
eoni@fvst.dk

Antimicrobial resistance is closely related to the antimicrobials used. Consequently, Denmark has focus on crude consumption and prudent use of antimicrobials. The consumption in pigs and cattle was reduced by 17% between 2009 and 2014. The aim is another 15% reduction by 2018. For critical important antimicrobials, the pig industry voluntarily introduced a ban on the use of 3rd/4th generation cephalosporin in 2010. The cattle industry introduced a similar ban in 2014. Denmark has a low antimicrobial consumption for production animals (44.9 mg/PC). The pigs account for approx. 78 % of the total consumption amounting to approx. 105 ton for production animals in 2015. Important for the achieved successes is a strong cooperation between sectors, authorities on human health and veterinary health, universities, pharmaceutical industry and the veterinary association. There have been some concerns for welfare of animals as we went along with the reduction. But there has not been seen any evidence of a decreased animal welfare or increase in infection prevalence. Some of the cornerstones in the Danish experience are that all antimicrobials are prescription-only medicines. In 1996 the veterinarians were limited to non-profit sales, and Veterinary Advisory Service Contracts of herds of cattle and pigs came into force. The contracts establish a 1:1 relationship between farmer and veterinarian. In 2002, the use of critical important fluoroquinolones was restricted by legislation.

**Yellow Card initiative.** The Danish experience is that data on the antimicrobials consumption on herd, species and age-group level is crucial for reduction. The first Danish target was a 10% reduction over three years. This was achieved by addressing the pig farmers with the Yellow Card initiative. The current 15% reduction is pursued by lowering the thresholds on herd level for the use in pigs. Furthermore,
prudent use guidelines are part of Danish the risk management strategy. The first treatment guideline was made for pigs in 2005; a revision is carried out in 2016.

*Recent developments.* A new model of the Yellow Card Initiative has been introduced in 2016 to reduce the consumption of antimicrobials that are particularly necessary to continue to treat infections in humans. This is done by weighting the current animal daily dose with selected factors. For instance, the critical important antimicrobials are given 10 times weight, so the threshold will be reached very soon if used.
Microbial colonisation is essential for the development of the gut immune system in chickens

S. Lettmann¹, S. Röll¹, S. Härtle¹, B. Schusser², C. Schouler³, P. Velge³ and Bernd Kaspers¹ ¹Department of Veterinary Sciences, LMU Munich, Germany; ²Biotechnology of Reproduction, TUM, Germany; ³French National Institute for Agricultural Research (INRA), France kaspers@lmu.de

Development of the immune system is controlled by genetic programmes and environmental cues. Microbial colonisation of the gut after birth is critical for immune system development as shown in mice reared under germ free conditions. While mammals acquire the initial gut flora from their mother this is not the case in modern poultry production. Layer and broiler chickens are hatched under nearly sterile conditions and reared without contact to a maternal flora. Whether this impacts on the development and functional maturation of the immune system is largely unclear.

We therefore set up a series of experiments to compare immune system development in sterile (germ free), mono- and tetra-reconstituted and SPF birds. A fifth group of birds was raised in the presence of a maternal flora reproducing the natural conditions in most galliforms. While no significant differences were observed in the development of selected parameters of the innate immune system between the groups, striking differences were observed in the adaptive immune system. Germ free birds had highly reduced numbers of B-lymphocytes in the gut. Similarly, intestinal T-cells were reduced in numbers as revealed by immuno-histology and qRT-PCR quantification of CD4 and CD8α transcript abundance. Most strikingly, germ free birds lacked germinal centres in caecal tonsils the essential compartment for immunoglobulin class switching and the generation of high affinity antibodies. A similar phenotype was observed in birds reconstituted with one probiotic E. coli strain while birds reconstituted with four different probiotic bacterial strains showed advanced immune system development, nearly matching SPF birds.

Reduction in lymphocyte numbers in germ free birds was paralleled by a complete absence of IgA production in the gut and blood. IgA production was partially restored by mono-reconstitution and further enhanced in tetra-reconstituted and in SPF birds. However, development of the B-cell system was greatly retarded in all groups in comparison with birds that acquired a maternal flora on the day of hatch. These birds showed significantly higher blood immunoglobulin concentrations and IgA concentrations in the bile and gut. Interestingly, neither B-cell maturation in the bursa of Fabricius nor circulating B-cell numbers were affected. These data predict that molecular cues induced by microbial colonisation attract circulating lymphocytes into the mucosal tissue and regulate mucosal immune system development. Lack of these cues may have a significant impact on the control of pathogen invasion and vaccine efficacy. Thus, timely development of a functional mucosal immune system induced by physiological colonisation with a maternal flora will enhance animal health, welfare and reduce antibiotic usage. Consequently, we should aim at identifying a defined bacterial flora providing essential molecular cues to fully stimulate immune system development and thereby animal health.

Antibiotic usage during early life perturbs gut development in pigs

Dirkjan Schokker
Animal Sciences Group, Wageningen University & Research, the Netherlands dirkjan.schokker@wur.nl

Early life perturbations, such as antibiotic usage, have long-lasting effects on the gut ecosystem. Evidence is accumulating in human and rodent studies, that individuals exposed to (oral) antibiotics in early life have a higher risk of developing immune related diseases, such as asthma and allergy. However, the underlying mechanism that a change in microbiota due to the use of antibiotics result in disease is not yet fully understood. Therefore, it is important to identify the factors that (co)determine intestinal development and microbial colonisation in early life of livestock species.
Here, a study was designed to investigate early life environmental perturbations on gut intestinal (immune) development and microbial colonisation. To this end, we divided piglets of 16 litters into 3 groups: group 1 was not treated; group 2 was treated with an antibiotic (day 4 after birth); and group 3 was treated with an antibiotic and simultaneously exposed to (stressful) routine management procedures (including tail docking, toenail clipping, and weighing). The short-term effects on gut development were measured 4 days later, at day 8 after birth, whereas long-term effects were measured at day 55 (4 weeks after weaning) and day 176 (slaughter). At these time-points, 16 piglets per treatment group were subjected to both community-scale analysis of the gut microbiota as well as genome-wide intestinal transcriptome profiling. At day 8, short-term effects, were observed in both the microbiota composition and diversity as well as transcriptomic profiling were the immune signalling pathways in the gut were modulated. These results suggest that immune programming between these groups is different. Long-term effects of the early life treatments were seen in the transcriptomic data at day 55 as well as on microbiota diversity at day 176.

By measuring multiple facets of the gut system in time, it is possible to unravel certain biological processes involved in gut health. These observed data are consistent with the hypothesis that early life changes in a developing gut system affect the gut microbiota composition and/or diversity, and concomitantly local gene transcription. These changes last throughout adulthood, and may result in higher risk for certain mucosal related diseases.

**Antibiotic therapy in poultry: friend or foe to the GI tract?**

C. Stephen Roney  
Poultry Diagnostic and Research Center, College of Veterinary Medicine, The University of Georgia  
sroney@uga.edu

The United States Food and Drug Administration's decision in 2014 to call for a voluntary withdrawal of certain antibiotics for growth and feed efficiency purposes in livestock capped off several years of the most publicised and often misunderstood topic in human food safety. For many years, commercial poultry in the US were reared almost completely with low levels of FDA approved antibiotics in feed to improve growth and feed efficiency and more recently as a prophylaxis for certain potentially devastating intestinal infections. The action of these antibiotics on the normal intestinal microflora has been poorly understood for many years but recent work has begun to reveal some of this information.

Researchers at the Poultry Diagnostic and Research Center at the University of Georgia, USA, have examined the effects of feed additives on the development on the ileal bacterial community of the broiler chicken. Using molecular ecology, utilising DNA-sequence heterogeneity of the 16S rRNA gene, they compared the differences among treatments with the common feed additive products bacitracin, virginiamycin and monensin, as compared to controls. These antibiotics were found to reduce the diversity of the ileal bacterial community and induced communities rich in clostridia throughout the life of the broiler chicken. The results indicate that some bacterial species, such as lactobacilli, were suppressed and suggested that many clostridia may be non-pathogenic in the broiler GI tract.

Microbial compositions of the broiler intestine as determined by the 16S rRNA gene analysis are compared to earlier studies using the more traditional culture based approach for identification.

**References**

Modulating the gut microflora to reduce antibiotic usage in livestock

Roberto Marcello La Ragione
Veterinary Pathology Centre, and Department of Pathology and Infectious Diseases, University of Surrey, UK
r.laragione@surrey.ac.uk

The average human or animal body harbours at least 10 times more bacterial cells than human/animal cells. However, relatively little is known about how these organisms contribute to health and disease. Moreover, bacteria are just part of this vast community, collectively known as the microflora. This community consists of up to 1,500 species of microorganisms including bacteria, fungi, parasites and viruses. It has recently been hypothesised that this vast community may play an important role in regulating animal and human responses to infectious and non-infectious diseases, in addition to playing a pivotal role in nutritional status.

Infectious diseases are responsible for significant economic losses in the livestock industry and have implications with regard to animal welfare. Furthermore, a number of livestock pathogens are zoonotic. Traditionally infectious diseases in livestock have been controlled through the use of vaccination, biosecurity measures and antibiotics. However, with the increased awareness of the emergence of antimicrobial resistance, alternative control strategies are urgently required. One such alternative may be to use prebiotics and probiotics to modulate the gut microflora. Probiotics are classified as live microbial feed supplements; often members of the normal flora. Lactobacillus-based probiotics have been reported previously as protecting against infection with common enteric pathogens in livestock. Prebiotics are non-digestible in the upper gut, however, they are fermented in the large intestine. Diets enriched with prebiotic oligosaccharides, such as galactooligosaccharide (GOS), have been shown to increase the number of lactic acid bacteria (LAB) such as bifidobacteria and lactobacilli and/or their fermentation products in the colon and thus may stimulate beneficial bacteria and therefore selectively modulate the gut microflora.

Despite the widespread use of prebiotics and probiotics in humans and animals the mechanisms of action of these novel interventions remain to be elucidated. Therefore, the studies described here aimed to evaluate the efficacy and mechanisms of action of the prebiotics and Lactobacillus-based probiotics in mitigating Brachyspira pilosicoli and Salmonella Typhimurium infection in livestock species. The studies utilised a number of novel in vitro and in vivo models, including 3D cell culture, in vitro organ culture (IVOC) and whole animal studies to study how these interventions exerted their effect on the pathogens. Furthermore, the studies evaluated the host response to the prebiotics and probiotics, including evaluating effects on the normal gut microflora. The studies demonstrated that probiotic bacteria and prebiotics can antagonise aspects of the pathobiology of Brachyspira pilosicoli and Salmonella Typhimurium and modulate the gut microflora. Therefore, they may be effective in the control of bacterial pathogens in livestock.

Factors affecting the gastro-intestinal microbial balance and the impact on the health status of pigs

Paolo Trevisi and P. Bosi
Department of Agricultural and Food Sciences, University of Bologna, Italy
paolo.trevisi@unibo.it

The responsible use of antimicrobials in both human and veterinary medicine is one of the main EU policy areas relevant to tackling antimicrobial resistance. As reported in the recent document published from EU Commission (2015/C 299/04) on the prudent use of antimicrobials in veterinary medicine, the ‘appropriate use’ approach needs to be twinned with preventive intervention based on a well-designed management strategies able to maintain/favour the pig health, by promoting the natural defences development to increases the resistance against pathogen infections. In pigs, the largest part of antibiotics is used to treat the post weaning diarrhoea (PWD) occurrence. The growing evidences on the prominent role of gut microbiota to stimulate the maturation of the gastro-intestinal tract (GIT) and on the key role in the prevention of pathogen colonisation of the GIT of piglets, are the main reason that drove the set-up of the FA1401 – PiGutNet network, supported by the COST Action programme. Today, there is not a consensus on the definition of ‘balanced or favourable microbiota’, even because in the last few years the knowledge on the host-microbiota-environment interplay disclosed a very complex
picture on this field. The available information concurs to define the modern vision based on the development of a continuity concept in which sow, piglet and growing pig are connected, highlighting the need to move the attention also on the farrowing/lactating period, when the early settlement/modification of a non-pathogenic microbiota can affect the health of the animals along all their life. Moreover, the advancement in genomic and phenomic field provides an enormous amount of information, that, are a milestone to defragment this complex picture, even thanks to the progress on the genome/metabolome wide association study approach, based on properly designed bioinformatics tools. The work to define all the factors affecting the GIT microbiota and its metabolism, clarifying their relevance in practice, is still in progress and is the first step to hypothesise effective strategies to manage/restore the intestinal microbiota after an external perturbation, such as the early administration of an antibiotic or a bacterial infection disease.

Proteolytic fermentation and the effects on intestinal health in broilers

Francois J. Nell1,3, S.W.C. van Mil2, R.P Kwakkel1 and A.K. Kies1,3
1Animal Nutrition Group, Department of Animal Sciences, Wageningen University & Research, the Netherlands; 2Department of Molecular Cancer Research, University Medical Center Utrecht, the Netherlands; 3Animal Nutrition and Health, DSM Nutritional Products, the Netherlands
francois.nell@dsm.com

Due to the growing world population, protein resources are becoming more scarce in the next decades. Because higher-quality protein sources will primarily be used in human nutrition, only lower-quality protein sources will be available for animal nutrition. Fast growing (monogastric) animals demand high-quality protein, however. The result will be that less-digestible proteins will be fed to animals, which in combination with overfeeding of protein may result in proteolytic fermentation in the distal part of the small intestine as well as the caeca of broilers. Proteolytic fermentation is connected to health of animals; during this fermentation, metabolites are produced resulting in poor health, raising the requirement for antibiotic treatments. The mode of action of this relationship is presently unknown. The metabolites and toxins can potentially result in damage to the gut microstructure, activation of the immune system, have an impact on protein and energy metabolism and potential organ damage. Modern techniques, like metabolomics and microbiomics, enable us to study this mechanism in more detail. A better understanding of what intestinal health is and how it can be affected, permits us to take dietary measures, e.g., different food processing or use of specific additives, to improve animal's health, and consequently its wellbeing and performance, while reducing the requirement for medical treatment with antibiotics.

We tested the feasibility of untargeted metabolomics as a method to identify differences in metabolites produced by broilers fed highly digestible diets vs. less digestible diets. Serum samples were analysed by direct infusion mass spectrometry. To visualise the metabolic variations among different groups partial least squares-discriminant analysis was performed. The scores plot shows a clear separation between the serum metabolite profiles of the treatment groups. Amongst the most increased metabolites are indole and chorismic acid, two metabolites exclusively produced by gut bacteria but not by the host. These metabolites are typically considered as potentially harmful. The results indicate that the metabolites of fermentation can be affected by varying the digestibility of a broiler diet. A feasible method to observe the detail of these metabolic variations can be untargeted metabolomics.

ResPig: impact of a structural health approach on antibiotic use on pig farms

Victor N.A.M. Geurts1, L.A. Kaalberg2 and A.L.M. Cruissen1
1MSD-AH Intervet Nederland, the Netherlands; 2Veterinary Clinic ‘t Wijdseland, the Netherlands
victor.geurts@merck.com

Nowadays, pig farms face more complex diseases, such as porcine respiratory disease complex. These disease entities are often caused by multiple infections combined with suboptimal conditions. Poor understanding of the risk factors for infectious diseases often results in the unnecessarily prolonged use of antibiotic treatments. This is also allowing the development and selection of more antibiotic-resistant
bacteria. To reduce the antibiotic use in order to reduce antibiotic resistance, the Dutch government, veterinarians and farmer organisations developed an action plan. Transparency in the antibiotic use, creating awareness of the risks of antibiotic use, improvement of the health status on pig farms, and reduction goals were part of this action plan. Optimising farm management, including preventive vaccination, is an essential tool to improve/maintain the health status of pig farms. However, changing farmer’s behaviour and his way-of-working is the most challenging task for veterinarians. Demonstrating the effect of an intervention on health status and antibiotic use helps to overcome that challenge. This study describes how MSD-AH’s ResPig programme was used for that purpose.

ResPig is a diagnostic and monitoring programme for veterinarians involving regular cross-sectional serological and antigen investigations for the presence of PRRSV, PCV2, Actinobacillus pleuropneumoniae, Mycoplasma hyopneumoniae, influenza and Haemophilus parasuis. It includes also an objective scoring system for possible risk factors (environment, management, housing, biosecurity) for the investigated diseases. This helps the veterinarian towards a structured approach of respiratory problems with restrictive use of antibiotics. Per infection a health score is calculated based on the clinical history, vaccinations and the test results. The score can vary from 0 to 3 with intervals of 0.5. Each score is defined so that the farm can be compared over time but also with other farms. Definition per category: (0) SPF, pathogen not found and serology negative; (0.5), no clinical symptoms, pathogen not found and/or (partial) + serology; (1.0), no clinical symptoms, pathogen found or present and + serology; (1.5), possible clinical symptoms, pathogen found and/or + serology; (2.0), pathogen found and related with the clinical symptoms and + serology; (2.5), pathogen found and related with the clinical symptoms, herd infection and + serology; and (3.0), pathogen found and related with chronical herd problem and + serology.

The effect of the implemented actions on the health status and antibiotic use (DDD) was visualised in a graphical way. 3 farms were evaluated. On each farm one specific intervention was implemented and evaluated: 1: Biosecurity: proper cleaning and disinfection of transport lorry with enough downtime (in the past PRRSv negative piglets became immediately PRRS positive on the fattening farm); 2: passive immunisation against Bordetella bronchiseptica of piglets via sow vaccination (piglets suffered from early infections that caused recurred coughing and secondary infections); and 3: Porcilis PCV M Hyo vaccination of piglets that are infected with PCV2 en M hyo during the finishing phase.

Clinical, vaccination and test result data from ResPig make it possible to calculate a health score for respiratory diseases. However, the score category depends on the quality of the clinical history given by the practitioner/farmer, health diseases on and between farms could be followed during time. All three interventions resulted in an improvement of the health scores and were effective in reducing antibiotic us on those farms. The graphical display of the health score including implemented measurements and antibiotic score was highly appreciated by veterinarians and farmers and was very useful for demonstrating and convincing farmers which interventions were effective in reducing antibiotic...
use and improving health. This is also in line with a previous ResPig survey where a majority of the users stated that the structural health approach was helpful for reducing antibiotic use including saving money.

The nutritional cost of the immune response in pigs

Elodie Merlot and N. Le Floc’h
INRA, UMR 1348 PEGASE, France
elodie.merlot@inra.fr

In growing animals, microbial challenges are associated with a decrease in feed intake and growth. Experimental data show that the slowing down of growth cannot be explained by the sole decrease in ingestion, suggesting that the immune response must be costly in nutrients. Immune defences include maintenance of resting sentinel leukocytes and mucous barriers. Upon immune activation, mucosal epithelia are often dramatically challenged, haematopoiesis of innate immune cells is increased, lymphocytes undergo clonal expansions, and leukocytes metabolism increases to produce various effector responses (immunoglobulin synthesis, oxidative burst, etc.). The cost of these effector responses is uneasy to estimate. However, considering the marginal mass of the immune system regarding the whole body mass, it is doubtful that the immune system would be a significant consumer of nutritional resources, even upon severe immune activation.

Many immune responses are accompanied by an acute phase response, which involves non immune tissue. It is characterised by the synthesis of acute phase proteins in the liver, fever, accelerated whole-body protein turnover, and high rates of hepatic gluconeogenesis. Clearly, the acute phase response is a process that is both nutrient liberating (skeletal muscle catabolism) and nutrient consuming (acute phase protein synthesis, fever). Experimental data show that inflammation alters the metabolism of amino acids in pigs. Some of them, such as histidine, valine, threonine, arginine or tryptophan, displayed more severe and long lasting alterations, maybe related either to their role as a substrate for gluconeogenesis or ketogenesis, either as specific precursors/substrates for immune processes. Glucose metabolism is also severely affected. Based on this knowledge, various nutritional strategies have been tested, to adequately feed the immunologically challenged pig, limit inflammation and minimise growth deterioration. This presentation focuses on two of them: moderate feed restriction displayed mixed results, while tryptophan supplementation is more promising.

Costs of poultry and pig diseases, with reference to anti-microbials

Helmut W. Saatkamp¹, A.K. Kies² and J.L. Roskam¹,
¹Business Economics, Department of Social Sciences, Wageningen University & Research, the Netherlands; ²Animal Nutrition and Health, DSM Nutritional Products, and Department of Animal Sciences, Wageningen University & Research, the Netherlands
helmut.saatkamp@wur.nl

Livestock production implies livestock diseases which cause reduced production efficiency and associated costs. These costs include losses (resulting from impaired production) and expenditures (for treatment and prevention). There are a number factors that impact the costs of diseases, quite often farm specific. Consequently, this also holds for the economic impact of measures aimed at reducing the disease impact, such as the use of anti-microbials (AM). Reduction of AM use and increase in responsible AM use is an important objective of national and international authorities. Therefore, a better understanding of factors influencing the costs of livestock diseases as well as the economic value of AM is important for well-targeted policy measures. This presentation aims to provide some basic insights in these matters with regard to broiler and pig production.

Costs of important broiler and pig diseases. Using existing literature, Gocsik et al. (2014) estimated the costs of some important broiler diseases in different housing and management systems in The Netherlands. Large differences were observed between diseases: the increase in production costs due to intestinal diseases (coccidiosis, E. coli and necrotic enteritis) could exceed 10%, whereas for respiratory diseases this most likely would be around 1% (which still can be of importance, considering the very small margins). The pattern of disease risks was partially influenced by the housing system.
Animal friendlier outdoor housing resulted in higher risks of intestinal diseases and lower risks of respiratory and locomotive diseases. Finally, although the absolute disease costs increase with outdoor housing, the relative disease costs, compared to housing costs (which are increased with outdoor housing) do not increase. Another important result was, that approximately 75% of the disease costs result from increased feed costs; this part is rather obscure because it is not explicitly visible as ‘veterinary costs’. Similar observations were found by Groen (2016) with regard to pig diseases.

Implications for responsible use of AM. The above stresses the importance of the statement: the costs of disease do not exist. Moreover, the visibility of disease costs differs per disease (losses versus expenditure) and the value of disease costs can differ per farm type and farmer. Obviously this has implications for measures to reduce disease risks and costs. One of such measures is use of AM. In this regard, two economic aspects are important: (i) the (absolute or relative) costs of AM-use; and (ii) the marginal value (MV) of AM use. Roskam et al. (forthcoming) show the importance of application of these economic aspects in farm advice and policy making on AM use by qualitatively elaborating various farm scenarios. One scenario concerned so-called over-use of AM. In such cases, the MV of AM use is (close to) zero, since physical production is not affected. However, because of the low costs, AM use is a relatively cheap insurance against disease hazards even if they have a very small likelihood of occurrence. Incentives to reduce AM use should therefore focus on the farmer, i.e., his risk awareness and attitude, and risk management. Efforts to improve farm conditions to reduce the need of AM-use will only have a small potential to achieve results.

The other extreme scenario concerned quite unfavourable farming conditions resulting in a high likelihood of occurrence of the abovementioned disease costs. In such conditions, the potential MV of AM-use is very high: depending on the type of disease this MV can be up to 10% of the production costs (or even higher in case of large late mortality). With the current low costs of AM-use, (abundant) use of AM is completely sensible from an economic point of view: AM costs are more than compensated by the reduced economic costs of the disease, i.e., the high MV of AM use. Attempts to reduce AM use in such cases should particularly focus on improvement of the farm conditions, and should include alternatives for AM use, both structural (increased hygiene, i.e., investments) and ad hoc (alternative treatments). A major problem can be that investments and costs of most of these alternatives are outweighed by the economic impact of the current AM use.

In conclusion, occurrence of costs of livestock diseases is partially determined by the type of disease, and is partially farm specific. Consequently, so are the benefits of counteractive measures, including AM use. Therefore, in order to have the highest pursuit of success, measures to reduce AM use and/or to increase responsible AM use should be made as tailor-made as possible to the conditions and specific features of the farm and the farmer.
TUESDAY 27 SEPTEMBER 2016

PARALLEL SESSION 3
THE STAKEHOLDERS’ VIEW ON ANTIBIOTIC USE AND RESISTANCE

Veterinary antibiotics: a perspective on usage, innovation incentives and effective policies

Carel du Marchie Sarvaas
Executive Director, HealthforAnimals (Global Animal Medicines Association)
carel@healthforanimals.org

Usage. The presentation will address the lack of information about the volume and nature of use of antibiotics around the world. It will consider the latest data, address misconceptions and review the latest developments related to surveillance. It will consider the latest scientific reports about the possible link between the spread of AMR and use in agriculture. Whilst agriculture must play a role in decreasing AMR, there should be no doubt that the core of the solutions remains with human use.

The presentation will consider the role and responsibility of animal medicines producers. What has been done over the last two decades? The veterinary sector plays an active role in fighting anti-microbial resistance. Animal medicines producers and their national, regional and global associations have been active contributors to judicious/responsible use for over two decades. Significant thought and resource have been devoted to use, control and application of antimicrobials that has resulted in numerous actions toward better control of the issue.

Innovation incentives. The private sector is committed to innovation in vaccines, antibiotics and their alternatives. Many hundreds of millions of R&D funds are spent to develop new products and approaches. The presentation will consider how best to incentivise the development of products to help combat antibiotic resistance. What incentives have been tried and what has worked? Which approaches are ‘realistic’ and can help to maximise return on investment and have the greatest impact on public resources. What incentives have been tried? Unlike the human health, where billions of public support are offered to develop new products, there is no tradition of public finance/government support in animal health. What has worked?

The presentation will put forward 9 actionable proposals related to:
- current AB usage
- return on investment
- regulatory predictability
- global convergence of regulatory processes
- vaccines
- alternatives
- data protection
- performance metrics
- financial incentives

Effective policies. The WHO Global AMR Action Plan provides a holistic approach to combating AMR. Some recent policy initiatives related to antibiotic resistance have been well construed. Some ideas have not, and will have little or even negative effects. The presentation will consider what policies will work and which won’t and why. It will address the lack of science base used to back some policy proposals. It will consider policies like labelling initiatives, taxes, surveillance, bans and other policies. It will suggest policies that will work.
The continued availability and use of antimicrobials for animals is essential to ensure good animal health and welfare, by minimising the effects of disease. However, there is a risk that, as with their use in humans, the inappropriate use of antimicrobials in animals may contribute to the development of antimicrobial resistance (AMR), which can negatively affect both animal and human health. Therefore, veterinary surgeons have a responsibility to ensure they fully understand their role and take the necessary steps to safeguard animal and human health. It is also recognised that because of the often complex interrelationships of AMR between humans, animals and the environment, tackling the crucial issue of AMR in the global arena will require a multidisciplinary approach with medical, veterinary and environmental health professionals working collaboratively underneath the banner of ‘One Health’.

This presentation will explore the role of the veterinarian within this context. It will look at how we use antibiotics judiciously within our farmed species to ensure disease impacts are minimised, within a sustainable farming system. For example, the use of herd level data to inform responsible prescribing, and subsequent interrogation of production systems to consider alternative strategies focused more on disease prevention rather than treatment, through improved animal husbandry, improved immune status of the animal via vaccination, maximising biosecurity and optimising environmental factors. It will also look at how we can then utilise herd level data to build up an accurate picture of national and sector specific usage. This will help in the setting of achievable goals in line with reduction in usage in line with targets as highlighted in the O’Neill report (‘Tackling drug-resistant infections globally’) and enable us to identify gaps exist in our diagnostic capabilities to help us reach those targets.

Veterinarians are recognised as a trusted means for delivering key policy messages to their clients. Working in collaboration with medical colleagues and industry partners on how we can provide resources that can be used to inform animal keepers and society at large of the need to conserve these effective medicines for the next generation.

Possible measures to reduce antimicrobial use in livestock and fish; a veterinary perspective

Nancy De Briyne* and D. Iatridou
*Deputy Executive Director, Federation of Veterinarians of Europe (FVE)
nancy@fve.org

At the beginning of 2016, following a request by an ad hoc joint European Food Safety Authority (EFSA)-European Medicines Agency (EMA) Working Group, FVE made a survey to provide input on antimicrobial use in food-producing animals in Europe and on possible measures to reduce antimicrobial use. The survey considered the following species: cattle, pigs, poultry, sheep/goat, horses, aquatic animals, rabbits, and bees. Six questions were asked for all main food-producing species as requested by EFSA-EMA working group. An additional question concerning the use of colistin complemented the questionnaire. The information provided in the FVE report was based on scientific publications, governmental reports and other formal data and complemented by expert opinions from many veterinarians working with the species concerned. In general, it was found that limited formal data exist with detailed analysis on the antimicrobial use of the main indications/production systems, especially for the minor food-producing species such as small ruminants and rabbits. Therefore, much of the report is based on expert opinion.

It should be noted that there are great differences between husbandry and management conditions of food-producing animals in Europe between countries. This also applies to the relative share of the various animal species/sub-species kept, climate, epizootiology, the infectious disease and the availability of veterinary antimicrobial products and alternatives. Nevertheless, for all animal species applies that ‘prevention is better than cure’. Prevention is the best way to reduce the use of antimicrobials, improves production and increases food safety. Prevention of diseases can be done through a wide choice of tools such as improving biosecurity, good housing and ventilation, good
hygiene, appropriate nutrition and robust animals, regular veterinary visits to monitor animal health and welfare and to develop herd health plans, use of diagnostics, both to diagnose the disease as to do antimicrobial sensitivity testing, vaccination and responsible use of veterinary medicines.

While it is emphasised that the results may not necessarily reflect the total picture in Europe and more thorough analysis per species/country/production systems/etc., might be needed, they are indicative and already well acknowledged and appreciated as a valuable source of information.

Reducing the need for antibiotics through animal nutrition

Predrag Peršak
Chairman of the Animal Nutrition Committee, European Feed Manufacturers’ Federation (FEFAC)
predrag.persak@pehra.hr

In the present and emerging threat of AMR, there is an essential request to all sectors involved in animal production to find measures and ways of how to fight against it. Only by this multi-sectorial approach, with full understanding of all parts, significant and achievable measures can be defined and implemented in one joint strategy. Animal nutrition and feeding are a very important and indispensable part of that strategy. Knowing the importance of animal nutrition through that chain, European compound feed producers joined in FEFAC, completely understand the importance and their responsibility in achieving that goal. A clear indication of that commitment is communicated through several important documents that reflect the focus direction and determination in its realisation. One of the latest is FEFAC Vision on Animal Nutrition, a document presented on the XXVII FEFAC congress in April 2016 (see http://www.fefac.eu/publications.aspx?CategoryID=3666), in which it is clearly stated that animal nutrition is a multifunctional science delivering solutions to a sustainable livestock sector. An evolving approach to animal nutrition from static to dynamic science is one of the crucial pillars in defining strategies of fighting AMR. A change in approach of making feed for animals, including defining requirements in specific and individual way, using modern nutritional diagnostics tools and using variety of feed ingredients and available technologies, give us strong weapons in that fight where the goal is to have healthy and productive animals with as less as possible used antimicrobials or without any. And most importantly, it is something that is already being done.

The links between animal health, welfare and antibiotics use from a public health perspective

Sascha Marschang
Policy Manager for Health Systems, European Public Health Alliance (EPHA)
s.marschang@epha.org

Antibiotic resistance is a cross-border health threat that calls for effective and coordinated collaboration between stakeholders in different sectors. The One Health approach to AMR recognises this. Today, various actors at international, European and national level promote a holistic view that calls attention to the interactions between human health, animal health/agriculture and the environment. EPHA’s campaign on AMR advocates a strong role for binding legislation at EU level and European funding to enable member states to implement national action plans on AMR and build up capacities. This is important because detrimental actions undertaken in one country can easily have a negative impact on more advanced countries, too. A number of existing good practices in the agricultural sector can be adapted to the specific contexts of other member states. Increasing animal welfare and reducing antibiotics use are actions that can boost public health at large.
Public health points of view regarding antibiotic resistance

Aura Timen
President, Section Infectious Disease Control, European Public Health Alliance (EUPHA)
office@eupha.org

The European Public Health Association (EUPHA), founded in 1992, is an international, multidisciplinary, scientific organisation, bringing together around 14,000 public health experts for professional exchange and collaboration throughout Europe. Being an umbrella organisation for public health associations and institutes in Europe, EUPHA’s members consist of 40 national associations of public health, 18 institutional members and 9 European NGOs. Sharing the common goal of health and well-being, EUPHA aims at narrowing health inequalities for all Europeans. EUPHA defines public health as: ‘the science and art of preventing disease, prolonging life and promoting health and well-being through the organised efforts and informed choices of society, organisations, public and private, communities and individuals, and includes the broader area of public health, health services research, health service delivery and health systems design’. Threats to health – short, middle or long term – occurring across all Europe are major concerns of EUPHA. Examples of those are chronic diseases, obesity, emerging infections, and demographic changes. Antimicrobial resistance is one of these and its unprecedented magnitude and unpredictable further development require prompt attention.

Antimicrobial resistance is according to the WHO, ‘one of the biggest threats to global health today. It can affect anyone, of any age, in any country’. EUPHA’s efforts are directed to raising awareness of the problem and providing a platform for exchanging information between scientists, policy makers and frontline professionals. We approach this threat during the yearly European Public Health Conference from various areas of expertise, which are represented in the sections of EUPHA, such as communicable disease control, health promotion, environmental health, public health ethics, and health economics. During the past years, we addressed dilemmas in the human veterinary interface repeatedly and pointed out to the large differences in antibiotic use both in humans and animals in the European countries. Efforts to further reduce veterinary use of antibiotics face the challenge of identifying the ‘optimal mix of instruments to meet the final aim of an economically profitable animal production chain with a prudent use of antimicrobials’. Furthermore, we need to have a better understanding of the relationship between man and the whole environment when it comes to controlling antimicrobial resistance. Yet importantly, the scientific community, which EUPHA is a part of, needs to keep engaging in systematic communication efforts with the political environment to provide the evidence for science based policy decisions with positive consequences for public health as a whole.

The future of broiler breeding in an ever-changing world

Mitchell Abrahamsen
Senior Vice President R&D, Cobb-Vantress, USA
mitch.abrahamsen@cobb-vantress.com

With the increasing movement away from the use of antibiotics in poultry production, the industry is faced with new challenges regarding production efficiencies. In addition, the pressure to not use antibiotics has raised complex questions regarding the health and welfare of the birds in production, in particular, as it relates to sick animals. The industry has learned that efficient antibiotic free production (ABF) can be achieved, but it does typically result in higher production costs. The challenge for breeding companies it to develop improved products that can meet the desired production efficiencies and animal health and welfare standards, without incurring the costs typical of ABF production. This challenge will require refocused efforts for evaluation and selection of breeding stock for improved performance in the face of increasing microbial challenge. In particular, selection programmes targeted at improving intestinal health and innate immune responses will be critical component for developing improved products in response to the changing market needs.
Interventional genomics for the prevention of antibiotic resistance

Alex W. Friedrich
Medical Microbiology and Infection Prevention, University Medical Center Groningen, the Netherlands
alex.friedrich@umcg.nl

Outbreaks of multidrug resistant (MDR) bacteria in hospitals and communities worldwide have become increasingly common over the past decades, posing a serious threat to quality of care in modern healthcare. Despite the apparent emphasis of media on reporting outbreaks of MDR Gram-positive bacteria, such as methicillin resistant *Staphylococcus aureus*, outbreaks of MDR Gram-negative bacteria are rapidly increasing in number as well as in impact on quality of care. Next to the extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli*, especially carbapenem-resistant (CR) *Klebsiella pneumonia*, and CR and non-fermenting bacteria, such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, are becoming one of the leading problems of hospital acquired infections with reduced therapy options.

Measures against the spread of resistance are today focused on individual health care institutions, because they are regarded as a source of resistance. Healthcare institutions however, do not stand alone when it comes to controlling resistance because will follow their carriers between hospitals and healthcare facilities. Antibiotic resistance follows the patient flow, the mapping of this patient flow gives an idea of the health care network with ‘natural health care regions’.

Outbreak control and preventive measures rely on epidemiological analysis. In case of MDR bacteria classical epidemiology, relying on place, time, person and bacterial species is not discriminatory enough to understand hospital epidemiology of these highly diverse microorganisms. The information we need to acquire for proper infection control is hidden in the genome sequence. Therefore, sequence-based typing techniques have become more and more important the last years. Today, sequencing of the whole bacterial genome provides important new insights in how these bacteria differ from each other including resistance and virulence factors. However, the classic typing approach is usually only subsequent to the identification of carriers with a diagnostic waiting-time for the patient. Whole genome analysis allows today the *ad hoc* development of outbreak-specific diagnostics, enabling to screen only for carriers of only the outbreak strains, and allows using the regional network organisation to adapt preventive microbiological screening in the healthcare region, tackling down the outbreak-strains in an initial phase of regional spreading. Furthermore, metagenomics approaches for detection are developed in order to improve diagnostic approaches allowing the analysis of polymicrobial infections and therapy follow-up diagnostics. This direct use of interventional genomics can lead to improvement of real-time infection prevention and individualisation of antibiotic therapy.

Animal production without any antimicrobials: potential scenario or license for euthanasia?

Jaap A. Wagenaar1,2,3,4, D.C. Speksnijder1 and D. Heederik4,5
1Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, the Netherlands; 2Wageningen Bioveterniary Research, Wageningen University & Research, the Netherlands; 3WHO Collaborating Center for Campylobacter and OIE Reference Laboratory for Campylobacteriosis; 4Netherlands Veterinary Medicines Authority, the Netherlands; 5Institute for Risk Assessment Sciences, Utrecht University, the Netherlands
j.wagenaar@uu.nl

Antimicrobial use (AMU) in animals poses a potential risk for public health as it contributes to the selection and spread of antimicrobial resistance (AMR) in humans. Therefore, it is important that prudent use of antimicrobials in animal production should be promoted around the globe. One of the elements of prudent use promotion is to ban unnecessary use and overuse. However, it might be complicated to define and distinguish unnecessary from required or permissible use. It is generally accepted that the use of antimicrobial growth promotors (AGPs) is not necessary, as proven management alternatives are available, which yield comparable economic profits. Prophylactic use is also considered as overuse as it is used in the absence of manifest infectious disease and the use lacks a prior specific diagnosis. For metaphylactic use, this is less clear under certain circumstances.
For antimicrobial use that is applied as therapeutic intervention it is not always clear whether the use is justified or not. A proportion of the therapeutic use is needed to cure animals that would otherwise not be able to overcome the infection without unacceptable negative effects on animal health and welfare. The proportion of animals this refers to is usually not known. However, when therapeutic application exceeds the amount needed to cure an infection, also therapeutic application can be to some extent defined as overuse. For instance, when a course involves more time than required. In order to reduce AMU in animal production, this threshold of minimal required use becomes an increasingly delicate balance which needs a thorough and continuous consideration and evaluation of the benefits of lowering AMU versus the risks related to animal health and welfare.

The Netherlands Veterinary Medicines Authority (SDa), annually reports and benchmarks the distribution of AMU at farm level of poultry, pig, dairy and veal farms in the Netherlands. Depending on the animal sector, a certain fraction of the farms has shown to be able to produce with a very low use pattern of antimicrobials over prolonged periods of time, and in some cases even without using antimicrobials at all. So far, there are no major indications for a decline in animal health and welfare in the Dutch livestock sectors, however, a close watch on these parameters remains necessary.

Although some stakeholders propose antibiotic free production (AFP) for the whole animal production sector in the Netherlands, the desired use level is set by the SDa and does not equal ‘zero-usage’. During production cycles of animals, there will always be risks for the introduction and spread of infectious agents (either primary bacterial infections or viral infections followed by secondary bacterial invaders), despite all kinds of preventive measures implemented. A zero-use policy would lead to unwanted animal health and welfare problems. There are anecdotic cases from Denmark, the USA and the Netherlands suggesting that diseased animals are deprived from antimicrobial treatment or that animals are euthanised just to prevent antimicrobial use. This is an unwanted situation that should be recognised by the stakeholders. A zero-use policy, as seen at some farms, cannot be applied to the entire production sector. AMU reduction should focus at structurally high using farms and efforts should be directed to lower AMU as far as possible, but without exceeding the threshold of impairing animal health and welfare.
PARALLEL SESSION 4
ONGOING RESEARCH TO CONTROL ANTIBIOTIC USE AND RESISTANCE

A rapid procedure of PCR and a microarray lateral flow test for detection of antibiotic resistance genes

Aart van Amerongen1, R. Slendebroek1, K. Veldman2, D. Mevius3 and J. Wichers1
1BioSensing & Diagnostics, Wageningen University & Research, the Netherlands; 2Wageningen Bioveterinary Research, Wageningen University & Research, the Netherlands; 3Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, the Netherlands
aart.vanamerongen@wur.nl

Antibiotic resistance is a worldwide threat not only to human but also to animal health. Since animals are a source of antibiotic resistant microbial species, especially food-producing animals pose a substantial problem to the prevention of transfer of such species to humans. In the human clinical practice, a number of diagnostic methods is being applied to detect antibiotic resistant microorganisms, such as conventional phenotypic culturing, genotypic methods (qPCR, microarrays, next generation sequencing), and mass spectrometric approaches. Often these methods are too expensive, too time-consuming and/or too facility-demanding for use in the veterinary practice. Preferably, acute infections in agricultural animals should be investigated at short notice, if possible on-site. This would allow the detection of antibiotic resistance genes, if desired combined with information on the pathogenic microorganism(s). Timely diagnosis would enable veterinarians to start a dedicated antibiotic treatment on the same day. Therefore, we are developing a rapid procedure in which the result of a multiplex PCR is assessed by a user-friendly test. We started to set up a 30-minutes multiplex PCR to detect carbapenemase-coding genes (NDM-1, OXA-48, KPC and VIM) in combination with the emerging MCR-1 gene (colistin resistance). The latest results will be shown in the presentation.

Our group is focused on the development of new multi-analyte diagnostic platforms such as the lateral flow microarray immunoassay (LMIA) and the microarray-ELISA (MELISA). Generally, a rapid LMIA consists of 25 assay spots. With duplo measurements up to 8 different antigens can be detected within 15 minutes. In MELISA multi-analyte detection is performed in the wells of ELISA plates. A well can easily accommodate an 8x8 array (64 spots) with which it is possible to measure up to 25 different antigens in one sample simultaneously. These diagnostic platforms allow applications in various settings: the lateral flow test is a user-friendly format and can be used on-site/Point-of-Care, while the microarray-ELISA is very well suited for an automated work flow in laboratory settings. For LMIA dedicated readers are being developed, while MELISA results can be obtained by using existing readers. Initial results of the detection of the five antibiotic resistance genes will be shown such as with a conventional lateral flow test having antibody-sprayed lines and an LMIA having antibody printed spots applied with the Scienion arrayer S3. In addition to a user-friendly performance, it is clear that the successful commercialisation of these diagnostic platforms requires the presence of signal/image readers and dedicated software packages. However, a very crucial step is the manufacture of the tests by the large-scale printing of capture ligands and the precise cutting of the nitrocellulose membranes to strips having an intact microarray of spots. It will be explained that the economically viable production of the LMIA and MELISA platforms is feasible.

Host defence peptides: natural anti-infectives?

Henk P. Haagsman
Department of Infectious Diseases and Immunology, Utrecht University, the Netherlands
h.p.haagsman@uu.nl

While antibiotics have been indispensable for the treatment of bacterial infections, the increase in antibiotic resistance and lack of new antibiotic compounds, greatly limits the available treatment options. Therefore, development of novel anti-infective therapies is crucial for the treatment of bacterial infections in the future. In the search for new anti-infective templates, host defence peptides, such as cathelicidins, have gained a lot of interest. These short cationic peptides have been shown to elicit broad-spectrum
antimicrobial activity against both Gram-positive and Gram-negative bacteria and are thought to have beneficial immunomodulatory functions as well. Of these cathelicidins, the chicken cathelicidin-2 (CATH-2) has been shown to be an interesting template, with strong antimicrobial activity against a wide variety of bacterial pathogens and potent immunomodulatory activities.

The antibacterial mechanism of action of CATH-2 was investigated using a variety of different microscopic and biochemical methods with Escherichia coli and methicillin-resistant Staphylococcus aureus (MRSA). The (inner) membrane was shown to be an important target of the peptide, as the peptide caused binding and permeabilisation. Transmission electron microscopy demonstrated that CATH-2 induces dose-dependent morphological changes. Interestingly, sub-minimal inhibitory concentrations (sub-MIC) of the peptide resulted in intracellular changes (relocalisation of DNA and ribosomes), whereas MIC values had detrimental effects on the bacterial membranes. Immunogold labelling of these electron microscopy sections demonstrated that already at low concentrations CATH-2 translocated intracellularly without visible membrane permeabilisation. Additional time killing studies demonstrated that most effects occurred within the first minutes after incubation, highlighting the rapid actions of the peptide.

CATH-2 exhibits many immunomodulatory activities. In order to investigate its efficacy as an immunomodulatory agent in vivo the stable D-amino acid analogue of CATH-2 (D-CATH-2) was administered to chickens in ovo at 3 days before hatch. First, in ovo live imaging of zirconium-89 labelled CATH-2 peptide by positron emission tomography was carried out to study the uptake by chicken embryos, a technique to study peptide distribution in vivo. By in ovo injection of labelled CATH-2 peptides, uptake of the peptide by the embryo was seen from 4 h after injection onwards followed by accumulation in the gastrointestinal and respiratory tract. D-CATH-2 administration in ovo partially protected chicks against a systemic Salmonella infection or a respiratory E. coli infection in the first week posthatch. In the salmonellosis model, mortality was 50% reduced by administration of the peptide and the number of birds with clinical symptoms was even 69% less than in control infected chickens. In the colibacillosis model, the reductions in mortality and morbidity were 30% and 52%, respectively.

It is concluded that prophylactic treatment with immunomodulatory cathelicidin-derived peptides may reduce the likelihood of bacterial infections of young animals. The elevated threshold above which infections occur may help to reduce the use of conventional antibiotics. Acknowledgements. This work was financially supported by the Immuno Valley ALTANT ASIA 2 programme of the Dutch Ministry of Economic Affairs.

Unlocking the medieval medicine cabinet to help fight antimicrobial resistance

Stephen P. Diggle  
Centre for Biomolecular Sciences, School of Life Sciences, University of Nottingham, UK  
steve.diggle@nottingham.ac.uk

Plant-derived compounds and other natural substances are a rich potential source of compounds that kill or attenuate pathogens that are resistant to current antibiotics. Medieval societies used a range of these natural substances to treat conditions clearly recognisable to the modern eye as microbial infections, and there has been much debate over the likely efficacy of these treatments. Our interdisciplinary team, comprising researchers from both sciences and humanities, identified and reconstructed a potential remedy for Staphylococcus aureus infection from a 10th century Anglo-Saxon leechbook. The remedy repeatedly killed established S. aureus biofilms in an in vitro model of soft tissue infection and killed methicillin-resistant S. aureus (MRSA) in a mouse chronic wound model. While the remedy contained several ingredients that are individually known to have some antibacterial activity, full efficacy required the combined action of several ingredients, highlighting the scholarship of premodern doctors and the potential of ancient texts as a source of new antimicrobial agents.
Disarming pathogens: an evolution-proof approach to manage infections?

Rolf Kümmerli
Department of Plant and Microbial Biology, University of Zurich, Switzerland
rolf.kuemmerli@uzh.ch

There is currently much interest in therapeutic approaches that inhibit the expression or functioning of bacterial virulence factors. Virulence factors are structures and molecules that cause host damage, and include (i) flagella and pili to adhere to host tissue, (ii) enzymes and siderophores to scavenge nutrients from the host, and (iii) toxins to attack host tissue. Approaches that target these traits are called anti-virulence treatments, and there is great promise that disarming rather than killing pathogens is an efficient and evolutionarily robust way to manage infections. In particular, it is thought that anti-virulence treatments exert weaker selection for resistance than conventional antibiotics because pathogens are not killed directly. However, empirical evidence for the claim of evolutionary robustness is sparse and controversial.

Here, I present evolutionary concepts and experimental data to examine when anti-virulence drugs can be evolutionarily robust and when not. In our experiments, we focused on the bacterium Pseudomonas aeruginosa, a multidrug-resistant opportunistic pathogen of humans and animals. As anti-virulence strategy, we applied treatments that interfere with siderophores, molecules secreted by this pathogen to scavenge iron from host tissue. Using a combination of experimental evolution, phenotype screening and whole-genome sequencing, we show that anti-virulence treatments can indeed be evolutionarily robust, but the robustness is dependent on the specific mode of action of the drug. When treating bacteria with flucytosin, a drug that inhibits siderophore synthesis within the cell, resistance can readily arise and spread, through enzymatic drug inhibition within the cell. Conversely, no signs of resistance evolution were observed when treating bacteria with gallium(III), a metal ion that neutralises secreted siderophores. We argue that resistance does not evolve in this case because gallium acts outside the cell, where standard resistance mechanisms, such as drug efflux and degradation, cannot operate. Taken together, our work highlights that anti-virulence approaches are not evolution proof per se, instead it is the mode of action, especially the extra-cellular inactivation of virulence factors, that confers evolutionarily robustness.

Human contact with dogs and the risk of carriage of antimicrobial resistant E. coli

Emma Ormandy1, G. Pinchbeck1, A. Wedley1, S. O’Brien1, S. Dawson2 and N. Williams1
1Department of Epidemiology and Population Health, Institute of Infection and Global Health, University of Liverpool, UK; 2School of Veterinary Science, University of Liverpool, UK
e.e.ormandy@liverpool.ac.uk

The increasing prevalence of antimicrobial resistant and extended-spectrum β-lactamase (ESBL)-producing Escherichia coli is recognised as a global issue in both human and veterinary medicine. Previous studies have indicated faecal carriage of ESBL-producing E. coli in up to 25% patients within veterinary small animal hospitals. Given the close contact between people and pets, these animals may play an important role in the transfer of antimicrobial resistance. There is currently a paucity of data on carriage rates of these bacteria in populations with high levels of contact with animals.

A cross-sectional study of employees at 69 randomly selected veterinary practices, kennels and rescue centres was conducted, with a faecal swab collected and questionnaire completed by the participant. Faecal samples were cultured on selective and non-selective media in order to isolate antimicrobial-resistant E. coli. Confirmation of an ESBL phenotype in isolates resistant to third generation cephalosporins was undertaken using the double disc diffusion method. All isolates underwent susceptibility testing with a panel of seven antimicrobials and isolates which were identified phenotypically as ESBL or AmpC producing, were examined by PCR for the presence of blaCTX-M, blatem, blashv, blaOXA and blaAmpC genes. In total, 229 faecal samples were collected; the prevalence of carriage of antimicrobial resistant E. coli was 74.0% (95% CI 68.3-79.7%), with resistance to ampicillin (56.4%; 95% CI 49.9-62.9%), tetracycline (43.2%; 95% CI 36.8-49.6%) and augmentin (37.9%; 95% CI 31.6-44.5%) most prevalent. Carriage of multidrug-resistant (resistant to three or more antimicrobial classes) E. coli was identified in 25.1% (95% CI 19.5-30.7%) of participants. Eight isolates from seven samples (3.1%; 95% CI 0.9-5.2%) were identified phenotypically as ESBL-producing E. coli
and six of these isolates were shown to carry blaCTX-M genes, with Group 1 genes most prevalent (n=5). Whole genome sequencing will also be undertaken to further explore the genetic background, resistance genes and mobile genetic elements present in these isolates.

This is the first study investigating the carriage of antimicrobial resistant and ESBL-producing *E. coli* in this population in the UK and highlights the high levels of carriage of antimicrobial resistant bacteria that could have significant implications for the health of individuals within the veterinary field.

The influence of farm-specific characteristics and production management on antibiotic usage in conventional broilers

Tommy Van Limbergen
Department of Obstetrics, reproduction and herd health, Ghent University, Belgium
tommy.vanlimbergen@ugent.be

No abstract received.

Impact of facilitated structural animal health management on antimicrobial use and animal health parameters in Dutch dairy farming

David Speksnijder1,2, H. Graveland1, D. Heederik3, T. Verheij4 and J. Wagenaar1,5
1Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, the Netherlands; 2Veterinary Clinic Tweestromenland, the Netherlands; 3Institute for Risk Assessment Sciences, Faculty of Veterinary Medicine, Utrecht University, the Netherlands; 4Julius Centre for Health Sciences and Primary Care, University Medical Center Utrecht, the Netherlands; 5Wageningen Biovitaminery Research, Wageningen University & Research, the Netherlands
d.c.speksnijder@uu.nl

Antimicrobial use (AMU) in production animals has a potential impact on antimicrobial resistance (AMR) in human pathogens and, therefore, there is a global effort to reduce AMU in animals. Coercive nationwide policy measures have reduced AMU in production animals in the Netherlands with 65% in 7 years. However, large variations in AMU between individual farms suggests possibilities for further reduction, in particular for the higher users.

Structural animal health planning (SAHP) might help to improve animal health by reducing the prevalence of infections and therefore AMU. It is important that the relevant advisors are involved in SAHP and present uniform and feasible advices which receive farmers’ commitment. We conducted a trial to assess the effect of SAHP on conventional dairy farms in the Netherlands. The study period was between March 2014 and April 2016. 39 dairy farmers with their advisors (veterinarian and nutritionist) voluntarily participated. A requirement for participation was a yearly average AMU between 3-6 DDDA (defined daily dose animal) which is above the country average. Farms were randomised into an intervention and control group. Veterinarians from intervention farms received a course on communication and advisory skills, while nutritionists and intervention farmers followed an E-learning module on SAHP developed as part of the project. A professional facilitator guided with the farmer and involved advisors during a 3-4 h session on the farm. The objective was to develop a farm specific action plan for a one-year period, containing SMART-defined goals and associated activities. During the trial, the facilitator regularly contacted the team to monitor progress. After one year, a final session was organised to evaluate the process and develop an action plan for the next year.

In the second year, the control farms turned into intervention farms and the original intervention farms were followed to see if the SAHP can be sustained without a facilitator. In the first year of the study, intervention farms had a 19% reduction in AMU; controls a 15% (*P*=0.625) while no significant adverse effects on animal health were visible in both groups. The level of compliance to the action plan was positively correlated to the percent reduction in DDDA (*P*=0.036) on intervention farms in year 1 and had the tendency to be associated with the quality of cooperation in the advisory team (*P*=0.091).
These preliminary findings may indicate that constructive collaboration between farmer and involved advisors in SAHP can positively impact animal health and AMU.

**Antibiotic resistance in aquatic environments: from the evidences to the mitigation measures**

Célia M. Manaia  
Centre for Biotechnology and Fine Chemistry (CBQF), Catholic University of Portugal, Portugal  
cmanaia@porto.ucp.pt

Despite of being a natural property of bacteria, antibiotic resistant bacteria and their genes (ARB&ARG) are nowadays considered important environmental contaminants. Differently of chemical contaminants, biological contaminants have the potential to multiply under appropriate conditions, a fact that has the potential of increasing the severity of the risks associated with ARB&ARG of human and animal origin released in the environment. Although it is at the clinical settings that antibiotic resistance shows the most threatening effects, distinct environmental reservoirs and human practices have been identified as potential enhancers on the ARB&ARG propagation and eventual transmission to humans. Animal production facilities, the amendment of agriculture soil with manure, hospital and municipal effluents are of those some examples.

Besides one of the most important bacterial habitats, water has a pivotal role on the transport of all kind of contaminants, from chemical compounds to nanoparticles or bacteria. Hence, water is both a reservoir and key path for the dissemination of ARB&ARG. In wastewater, different types of contaminant, including antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes are continuously brought together and are not totally removed during wastewater treatment. These and other contaminants are, therefore, released in natural water bodies leading to the continuous environmental contamination, for which boundaries seem to be inexistent. Some examples of this situation will be presented and discussed.

It is increasingly consensual that in order to face the widespread environmental contamination with antibiotic resistance it is necessary to identify major current limitations and plan some key actions to be promoted by the scientific community and policy making entities. These aspects will be discussed.

**Does application of antibiotic molecules to farm soil significantly increase resistance genes abundance?**

Pascal Simonet¹, J. Nesme¹ and E. Topp²,³  
¹Environmental Microbial Genomics group, Lab. Ampere, UMR CNRS 5005, École Centrale de Lyon, Université de Lyon, France; ²Agriculture and Agri-Food Canada, Canada; ³Department of Biology, University of Western Ontario, Canada  
pascal.simonet@ec-lyon.fr

In a context where pathogens acquire resistance to more and more antibiotic treatments in hospital settings, a number of questions are raised regarding mobilisation, acquisition and dissemination of antibiotic resistance traits between and within bacterial populations. Since antibiotic resistance genes (ARG) are common features of many saprophytic soil bacteria a significant ARG reservoir is readily accessible to previously sensitive pathogens by means of horizontal gene transfer (HGT). It is known since decades that environmental bacteria and pathogens share resistance genes hence the importance to clearly establish transmission routes of such traits in order to prevent their dissemination.

Crop fertilisation using manure from animals previously treated with antibiotics is a major anthropogenic entry point of these pharmaceuticals in soils. A number of risk-assessment studies focused on the effect of manure fertilisation on soil bacterial community and showed that such practice can indeed increase ARG abundance. Such studies are however most often depicting short- to mid-term impact and cannot distinguish what is leading increase in resistance genes abundance. Our objective was therefore to assess if long-term (1999-present) impact of repeated antibiotic molecules input would have a similar
impact on soil bacterial communities, in absence of manure. Can similar concentrations of antibiotic select for resistance traits and increase their dissemination potential in absence of organic matter and intrinsic bacterial community added to soil via manure? To test this hypothesis, we analysed environmental DNA extracted from soil samples under crop rotation and treated yearly with veterinary antibiotics (tylosin, chlortetracycline and sulfonamides) for 15 years (1999-present). Antibiotic molecule concentration added to soil each year represented similar amount to what is conventionally found in antibiotic-treated animal manure. Contaminated soil plots examined in this analysis represents a 12 years time frame (2001-2012) of this long-term experimental setup. No correlation between increased antibiotic concentration added to soil and antibiotic-resistance gene abundance could be established with our results and the soil bacterial community is not significantly affected by such treatment.

Investigation of ARG and mobile genetic element promoting gene transfer between bacterial lineages (i.e., from environmental bacteria to pathogens) has also been analysed. It shows that co-abundance is frequent in sequence datasets obtained from soil samples. However, new sequence analysis methods are needed to determine if co-abundance is indeed a sign of co-occurrence on the same molecule, explaining increased transfer potential and successful ARG dissemination.

**Antibiotics in aquaculture: facts and alternatives**

*Igor Hernandez* and *A. Lasagabaster*
Food Research Division, AZTI-Tecnalia, Spain
ihernandez@azti.es

The importance of aquaculture is growing and has an obvious social and economic impact. Fish farming has inherent problems, such as organic material accumulation, which allow fast fish pathogens spread in the facilities. Historically, most used antibacterial strategy includes antibiotics, but, at present, the use of antibiotics is strictly controlled, consumers demand products free of antibiotics, and public health problems can derive from antibiotic resistant bacteria occurrence, mainly multi-drug resistant bacteria. Also, a negative influence in animal welfare has been described, increasing the fish stress-related metabolism and, in some cases, reducing the growth rate.

Various alternative strategies have been developed in last years, including vaccination, growth promotors, essential oils, pre- and probiotics and, in last years, bacteriophages. These strategies have been considered by different authors, reporting their advantages and limitations. The main advantages are related with animal welfare and the absence of transmissible resistance. No antibiotic strategies would allow reducing the impact of fish-farming facilities on the environment and lead to a more sustainable industry. Disadvantages and limitations are depending on the selected antibacterial agent and on the application strategy. For example, few of these alternatives are applicable during hatchery or fry growth, some of them are only effective depending on the fish-farming strategy and, in some cases, presented results are ambiguous or not robust. At this moment, industry and consumers are demanding new antibacterial agents and application strategies with high specificity, adapted to new production facilities and strategies, and with allow environmental and health impact. Our mission is to find, evaluate and produce them.

**Selection for, dissemination of and exposure to antibiotic resistant bacteria in the natural environment**

*William H. Gaze*
European Centre for Environment and Human Health, University of Exeter Medical School, University of Exeter, UK
w.h.gaze@exeter.ac.uk

The natural environment is increasingly recognised as playing a significant role in the ecology and evolution of antibiotic resistance. This ‘environmental dimension of antimicrobial resistance’ integrates a variety of processes including selection for resistance by antimicrobial residues in soil and water, dissemination of resistant organisms at a landscape scale and human exposure and environmental
transmission of resistant bacteria in environmental compartments. Antibiotics and biocides are present in environmental matrices from low ng/l – mg/l concentrations depending on location, compound and matrix. Recent data suggests that selection for resistance occurs within this selective window and novel data will be presented illustrating the use of laboratory microcosms to define minimal selective concentrations (MSCs) for a range of antimicrobial compounds. Understanding spatial variation in the prevalence of antibiotic resistance gives insights into the drivers of increased prevalence in natural and farmed environments. Research by Gaze and colleagues has demonstrated that prevalence of antibiotic resistance varies dramatically at a river catchment scale and that this prevalence is predictable based on readily available geospatial and water quality data. Arguably the most important issue from an anthropocentric perspective is whether selection for and dissemination of antibiotic resistance in the natural environment poses a risk to human health. New research by Gaze et al. has assessed the risk of human exposure to resistant bacteria in receiving waters (i.e., coastal bathing waters) and whether this exposure results in measurable negative health outcomes in terms of transmission of antibiotic resistance to the gut microbiome of exposed individuals. We estimate that over 6 million exposure events to 3rd generation cephalosporin resistant E. coli occur in UK bathing waters each year and that in a cohort study of individuals exposed to coastal bathing waters gut carriage of CTX-M bearing E. coli was 4 times higher than in controls. Thus we present data demonstrating that selection for antibiotic resistance in the aquatic environment is likely to occur, that anthropogenic factors affect prevalence of antibiotic resistance at a catchment scale and that the presence of resistant bacteria in the environment poses a very real exposure risk to humans that results in measurable transmission to the gut microbiome.
Antimicrobial stewardship is the term increasingly used in medicine to describe the multifaceted approaches required to sustain the efficacy of antibiotics and to minimise the emergence of resistance. Rather like controlling climate change, stewardship requires multiple interventions and approaches which individually may have small effects but which cumulatively will likely have a significant impact. A stewardship approach may not only buy time during which new antibiotics or interventions can be developed but which will help to preserve any future antibiotics that will be developed. The concept of stewardship is still developing and may have different meanings or practices to different groups. For example, antibiotic stewardship in large human hospitals may involve multidisciplinary teams of experts but a stewardship approach is needed by anyone involved in using antibiotics.

The term Good Stewardship Practice (GSP) has been introduced [1]. GSP takes a continuous improvement and dynamic approach to addressing resistance and sustaining the future of antimicrobial therapy which can be documented and assessed in veterinary practice standards and procedures at many different levels. GSP integrates the current best knowledge about how to use antibiotics optimally in particular infections and circumstances.

Important elements of antimicrobial stewardship are the integration of the following: development and implementation of practice guidelines; the use of pharmacokinetic and pharmacodynamic understanding in drug dosage; clinical microbiology data; resistance and use surveillance; infection control practices; national and international regulations; education about infection and antibiotic use; and owner compliance. Page et al. [2] have promoted a ‘5R’ approach to stewardship. These 5Rs are: 1. Responsibility by the veterinarian for use of an antibiotic in a cost-benefit analysis that balances the benefit with understanding of adverse consequences; 2. Reduction of use wherever feasible, including aspects such as immunisation, biosecurity, and reduction of duration of treatment; 3. Refinement, through integrating all the elements important in treatment of an individual (‘right drug, right dose, right time, right duration’); 4. Replacement wherever the evidence points to a superior alternative; and 5. Review, a continuous improvement process of reassessment of antibiotic use practice.

The resistance crisis has unleashed a wave of critical analysis of how veterinarians and physicians have been using antibiotics in the last 50 years, and identified numerous areas for improvement in all of the elements of antibiotic stewardship. Bacteria can change, but so can we.

References
group was established to oversee all stewardship activities and allow learning from work in humans to be shared with colleagues in animal health.

Healthcare in Scotland is delivered via 14 regional National Health Service (NHS) boards which provide both hospital and community based care. A key success of SAPG has been utilising this infrastructure to support stewardship with the national group operating as a consortium to give ownership by the regional Antimicrobial Management Teams (AMTs). A multi-professional approach is also important along with management support at national and regional level and links with colleagues in Infection Control and Patient Safety. Focusing on primary care, both our national group and regional AMTs include primary care representatives. Although GP Practices are independent contractors they receive information, education and advice on all aspects of prescribing from Pharmacist Prescribing Advisers employed by their regional NHS board.

SAPG have developed a range of interventions utilising surveillance of antimicrobial use data, quality improvement programmes and education of healthcare staff and the public to improve antibiotic use. Some of these interventions will be described focusing on those in primary care as these will provide learning that may be applicable to the veterinary context.

The availability of national surveillance data collected via prescriptions dispensed by community pharmacists and the use of a unique patient identification number on all prescriptions has allowed us to monitor use of antibiotics at national, regional and GP practice level and provide information on specific patient populations. We have developed a suite of prescribing indicators for inclusion in national annual reports and that also allow Prescribing Advisers to share local prescribing information with GPs to identify areas for improvement.

A key objective in the early years of SAPG was to reduce the use of antibiotics associated with high risk of Clostridium difficile infection, as this was a significant issue in the UK between 2005 to 2010. This meant restricting prescribing of cephalosporins, quinolones and co-amoxiclav by GPs and promoting use of recommended agents. Data has shown year on year reductions in broad spectrum antibiotic use and an increased proportion of prescriptions for recommended antibiotics.

Following this progress with ‘what to prescribe’ SAPG’s attention then moved to ‘whether to prescribe’ as both the literature and local audit work suggested that the majority of GP consultations for acute infections are for respiratory tract infections, the majority are self-limiting but many patients still receive an antibiotic. This has been tackled through use of a national target to reduce unnecessary use, an education resource to support GPs via a facilitated learning session and most recently providing personalised feedback reports including benchmarking information.

Antimicrobial stewardship in livestock: principles and practice

Guy H. Loneragan
Department of Animal and Food Sciences, Texas Tech University, USA
guy.loneragan@ttu.edu

Antimicrobial stewardship can be framed within the aspirational goal that we need to preserve the efficacy of antimicrobials for the benefit of others both in the present and the future (or some variant thereof). Setting an aspirational goal has distinct advantages in that it facilitates values-based actions at the individual and aggregate level, and encourages creative thought and innovation of how not to use antimicrobials (rather than focusing primarily on how to use them judiciously). Yet setting an aspiration goal has distinct challenges in that it may not be achievable, and in the case of antimicrobial stewardship, evidence-based approaches may not be universally accepted and competing values (such as reducing antimicrobial use and promoting animal welfare) may be weighted differently by various actors. As such, practices advancing the aspirational goal ought to be based on the best available evidence, framed within subjective and normative values, and importantly, must be perceived to be effective and practical by those responsible for their implementation. Disagreements on what is perceived to be ‘evidence’, and what is effective and practical are common and will remain so. In the United States, antimicrobial stewardship in livestock is proceeding incrementally in some instances and in others, major changes to production practices are being (or will be) mandated. In this presentation, various cases studies will be presented.
Antimicrobial stewardship in livestock: experiences in dairy farming

Andy Millar
Purata Farming Ltd., New Zealand
andy@puratafarming.nz

Purata Farming Ltd., a New Zealand-based, multi-farm dairy business, milking 14,000 cows, has a proactive health, welfare and milk quality strategy, led by in-house veterinarians supporting the goal of ‘Farming for Tomorrow’. From 2014, Purata has developed and worked under a bespoke antibiotic stewardship plan (ASP) in line with this future focused goal. The ASP addresses the ‘5Rs’ as described by Page [1]:

- Reduction, how can we use less antimicrobials?
- Refinement, how do we use antimicrobials more effectively?
- Replacement, are there other approaches that are equally (or more) effective?
- Responsibility, how do we get shared ownership and accountability?
- Review, how do we measure current state, monitor progress and improve the system?

The ASP incorporates 7 core principles: (i) the impacts of use are considered by any of the Purata team using antimicrobials; (ii) prevention of conditions that could require antimicrobial therapy is a priority; (iv) strategies are employed to reduce the number of animals given antimicrobials wherever this will not compromise animal health or welfare; (v) optimisation of antimicrobial dose rates and regimes; (vi) antimicrobials considered more important in human medicine are not used as first line treatment and only employed where likely to deliver superior outcomes; and (vii) all practical measures are taken to prevent antimicrobial residues entering the human food chain.

Supporting implementation of this plan was a programme of broad consultation and a structured approach to change management, as described by Kotter and Rathgeber [2]. Much of Purata’s stewardship plan has been incorporated into the quality assurance programme promoted by the local dairy processor (Synlait Milk Ltd.’s Lead with Pride Programme). In 2015, a similar approach was used as the basis of an online training forum to coach NZ dairy veterinarians to establish ASP with their clients [3]. Prior to establishing the ASP in practice, the course participants anticipated barriers to implantation as follows (weighting in brackets):

- changing established farmer behaviour (32%);
- providing a consistent message (20%);
- threat to veterinary business performance or competitiveness (17%);
- lack of definitive evidence (33% of participants);
- lack of resources (12%); and
- changing veterinarian and clinic staff behaviour (9%).

Preliminary results from a post implementation survey 1 year after the course suggest that farmer behaviour is not as hard to influence as anticipated (a relative weighting of 14%) and that lack of a consistent message across the profession (24%) and competition between veterinary businesses (18%) were relatively more significant barriers to implementation. While this preliminary data is not statistically significant, it suggests an opportunity for improved collaboration within the NZ dairy industry and vet profession exists.

References

Antimicrobial stewardship in companion animal practice: a pilot study in Canberra, Australia

Alison Taylor1 and M. Archinal2
1Kippax Veterinary Hospital, Australia; 2Manuka Veterinary Hospital, Australia
alisont@kippaxvet.com.au

There is an increasing awareness and recognition of a need for antimicrobial stewardship (AMS) in small animal practice. There has been quite a lot of thought given to how we should go about this. So, in Australia, more particularly in Canberra, we decided to take everything we could find, critically review it, develop a plan and get started on the implementation. We are quite aware that our AMS programme
will require regular review and refinement but we are very excited to have come as far as we have and hopefully be able to contribute our practical experience with this concept to the world stage.

Our AMS journey started about 18 months ago with a gradual and increasing collaboration of minds that were all trying to find a way to address the increasing and frightening problem of antimicrobial resistance. It was decided that to make a real difference, we needed a formalised programme and its successful start needed people to seriously want to be a part of it. We spent 12 months creating the programme and overcoming logistical hurdles, such as ethics committee approval and online aspects to the programme. Six months ago in March 2016 we were very proud to be able to launch the Australian pilot AMS programme to the Canberra veterinary community. Now we feel like we have a lot to share already about our experiences.

The AMS programme was developed and is overseen by a steering committee that includes a veterinary microbiologist, key members of the Australian Veterinary Association (AVA) – both the national and the regional organisations – and two local veterinary practitioners and practice owners. At the core of the programme is a set of prescribing guidelines for dogs and cats (the AIDAP or Australian Infectious Diseases Advisory Panel guidelines) that we refer to and recognise will need to evolve and be subject to change. Together we have created an AMS programme that involves each practice voluntarily signing up, selecting and appointing a practice champion, assigning one member to complete a practice survey on antimicrobial usage, each prescriber completing an online training programme and survey, the practice creating a practice policy and the delivery of ongoing education both in digital form and at discussion evenings. So far, we have had the great proportion of the local practices commit to and contribute to the programme. We have examined the initial survey data and plan to evaluate it again in 12 months in order to measure the effectiveness of the programme. Costs associated with the programme have been covered by the AVA. It would have been very difficult for the voluntary members of the AMS team to be able to achieve what we have without this support. Details of the costs involved will be presented in the presentation.

Since the launch of the programme, there have been a few challenges but the main one that had to be overcome was how to engage the local veterinary practices at the very beginning. Once they had committed their support, we then had to work out how to maintain the momentum within the practice and decide what forum of ongoing education would be attractive. We have provided support materials and some incentives to be part of the programme but feel that this is an area we still need to develop further. Key considerations as we move forward include:

- whether the cost of the programme is sustainable;
- whether what we have created is able to be relocated and implemented elsewhere;
- how we motivate others to get started on similar programmes in their jurisdictions.

We are looking to form industry partnerships partly from a funding perspective but also to spread the message of antimicrobial stewardship as far as possible. The first partnership that we are trying to create is with the microbiology laboratories. We believe that they can help promote the importance of laboratory testing when trying to decide on appropriate antimicrobial use. They are also important in the progressive reporting of results for our region before and during AMS activities. We are also looking to collaborate more with the local medical and government bodies in the spirit of a One Health approach. There have been many highlights for us already, notably the support that we have gained from local practitioners and the side effect of having a very important common goal for all practitioners to try and achieve. This is creating a collegial atmosphere that we have not seen for a long time which has to help in making this programme sustainable in the future.

Developing a global veterinary antimicrobial stewardship support programme

Stephen Page
Advanced Veterinary Therapeutics, Australia
swp@advet.com.au

Antimicrobial stewardship (AMS) describes the multifaceted and dynamic approaches required to sustain the clinical efficacy of antimicrobials by optimising drug selection and use while minimising the emergence of resistance and other adverse effects. The word stewardship implies the obligation to preserve something of enormous value for future generations, and resonates in a way that ‘prudent use’...
or 'judicious use' does not. Good Stewardship Practice (GSP) is the active, dynamic process of continuous improvement in antimicrobial use, and is an ethic applied by everyone involved in antimicrobial use - veterinary practitioners, laboratory diagnosticians, owners, drug regulators, and pharmaceutical companies.

The 5Rs of successful antimicrobial stewardship:

1. **Responsibility** – The appropriate use of antimicrobials is a shared responsibility between the prescribing veterinarian, who accepts responsibility for the decision to use an antimicrobial agent, and the livestock producer or companion animal owner, who is responsible for following all directions for use and implementing associated management changes. This approach safeguards the health and welfare of the animals whilst minimising the likelihood of any immediate or longer term adverse impacts on the individual animal, other animals, or on public health.

2. **Reduction** – Wherever possible, means of reducing the use of antimicrobials should be implemented. Infection control and prevention measures underpin animal health and welfare and are supported by meticulous hygiene, precision nutrition, biosecurity, vaccination, and expert animal husbandry, which when combined ensure infectious disease incidence (and need for antimicrobials) is minimised.

3. **Refinement** – Refined use means the right diagnosis, the right drug, at the right time, at the right dose, the right route, and for the right length of time. Information about each use of an antimicrobial agent should be recorded so that total use can be evaluated and future use fine-tuned.

4. **Replacement** – The use of antimicrobials should be replaced whenever available evidence supports the efficacy and safety of an alternative.

5. **Review** – Antimicrobial stewardship initiatives should be reviewed regularly and a process of continuous improvement adopted to evaluate compliance with initiatives and ensure that antimicrobial use practices reflect contemporary best practice.

AMS can be simple and pragmatic (and must be if it is to be adopted) but involves the interplay of many disciplines as outlined in the following Figure:

To facilitate the widespread and effective implementation of AMS programmes worldwide it is essential that barriers and enablers of adoption are recognised and pragmatic plans designed appropriately. There are pilot studies testing the adaptation and adoption of AMS plans. How can the learnings be disseminated? How can accumulating experiences be shared? A discussion about global support is the objective of the presentation, the suggestions and comments of all participants are essential ingredients in refining the final plan.

**References**

APPENDICES TO VETERINARY ANTIMICROBIAL STEWARDSHIP

Appendix 1

The history of veterinary antimicrobial stewardship

Stewardship of animals has been a fundamental task of veterinarians since the first veterinarians graduated in Lyon in the 1760s. Most recently the focus of stewardship has been positioned on antimicrobial use. What was the first published admonition of AMS made by Morley and colleagues in 2005? What published materials are missing from the following chronology?

2011 Nielsen, A.C., 2011. Formularies and other risk management strategies promoting responsible use. 2nd Conference on Responsible Use of Antibiotics in Animals, 14-16 November 2011, Egmond aan Zee, the Netherlands, p. 42.
Appendix 2
Judicious use principles

If antimicrobial agents are indicated and must be used, then it is important that they are used judiciously. There are many publications that describes the principles of appropriate (or judicious or judiciously).
prudent) use. Are there fundamental principles that can be extracted from published guidelines? The following guidelines have been analysed and 22 principles identified. What are the 22 principles? Are there any principles missing?

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WEDNESDAY 28 SEPTEMBER 2016

FINAL PLENARY MEETING
WHERE DO WE GO FROM HERE?

The global response to antimicrobial resistance

Mark Woolhouse
Centre for Immunity, Infection & Evolution and Usher Institute of Population Health Sciences & Informatics, University of Edinburgh, UK
mark.woolhouse@ed.ac.uk

After decades of neglect, antimicrobial resistance (AMR) has captured the attention and concern of the public health community and global leaders. In September 2016, a high-level meeting of the United Nations General Assembly (UNGA) to be held in New York City will discuss how countries can cooperate to preserve global access to effective antimicrobials. This will be only the third health issue (and the first One Health issue, integrating human, animal and environmental health) to bring together heads of state at the UNGA. It is a rare opportunity to set a global agenda to address this issue. It is proposed that (i) setting targets for reducing drug resistant infections, (ii) adequate financing for global action, and (iii) defining the global health architecture to address AMR, should be key elements of a UN plan. Here, I report on progress from the UNGA Special Session on AMR and suggest some possible next steps.

One Health; bridging the gap between surveillance and stewardship

Luke S.P. Moore
Department of Medicine, Imperial College London, UK
l.moore@imperial.ac.uk

Emergence of antimicrobial resistance is a natural phenomenon, yet at a societal level antimicrobial resistance selection has been driven by antimicrobial exposure in health care, agriculture, and the environment. To combat the threat to animal and human health posed by this, understanding the mechanisms and drivers of AMR is vital. Strengthening cross-sector surveillance of both antimicrobial resistance and drug consumption is essential to accomplish this, enabling targeted strategies to reduce antimicrobial resistance. Yet where such stewardship relies solely upon removing antimicrobial selective pressure, this only impacts resistance trends where resistance mechanisms impart a fitness cost at the cellular level; an effect not always apparent. Instead therefore stewardship must be considered comprehensively; by resistance mechanism, microorganism, antimicrobial drug, host, and context. This must run parallel to new drug discovery, and broad ranging multidisciplinary research interlinked across the health care, agriculture, and environment sectors. Intelligent, integrated approaches, mindful of potential unintended results, are needed to ensure sustained, worldwide access to effective antimicrobials.
POSTERS

P1  How do French free-range poultry farmers decrease the use of antimicrobials?
Cécile J.M. Adam, N. Fortané, C. Ducrot and M.C. Paul
INRA, UR346 Epidémiologie Animale, France; Université de Toulouse, INP, ENVT, UMR 1225, IHAP, France; INRA, UR1323 RITME, France

P2  Antibiotic stewardship programme for food businesses
Ines Ajuda, Y. Lu and T. Jones
Food Business Programme, Compassion in World Farming, UK

P3  In vitro antibacterial activity of essential oils and plant extracts on enterotoxigenic Escherichia coli strains isolated from the pig gut
Carmen M.S. Ambrosio¹, Natália Y. Ikeda¹, Severino M. de Alencar¹, Carmen J. Contreras Castillo¹, Andrea M. Moreno² and Eduardo M. Da Glória¹
¹Department of Agroindustry, Food and Nutrition - ESALQ, University of São Paulo, Brazil; ²School of Veterinary Medicine and Animal Science, University of São Paulo, Brazil

P4  In vitro evaluation of the potential of Aloe barbadensis Miller in association with synthetic antimicrobials in resistance development in bacteria responsible for clinical mastitis in dairy cows
O. Chacón¹, N. Forno¹, Carolina Araya-Jordán¹, A. Maddaleno¹, R. Muñoz¹, J. Cornejo² and B. San Martin¹
¹Laboratory of Veterinary Pharmacology, Department of Clinical Sciences, University of Chile, Chile; ²Food Science Unit, Department of Preventive Medicine, University of Chile, Chile

P5  Performance of antimicrobial susceptibility assays on clinical Escherichia coli isolates from animals
Skye Badger¹,², S. Abraham², S. Saputra¹, D. Trott¹,³, C. Caraguel¹ and D. Jordan¹,²,⁴
¹School of Animal and Veterinary Sciences, University of Adelaide, Australia; ²School of Veterinary and Life Sciences, Murdoch University, Australia; ³Australian Centre for Antimicrobial Resistance Ecology, Australia; ⁴NSW Department of Primary Industries, Australia

P6  The link between antimicrobials, animals and antimicrobial resistance: its ‘interdisciplinary’ controversies in the UK
Stephanie Begemann
NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, Institute of Infection and Global Health, UK

P7  Supplementation of phytogenic feed additives (NSOAB9®) reduces faecal shedding of Lawsonia intracellularis and Brachyspira hydysenteriae in fattening pigs: a strategy to reduce antibiotics use?
Amine Benarbia¹ and O. Tsuguaki²
¹Nor-Feed SAS, Beaucouzé France; ²Bussan, Japan

P8  Feather analysis as a tool to monitor antibiotic use in poultry production
Bjorn B.J. Berendsen, L.J.M. Jansen, Y.J.C. Bolck and T. Zuidema
RIKILT Wageningen University & Research, the Netherlands

P9  Raising pigs without antibiotics, thanks to algae
M. Gallissot¹, P. Gréau² and Olivier Blan nic¹
¹Olmix SA, France; ²Breizh Algae, France

P10  The impact of antibiotic use in Australian piglets — resistance in domestic and feral pig populations
Lechelle K. van Breda¹, A.N. Ginn²,³, O.P. Dhungyel¹, S. Partridge², J.R. Iredell² and M.P. Ward¹
¹Faculty of Veterinary Science, The University of Sydney, Australia; ²Centre for Infectious Diseases and Microbiology, The Westmead Institute for Medical Research, The University of Sydney, Westmead Hospital, Australia; ³Antimicrobial Resistance Reference Laboratory,
Dairy production-related factors that affect amount of cows' treatments with antibacterials for systemic and intramammary use

Marta Brscic1, A. Azzolin2, A. Scollo1 and F. Gottardo1
1Department of Animal Medicine, Production and Health, University of Padova, Italy; 2Private Veterinary Practitioner, Italy

What causes antimicrobial resistance besides antimicrobial treatment?

Elke Burow, B.-A. Tenhagen and A. Käsbohrer
Unit Epidemiology, Zoonoses and Antimicrobial Resistance, Department Biological Safety, Federal Institute for Risk Assessment, Germany

Measuring concentrations of chlortetracycline antibiotic and its metabolite epi-4-chlortetracycline in treated poultry faeces by liquid chromatography-tandem mass spectrometry

Javiera Cornejo1, E. Pokrant1, C. Avell1, K. Yevenes1, A. Maddaleno2, C. Araya2, C. Vergara1 and B. San Martin2
1Preventive Medicine Department, Faculty of Veterinary and Animal Sciences, University of Chile, Chile; 2Laboratory of Veterinary Pharmacology, Faculty of Veterinary and Animal Sciences, University of Chile, Chile

In-house validation of analytical methodology for the determination of florfenicol and florfenicol amine in poultry faeces by liquid chromatography-tandem mass spectrometry and evaluation of concentrations after oral treatment

Javiera Cornejo1, R. Riquelme1, E. Pokrant1, K. Yevenes1, A. Maddaleno2, C. Araya2 and B. San Martin2
1Preventive Medicine Department, Faculty of Veterinary and Animal Sciences, University of Chile, Chile; 2Laboratory of Veterinary Pharmacology, Faculty of Veterinary and Animal Sciences, University of Chile, Chile

Development and in-house validation of analytical methodology for the determination of tylosin in broiler chicken feathers by high-performance liquid chromatography with diode-array detection

Javiera Cornejo1, C. Carvallo1, E. Pokrant1, A. Maddaleno2, C. Araya2 and B. San Martin2
1Preventive Medicine Department, Faculty of Veterinary and Animal Sciences, University of Chile, Chile; 2Laboratory of Veterinary Pharmacology, Faculty of Veterinary and Animal Sciences, University of Chile, Chile

Understanding the culture of antimicrobial use behaviours in agriculture: a quantitative study of UK pig veterinary surgeons and farmers

Lucy A. Coyne
Institute of Infection and Global Health, University of Liverpool, UK

Multidrug resistance of avian pathogenic Escherichia coli (APEC) isolated from diseased chicken flocks in the Mekong Delta region of Vietnam

Nguyen Van Cuong, N.T. Nhung, J. Campbell and J. Carrique-Mas
Clinical Research Unit, Oxford University, Vietnam

Quantitative assessment of antimicrobial resistance in livestock during the course of a nationwide antimicrobial use reduction in the Netherlands

Alejandro Dorado-García1,2, D.J. Mevius2,3, J.J.H. Jacobs1,4, I.M. van Geijlswijk4,5, J.W. Mouton4,6, J.A. Wagenaar2,3,4 and D.J.J. Heederik1,4
1Department of Environmental Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, the Netherlands; 2Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, the Netherlands; 3Wageningen Bioveterinary Research, Wageningen University & Research, the Netherlands; 4The Netherlands Veterinary Medicines Authority (SDa) Expert Panel, the Netherlands; 5Department of Pharmacy, Faculty of Veterinary Medicine, Utrecht University, the Netherlands; 6Department Medical Microbiology and Infectious Diseases, Erasmus Medical Centre, the Netherlands
Disc diffusion antimicrobial susceptibility outcomes for bovine mastitis streptococci betalactams reveal an urgent need for evidence based breakpoints
M. Melchior1,2, H. Mulder1 and Monique Driesse2
1MBM Veterinary Diagnostiek, the Netherlands; 2Boehringer Ingelheim Animal Health, the Netherlands

Biofilm formation – an emerging therapeutic target?
X. Wu, R.R. dos Santos and Johanna Fink-Gremmels
Institute for Risk Assessment Sciences, Utrecht University, the Netherlands

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C. Soler1,2, Tim Goossens2, A. Bermejo2 and L. Fraile1,3
1Departament de Producció Animal. ETSEA, University de Lleida, Spain; 2Nutriad International N.V., Belgium; 3AgrotecnicioCenter, Spain

Effect of replacing in-feed antibiotic growth promotants with organic acid-based feed additives on growth performance, health, and gut microorganisms of weanling piglets
S. Long1, X. Piao1, Y. Wu2 and Yanning Han2
1State Key Laboratory of Animal Nutrition, China Agricultural University, China; 2Trouw Nutrition R&D, the Netherlands

Antibiotics for surgical prophylaxis in small animal veterinary practice in Australia
Laura Y. Hardefeldt, K. Bailey and G.F. Browning
National Centre for Antibiotic Stewardship, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Australia

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Maarten van der Heijden and C. Smits
Trouw Nutrition, the Netherlands

Identification of blaCMY-2 and blaCTX-M-1 carrying Escherichia coli in slaughterhouse broilers and broiler meat in Finland – whole genome sequencing reveals high genetic diversity with multiple MLST types
Annamari Heikinheimo, S.-M. Latvio, M. Päivärinta and M. Fredriksson-Ahomaa
Department of Food Hygiene and Environmental Health, University of Helsinki, Finland

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A.E. Heuvelink, Jobke van Hout and M.M.C. Holstege
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S. Le Gall-David1, Catherine Ionescu8, V. Meuric1,2, G. Benzoni7, S. Valière3,4, A. Guyonvarch7, J. Minet1,2, M. Bonnaure-Mallet1,2 and F. Barloy-Hubler5,6
1Equipe Microbiologie EA 1254, Université de Rennes, France; 2CHU, 35033, France; 3INRA, UAR1209, France; 4INRA, GeT-PlaGe, France; 5CNRS-UMR6290-IGDR, France; 6AMADEUS platform – Biosit, France; 7Invivo NSA, France; 8Pancosma SA, Switzerland

Responsible use of fluoroquinolones in farm animals; a mandatory prerequisite
L. Klostermann, Anno de Jong, B. Stephan, S. Tennagels, J.R. Vazquez, B. Martin, C. Christensen and Bernhard Stahl
Bayer Animal Health GmbH, Germany

Promoting of prudent use of antimicrobials in food-producing animals – experience from Finland
H. Helin-Soilevaara1, S. Nykäsenoja1, K. Kivilahti-Mäntylä2, L. Suojala3 and Liisa Kaartinen1
1Finnish Food Safety Authority (EVIRA), Finland; 2Finnish Medicines Agency (Fimea), Finland; 3Finnish Central Union of Agricultural Producers and Forest Owners MTK, Finland
In vitro evaluation of synergistic effects between commercial mixtures of essential oils and antibiotics on strains of Escherichia coli, Clostridium perfringens and Pasteurella multocida

Sylvain Kerros
Phytosynthese, France

Antimicrobial susceptibility of Escherichia coli and Salmonella isolates from healthy pigs in Australia: results of a pilot national survey

Amanda Kidsley1, S. Abraham2, J.M. Bell1, P. Mitchell3 and D. Trott1
1Australian Centre for Antimicrobial Resistance Ecology, University of Adelaide, Australia; 2Murdock University, Australia; 3Australian Pork Limited, Australia

Antimicrobial susceptibility of Salmonella spp. isolates from swine in Chile

C. Vergara, G. Asenjo, C. Araya, B. San Martin, J. Cornejo and Lisette Lapierre
Faculty of Veterinary, University of Chile, Chile

Status of antimicrobial surveillance in the APEC region

G. Asenjo, C. Vergara, C. Araya, B. San Martin, J. Cornejo and Lisette Lapierre
Faculty of Veterinary, University of Chile, Chile

Comparison between yeast administration at different timing on health status of weaned pigs in response to Escherichia coli F4ac infection

Diana Luise1, D. Priori1, V. Motta1, E. Auclair2, P. Bosi1 and P. Trevisi1
1Department of Agricultural and Food Science, University of Bologna, Italy; 2Phileo-Lesaffre Feed Additives, France

FDA’s regulatory approach to preserving the safety and effectiveness of medically important antimicrobials used in food-producing animals

Ron A. Miller1, R. Singh1, H.C. Harbottle1, S.A. Piñeiro1, S.S. Yan1, J.M. Gilbert1 and W.T. Flynn2
1Division of Human Food Safety, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, U.S. Food and Drug Administration, USA; 2Office of the Director, Center for Veterinary Medicine, U.S. Food and Drug Administration, USA

A comparison of clinical and economic outcomes when metaphylactically administering either a novel DNA immunostimulant or tilmicosin to beef calves at medium – high risk of developing bovine respiratory disease in the feedlot

Jason Nickell1, D. Keil1, T. Settje1, K. Rogers2 and D. Miles2
1Bayer Animal Health, USA; 2Veterinary Research and Consulting Service, LLC, USA

Development of a new bioluminescent murine model of bacterial wound infections

Abiodun D. Ogguni1, Z. Kopecki2, E.E. Hickey1, M. Khazandi1, A.J. Cowin2, S.W. Page3 and D.J. Trott1
1Australian Centre for Antimicrobial Resistance Ecology, School of Animal and Veterinary Sciences, The University of Adelaide, Australia; 2Future Industries Institute, The University of South Australia, Australia; 3Advanced Veterinary Therapeutics, Australia

Efficacy evaluation of a new water sanitiser for increasing the shelf life of seafood products

M. Khazandi1, P. Deo2, S. Ferro3, H. Venter2, H. Pi1, S. Crabb3, T. Amorico3, Abiodun D. Ogguni1 and D.J. Trott1
1Australian Centre for Antimicrobial Resistance Ecology, School of Animal and Veterinary Sciences, The University of Adelaide, Australia; 2School of Pharmacy and Medical Sciences, University of South Australia, Australia; 3Ecas4 Australia, Australia

Antibiogram, virulence traits, and genetic relatedness of methicillin-resistant Staphylococcus aureus isolated from bovine mastitic milk in Korea

Department of Veterinary Microbiology, College of Veterinary Medicine, Seoul National University, Republic of Korea
Comparison of oregano essential oil with antibiotic growth promoters on the performance of broilers
Qingyun Peng1,2, J. Li3, Z. Li2, Z. Duan2, Y. Lao2 and Y. Wu1
1School of Life Sciences, Sun Yat-sen University, China; 2Kemin Industries (Zuhai) Co., Ltd., China; 3School of Environmental and Biological Engineering, Liaoning Shihua University, China

Antimicrobial resistance prevalence in bacteria isolated from harbour (Phoca vitulina) and grey (Halichoerus grypus) seal pups stranded in the Netherlands: a first screening
1Sealcentre, the Netherlands; 2Department of Medical Microbiology, Izore Center for Infectious Diseases, the Netherlands; 3Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, the Netherlands; 4Faculty of Veterinary Medicine, Utrecht University, the Netherlands

Bacillus subtilis PB6 – a potential alternative for antibiotic growth promoters (AGPs)
Prakash Chandra Saini, S. Jayaraman, S. Sankaran and R. Chanthirasekaran
Kemin Industries South Asia Pvt. Ltd., India

Responsible use of antibiotics in pig production: an alternative approach to reduce mass metaphylaxis
Annalisa Scollo1,2, F. Gottardo2 and C. Mazzoni1
1Suivet snc, Italy; 2MAPS Department, University of Padova, Italy

A national near real-time antimicrobial surveillance in companion animals in the UK – a health informatics approach
David Singleton
Epidemiology and Population Health, Institute of Infection and Global Health, University of Liverpool, UK

Effect of a Bacillus subtilis probiotic on performance and gut health of broiler chickens
Natasja Smeets, S. de Smet and F. Nuyens
Kemin Europa NV, Belgium

CLOSTAT™ – a direct-fed microbial containing Bacillus subtilis PB6 for the replacement of antibiotic growth promoter in animal feed
BoonFei Tan, P.-S. Chan, A. Chua and E. Schoeters
Kemin Animal Nutrition and Health, Asia Pacific, Singapore

Antibiotic growth promoter replacement by a synergistic butyrate based product in piglets
Valentine Van Hamme1, A. Eto2 and L.B. Costa3
1Impextraco NV, Belgium; 2Impextraco Latin America, Brazil; 3Pontifícia Universidade Católica do Paraná (PUCPR), Brazil

Medium chain fatty acids to reduce antibiotic resistance
Ellen Van Meenen, W. Naeyaert, M. De Laet, J. Krijnen, G. De Clercq and K. Lannoo
Nuscience, Belgium

Canadian dairy producer perceptions towards antibiotic treatment and prevention of mastitis and on-farm record quality
M.E. Alexandrea Watters1, M.A. Godkin2, D.F. Léger3, J.B. Coe1, K.D. Lissemore1 and D.F. Kelton1
1University of Guelph, Canada; 2Ontario Ministry of Agriculture, Food, and Rural Affairs, Canada; 3Public Health Agency of Canada, Canada

Bactericidal activity of a novel antisense peptide-peptide nucleic acid against the cytidine monophosphate kinase of Staphylococcus aureus
Jang Won Yoon1,2, H.T. Lee1, D. Han1, J.B. Lee1 and Y.H. Park2
1College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Republic of Korea; 2College of Veterinary Medicine, Seoul National University, Republic of Korea
P51 Development of an enzyme-linked-receptor assay based on the carboxy-terminal of penicillin-binding protein BlaR for the detection of β-lactams in tissues of food-producing animals
G. Cheng, J. Peng, L. Huang, Y. Wang, H. Hao, D. Peng and Zonghui Yuan
National Reference Laboratory of Veterinary Drug Residues (HZAU) and MOA Key Laboratory for the Detection of Veterinary Drug Residues in Foods, Huazhong Agricultural University, China

P52 Utilising glycoside hydrolases to degrade bacterial biofilms and increase the efficacy of antibiotics
D. Fleming and Kendra P. Rumaugh
Department of Surgery and the Texas Tech University Health Sciences Center Burn Center of Research Excellence, Texas Tech University Health Sciences Center, USA
P1: How do French free-range poultry farmers decrease the use of antimicrobials?

Cécile J.M. Adam¹, N. Fortané, C. Ducrot and M.C. Paul
INRA, UR346 Epidémiologie Animale, France; Université de Toulouse, INP, ENV'T, UMR 1225, IHAP, France; INRA, UR1323 RITME, France
cecile.adam@clermont.inra.fr

Poultry ranks second in antimicrobial consumption worldwide. The diversity of industrial poultry production, with a wide range of species and farming systems, makes it difficult to analyse the use of antimicrobials. A transdisciplinary study, at the interface of sociology and epidemiology, was built in order to analyse the farmers’ perception on the use of antimicrobials, and the farming practices they identified as levers for decreasing antimicrobials. It focused on industrial free-range broiler chicken systems in France, which have been little studied so far. This quality-oriented production is associated with important environment, sustainability, biosecurity and animal welfare issues. 22 semi-structured interviews were conducted in 2015, using a pre-tested interview guide, with 12 farmers, 4 veterinarians and 6 technicians from three farmer organisations (FO) representative of the major French production areas. All the interviews – mean duration 2 h 15' - were recorded and fully transcribed. Analysis was completed following the principles of content analysis methods. Analysis of the interviews shows 3 major results. First, the farmers estimate more antimicrobials were used in the past. The interviews show that the more experienced farmers think that they would use antimicrobials when it was not essential. Second, they describe their current use of antimicrobials as low, in particular because they compare themselves to higher antimicrobials users like industrial indoor chicken farmers. They value reducing the use of antimicrobials because of economics incentives – they get an extra payment for breeding broilers without antimicrobials – but also for cultural reasons. This is indeed a way to adjust their farming practices to consumer demand, and thus gives a better image to agriculture, which is currently facing social criticism. Decreased use of antimicrobials is in accordance with their perception of a good quality production. Third, they managed this decrease by modifying a set of practices. They learnt to wait and analyse the situation, and accept a higher mortality. They also highlight how they changed their practices: improve water quality and farm hygiene, use herbal drugs, and observe more the animals. Farmers also rely on their health advisors: veterinarians and technicians support them in their strategy of reducing antimicrobials. Interviews show that FO play a major role in prescribing practices. In conclusion, interviews’ analysis show that quality productions could help identifying levers for change in optimising and reducing the use of antimicrobials. Poultry firms’ actors should also be taken into account by designing integrative programmes aiming at reducing antimicrobials consumption.

P2: Antibiotic stewardship programme for food businesses

Ines Ajuda, Y. Lu and T. Jones
Food Business Programme, Compassion in World Farming, UK
ines.ajuda@ciwf.org.uk

The industry engagement and initiative has been a driver for the development of different areas in animal production. A good example of this is the recent movement to go cage free in the United States and the United Kingdom, which represent commitments that are above legislation and very meaningful for animal welfare. Compassion in World Farming believes that food businesses can also be part of the movement for a more responsible use of antibiotics in animal production. The majority of the queries received by us from companies regarding antibiotic use are related to implementation of a plan and ‘how to tackle’ the issue. To our knowledge there is a substantial amount of information published about consequences of the misuse of antibiotics (in animal production and human medicine) and published guidelines for different sectors of the supply chain, but there are no specific guidelines published to help food companies to establish a more responsible use of antibiotics in their supply chain. In order to help food businesses to follow this journey the food business team at Compassion in World Farming has developed a roadmap to help food companies establish an Antibiotic Stewardship Programme in their supply chain. The roadmap is based on eight steps such as assessing motivations, defining priorities, measuring progress, educating and training as well as communicating. In each step specific examples are given and links provided, so companies can select the best solution for their specific case. There are also specific guidelines for companies that have control over the farms that supply them (for example manufacturers) and for companies that only supply from intermediary companies (for example food services). A pivotal part of this resource are the clear examples of how a good management on farm as well as higher animal welfare can help to reduce the use of antibiotics, helping to build the case for an improved animal health and welfare. Finally, the roadmap will be completed with three case studies from different stakeholders in the supply chain that have implemented antibiotic stewardships in their
businesses, clinics and organisations. With this tool the food business team member hope to help food companies to initiate the process of a more responsible use of antibiotics in their supply chain, helping to create an impact on ‘the bigger picture’.

P3: *In vitro* antibacterial activity of essential oils and plant extracts on enterotoxigenic *Escherichia coli* strains isolated from the pig gut

**Carmen M.S. Ambrosio**1, Natália Y. Ikeda1, Severino M. de Alencar1, Carmen J. Contreras Castillo1, Andrea M. Moreno2 and Eduardo M. Da Glória1

1Department of Agroindustry, Food and Nutrition - ESALQ, University of São Paulo, Brazil; 2School of Veterinary Medicine and Animal Science, University of São Paulo, Brazil
carmen.milagros8@usp.br; emgloria@usp.br

The diarrhoea in weaning pigs, mostly caused by enterotoxigenic *Escherichia coli* (ETEC) prevalence in the gastrointestinal tract, can result in deleterious effects on animal performance. To control ETEC outbreaks, antibiotics (AGP, antimicrobial growth promoter) have been frequently included in animal diet. However, the continuous use of antibiotics has caused the emergence of antibiotic bacterial resistance and its spread around the world. For example, nowadays, there is a great concern about the emergence of the plasmid-mediated colistin resistance (mcr-1) in *E. coli*, a mechanism of resistance to the last group of antibiotics, polymyxins (colistin). Due to this, some alternatives, such as essential oils and plant extracts, have been investigated and shown interesting *in vitro* antibacterial activity results. The aim of this study was to evaluate the antibacterial activity of some essential oils (EOs) and plant extracts on four ETECs that were isolated from the pig gut. An initial screening of antibacterial activity of eight citrus EOs (by-products of orange juice production), two propolis EOs and extracts (ethanolic, hexanic and isopropilic) from jalapeño (*Capsicum annuum*) and bacupari (*Garcinia gardneriana*) was performed by the disc diffusion method. Each EO was prepared at 90% using acetone as solvent. Extracts were dried and suspended in 30 ml of distilled water using Tween-80 as emulsifier when necessary. Colistin (15 µg/ml) was used as positive control. Only three citrus EOs exhibited antibacterial activity (Brazilian orange terpenes, orange oil phase essence and Tahiti lime oil phase) on ETECs and the analysis of variance (ANOVA) showed a significant difference in the antibacterial activity between them. Tukey’s test (P<0.05) and principal component analysis (PCA) showed that Brazilian orange terpenes (BOT) presented the largest antibacterial activity on ETEC isolates (27.8±2.0, 23.2±2.6, 25.1±1.0, 24.7±3.6 mm) showing a superior action to colistin. Moreover, the results also showed a significantly different sensitivity of the isolates to citrus oils, being the isolate *E. coli* U21 (K88 LT/STb/F18/STa) the most resistant. Thereby, BOT was tested to determine its MIC using survival curves and the resazurin test by the microdilution method, and MBC (by plating) on *E. coli* U21. The MIC and MBC of BOT were 3.7 mg/ml. CG-MS analysis of BOT showed that limone (81.37%), cis-limonene oxide (2.73%), and myrcene (2.61%) were the major compounds present. The results showed that some EOs that are by-products of orange juice production can be alternatives to control ETECs, but their use as alternative to synthetic antibiotics should be checked by *in vivo* evaluations.

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P4: *In vitro* evaluation of the potential of *Aloe barbadensis* Miller in association with synthetic antimicrobials in resistance development in bacteria responsible for clinical mastitis in dairy cows

O. Chacón1, N. Forno1, Carolina Araya-Jordán1, A. Maddaleno1, R. Muñoz1, J. Cornejo2 and B. San Martín1

1Laboratory of Veterinary Pharmacology, Department of Clinical Sciences, University of Chile, Chile; 2Food Science Unit, Department of Preventive Medicine, University of Chile, Chile
caroa@ug.uchile.cl

Increased bacterial resistance worldwide limits the therapeutic arsenal available to treat bacterial infections in food production animals. Therefore, research for new alternatives to improve the effectiveness of existing antimicrobials becomes essential. The use of association therapies might delay the selection of mutations conferring antimicrobial resistance to a particular drug, so the use of natural compounds with antimicrobial activity associated with synthetic antimicrobials might be an effective tool to slow down or decrease bacterial resistance. The objective of this study was to compare the potential for selection of resistant clones against five treatments: *Aloe barbadensis* Miller (*A. vera*); cloxacillin; cefitiofur; association *A. vera/cloxacillin*; and association *A. vera/cefitiofur*. The potential for selection of
resistant clones was assessed by 15 serial passages in bacteria that commonly cause clinical mastitis in dairy cows. Reference strains *Staphylococcus aureus* ATCC 29213 (MSSA) and *S. aureus* ATCC 43300 (MRSA) were used. The serial passages were conducted by exposing the bacterial strains to antimicrobial concentrations 3-fold lower and 3-fold higher than the MIC previously defined. Following incubation, the concentration of antimicrobial that allowed growth equivalent to the positive control without inhibitors was selected and 100 μl of it were transferred to Mueller-Hinton II broth free of antimicrobials to inoculate a new batch of tubes. This process was repeated for 15 serial passages. After 15 serial passages an increased in resistance of bacterial strains exposed to synthetic antimicrobials was recorded. MSSA had an initial MIC of 0.5 μg/ml for cloxacillin and 0.25 μg/ml for ceftiofur; after 15 serial passages MIC increased three times against cloxacillin (2 μg/ml) and one time for ceftiofur (0.5 μg/ml). MRSA showed a MIC of 2 μg/ml for cloxacillin and 0.5 μg/ml for ceftiofur; after 15 serial passages MIC increased 15 times against cloxacillin (32 μg/ml) and 127 times for ceftiofur (64 μg/ml). No increase in the MICs against *A. vera* extract (7 mg/ml), *A. vera*/cloxacillin (4 mg/ml + 0.03 μg/ml) and *A. vera*/ceftiofur was evident (4 mg/ml + 0.06 μg/ml), maintaining their initial MIC. In conclusion, the use of *A. vera* in association with synthetic antimicrobials delays the emergence of resistant strains, while allowing a decrease in the amounts of antimicrobials needed to treat clinical mastitis.

**P5: Performance of antimicrobial susceptibility assays on clinical *Escherichia coli* isolates from animals**

**Skye Badger**1,2, S. Abraham2, S. Saputra1, D. Trott1,3, C. Caraguel1 and D. Jordan1,2,4  
1School of Animal and Veterinary Sciences, University of Adelaide, Australia; 2School of Veterinary and Life Sciences, Murdoch University, Australia; 3Australian Centre for Antimicrobial Resistance Ecology, Australia; 4NSW Department of Primary Industries, Australia  
skye.badger@adelaide.edu.au

Surveillance is a critical component in the detection of emerging forms of antimicrobial resistance (AMR) threatening the health of humans and animals. In addition to active surveillance on commensal bacteria, a future Australian national AMR surveillance programme in animals will ideally include a passive component focusing on animal bacterial pathogens. Passive surveillance will likely involve capturing surveillance data generated by veterinary diagnostic laboratories (VDLs) as part of routine disease investigations. Most Australian VDLs use disc diffusion assays for antimicrobial susceptibility testing (AST). While disc diffusion is an inexpensive alternative to broth-microdilution assessment of minimum inhibitory concentration (MIC), broth-microdilution is regarded as the superior assay. There is insufficient evidence comparing the performance of disc diffusion and broth-microdilution assays for animal pathogens. As such it is not clear if disc diffusion susceptibility results are suitable for generating surveillance data in animals. This study aimed to compare the performance of disc diffusion relative to broth-microdilution in a large sample of pathogenic *Escherichia coli* isolated from companion animals. Clinical *E. coli* isolates (n=994) from predominately canine (n=514), feline (n=341), and other non-livestock species (n=139) were tested for susceptibility to twelve antimicrobials by broth-microdilution and disc diffusion methods using Clinical Laboratory Standards Institute (CLSI) protocols and clinical resistance breakpoints. Overall observed agreement between disc diffusion and broth-microdilution was greater than 90% for all antimicrobials, and greater than 95% for ten antimicrobials. Inter-test agreement measured by prevalence-adjusted-bias-adjusted kappa was >0.9 for all but two antimicrobials (cephalothin – 0.87; trimethoprim-sulfamethoxazole – 0.88). The relative sensitivity of disc diffusion compared to micro-broth dilution varied from 72-98%, and relative specificity was always >95%. The area under the receiver-operating characteristic curve was >0.9 for all antimicrobials. This study demonstrated that for most drugs the disc diffusion method is highly accurate for pathogenic *E. coli* from companion animals. However, variability in disc diffusion performance for some drugs (e.g., cephalothin, and trimethoprim-sulfamethoxazole) needs further investigation. Altogether, disc diffusion appears to have applicability in the passive acquisition of resistance data in a national surveillance programme provided individual laboratories can reliably implement the assays.
**P6: The link between antimicrobials, animals and antimicrobial resistance: its ‘interdisciplinary’ controversies in the UK**

**Stephanie Begemann**  
NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, Institute of Infection and Global Health, UK  
stephanie.begemann@liverpool.ac.uk

Although antimicrobial resistance (AMR) is a universal public health concern, great gaps remain in our current understanding of the magnitude of the problem. A major health concern and issue of controversy is the ‘inappropriate’ veterinary use of antibiotics in animals raised for human consumption. Both ‘scientific’ and ‘non-scientific’ literature present different versions of factors that contribute to the definition of AMR and the link with antibiotic use in animals. Not only is this a topic of scientific complexity and conflicting interests, it is also an issue of uncertainty, in which risk perceptions of various stakeholders are affected by different truth claims on what makes AMR and its link with antibiotic use in animals. This research uses the theoretical framework of science and technology studies (STS) to make visible the complex relations between AMR realisations in science, politics, the media and the public in the United Kingdom. Controversies on the topic will be analysed by considering where the issue is problematised, who is involved and what knowledge sources (persons, documents, objects) are drawn upon to make claims and counterclaims on the controversy. This approach enables the researcher to study the construction of the link on antibiotic use in animals and AMR beyond the established factors and ‘scientific facts’ listed by the official actors. The discussion on AMR is an ongoing process reducible neither to parliamentary politics, biomedical science, media or the public. This research will provide knowledge on the governance of scientific issues and how to deal with ‘interdisciplinary’ controversies.

**P7: Supplementation of phytogenic feed additives (NSOAB9®) reduces faecal shedding of Lawsonia intracellularis and Brachyspira hyodysenteriae in fattening pigs: a strategy to reduce antibiotics use?**

**Amine Benarbia**¹ and O. Tsuguaki²  
¹Nor-Feed SAS, Beaucouzé France; ²Bussan, Japan  
amine.benarbia@norfeed.net

Antibiotics used as growth promoter (AGP) in animal feed have several adverse effects either on human, animal or environment health. This is mainly due to residues and/or to the development on resistant strains of bacteria to antibiotic treatment. Many countries have banned the use of AGP in order to slow the development of resistance and reduce their adverse effects. This led to development of alternative to AGP. Some of the most promising are those based on plant and plant extract. Fattening in pig livestock represents a stressful period where antibiotics are used in order to control swine dysentery. This disease, mainly due to Lawsonia intracellularis (LI) and Brachyspira hyodysenteriae (BH), leads to serious economic loss due to the decrease of animal zootechnical performances and the use of veterinary drugs. In this trial, we studied the effect of feed supplementation with a phytogenic feed additive based on citrus- and saponins-rich plants as an alternative to control swine dysentery pathogens LI and BH. 120 day-old pigs were separated into 2 groups: the control group received standard diet and the NS group received standard diet supplemented with 3,000 ppm of the commercial solution NSOAB9®. Pigs were raised for 8 weeks. Faeces were sampled at the beginning of the trial (day 120) and at the end of the trial (day 180) in order to perform real-time PCR for quantification of LI and BH, and zootechnical parameters were recorded weekly. RT-PCR analysis showed that both level of LI and BH were equivalent in the two groups at day 120. After 8 weeks, the level of LI/BH significantly decreased in the NS group whereas the levels significantly increased in animals from the control group. Moreover, at the end of the trial the level of LI/BH was significantly lower in animals of the NS group compared to those of the control group. From the zootechnical point of view, animals of the NS group showed a higher feed intake compared to control group animals (+35%) with a lower feed conversion ratio (-20%). This resulted in a better average daily gain in animals from the NS group compared to the control group ones. Feed supplemented with the commercial solution reduced bacterial pressure and improved zootechnical parameter in fattening pigs. This strategy could be used in order to decrease antibiotics use during this period in order to slow the development of resistant strain and make antibiotic use more efficient in case of outbreak of the disease.
P8: Feather analysis as a tool to monitor antibiotic use in poultry production

Bjorn B.J. Berendsen, L.J.M. Jansen, Y.J.C. Bolck and T. Zuidema
RIKILT Wageningen University & Research, the Netherlands
bjorn.berendsen@wur.nl

Antibiotics are commonly used in the poultry industry to treat bacterial infections. In the combat against bacterial resistance policies aim for a reduction of antibiotic use in animal production. To be able to monitor antibiotic use, farmers are obliged to supply an up to date registration of all treatments when animals are offered for slaughter. For enforcement, tests are needed to detect antibiotic treatments having a long detection window and, preferably with the ability to antedate administration so to prevent improper and off-label use of antibiotics. We developed a procedure to easily and effectively detect antibiotic treatments in chickens. After poultry treatment, antibiotics are dispositioned to chicken feathers. These feathers can be easily obtained at the farm or during slaughter. Using a commercially available screening analysis (Charm Rosa; Charm Sciences, USA) that can be carried out on-site within a few minutes. Unexpected findings are followed up by an in-house developed confirmatory test using liquid chromatography coupled to mass spectrometric detection. Using this approach, we are able to detect antibiotic treatments effectively. We demonstrated that even the treatment of 2-day old chicks was detectable during slaughter, which demonstrates the power of the chosen approach. Furthermore, pilot studies show that in depth analysis yields information on the time and the way of treatment. Feathers are washed to remove excessive antibiotics on the feather surface and subsequently they are cut in six pieces of equal length. These segments are individually grinded and analysed. With this approach it is possible to distinguish between a registered therapeutic oral treatment, an off-label spray treatment, and an illegal prolonged sub-therapeutic treatment with enrofloxacin, a last resort fluoroquinolone antibiotic. This approach is a new and strong tool in the enforcement of new policies in the fight against off-label and supervacaneous antibiotic use.

P9: Raising pigs without antibiotics, thanks to algae

M. Gallissot¹, P. Gréau² and Olivier Biannic¹
¹Olmix SA, France; ²Breizh Algae, France
animalcare.ts@olmix.com

Antibiotic resistance is one of the top five public health concern according to a WHO 2015 report. Consequently, Olmix has developed a complete programme aiming at accompanying producers to decrease the use of antibiotics, achieve antibiotic-free production and thus limit the development of antibiotic resistance. This programme, adapted to each farm, is based on a global approach and relies on strong technical and management support together with the use of specific algae-based products. The programme was implemented in a farrow-to-finish pig operation of 840 sows in France. The farm was submitted to a detailed audit in December 2015, from which an action plan was prepared and agreed with all collaborators. The action plan aimed at reducing antibiotic use from 100% of the pigs being systematically treated with one or several antibiotics, to a maximum of 10% of the pigs being treated with only one antibiotic. Moreover, the farm manager aimed at improving piglets weaning weight. Several recommendations constituted the action plan. An improved cleaning and disinfection protocol was implemented concerning the rooms, the manure pit, the water distribution system and small equipment. Adjustments were made in the farrowing room (equipment for teeth grinding, heating lamps, etc.) in order to improve the comfort of newborn piglets. Biosecurity management was reinforced by a stricter quarantine programme, more control of inputs to the farm and an action against rodents. The use of 5 innovative marine algae-based products dealing with environmental hygiene, immunity, digestive welfare, mycotoxin risk and digestive efficiency, used in environment, feed or drinking water, completed these different measures. The action plan was implemented step-by-step from February to May 2016. Preliminary results of this case study show that the use of coccidiostat could be stopped, and antibiotic use was reduced by 94%. Indeed, only 5.16% of the piglets were treated with amoxicillin in the last monitoring of the results early July 2016. Moreover, global performance in maternity was improved. When comparing performance of the pre-trial period (January-February 2016) with the trial period (March-July 2016), +234 g at weaning was achieved, as well as an increased weaning rate (+0.51 weaned piglet/litter, +24 weaned piglets/week). The study continues in this farm in order to confirm the preliminary results obtained and further improve performance. The six-month feedback already shows that it is highly important to adapt the strategy to each situation, and confirms the need of a global approach to reduce the use antibiotics in farms.
P10: The impact of antibiotic use in Australian piglets – resistance in domestic and feral pig populations

Lechelle K. van Breda¹, A.N. Ginn²,3, O.P. Dhungyel⁴, S. Partridge⁵, J.R. Iredell⁶ and M.P. Ward⁷
¹Faculty of Veterinary Science, The University of Sydney, Australia; ²Centre for Infectious Diseases and Microbiology, The Westmead Institute for Medical Research, The University of Sydney, Westmead Hospital, Australia; ³Antimicrobial Resistance Reference Laboratory, Centre for Infectious Diseases and Microbiology Laboratory Services, Pathology West, Australia
lechelle.vanbreda@sydney.edu.au

Pre- and post-weaning diarrhoea in piglets caused by *Escherichia coli* has dramatic impacts on the Australian pig industry. Antibiotics are often used at weaning for treatment but *E. coli* can develop resistance over time. Australia’s geographically isolated location, strict biosecurity policies and restricted use in animals of antibiotics important in human medicine has contributed to Australia’s unique livestock resistance profile. Our aim was to determine the prevalence of antibiotic resistance in *E. coli* isolated from piglets in commercial herds in south-eastern Australia and compare this to a ‘control’ group of *E. coli* isolated from feral pigs in north-western Australia that has likely had no antibiotic exposure. A survey was conducted in 22 commercial piggeries in South Eastern Australia (New South Wales n=9; Victoria n=10; South Australia n=3) from September 2013 to May 2014. For each herd faecal samples (n=50) were collected from pens both with and without evidence of diarrhoea and spread onto Sheep Blood Agar and CHROMagar Orientation to isolate *E. coli*. Sampling of feral pigs has been described previously (Ward et al., 2013. Veterinary Microbiology 162: 921-929). Domestic (n=325) (5 pre- and 10 post-weaned) and feral (n=115) pig β- and non-β-haemolytic *E. coli* isolates were randomly selected and screened against 19 veterinary antibiotics (including antibiotics not permitted for use in Australia) using the Sensititre™ according to veterinary CLSI and EUCAST guidelines. Domestic *E. coli* isolated were screened for enterotoxigenic (ETEC) genes (F4, F5, F6, F18, F41 and Stα, Stb, Lt) commonly associated with piglet diarrhoea. In our study, >99% *E. coli* from both domestic and feral pig populations showed resistance to the antibiotics clindamycin, penicillin, tiamulin and tilmicosin. Low levels of resistance to important antibiotics ceftiofur (17/325; 5%) and gentamicin (24/325; 7%), were observed only in isolates from commercial piggeries. Of the 17 resistant isolates 14 lacked ETEC genes suggesting that resistance to third generation cephalosporin antibiotics (3GC) is circulating at higher proportions within non-pathogenic *E. coli* isolates at the farm level. Our study estimates baseline resistance data needed for monitoring changes in animals’ husbandry and antimicrobial use. It also highlights the importance of non-pathogenic *E. coli* as reservoirs of antimicrobial resistance genes. Resistance to last line veterinary antibiotics such as ceftiofur is concerning given the mobility of genetic material between bacterial species. Additional analysis is required to determine if similar resistance patterns are seen in finisher pigs, due to their relevance to potential zoonotic transmission [Ward et al., Veterinary Microbiology 162: 921-929].

P11: Dairy production-related factors that affect amount of cows’ treatments with antibacterials for systemic and intramammary use

Marta Brscic¹, A. Azzolin², A. Scollo¹ and F. Gottardo¹
¹Department of Animal Medicine, Production and Health, University of Padova, Italy; ²Private Veterinary Practitioner, Italy
martabrsic@unipd.it

Antibacterial treatments use in dairy cows is frequently a consequence of deficiencies in animal husbandry and management. This study aimed at monitoring consumption of antibacterials for systemic and intramammary use in 7 dairy farms in the Veneto region and identifying the main relationships between their use and milk quality and production-related factors due to the increasing interests towards a controlled use of antimicrobials in livestock. Antibacterial consumption data were gathered over 4 semesters (October 2013 to September 2015) using specific software that allows electronic management of the veterinarians’ drugs dispensary and registered treatments records (GeFaVet®, Dr. Aldrovandi, Italy). Data were expressed as the ratio between the number of treatment days over the average number of dairy cattle present on the farm. Data regarding production (effective daily and standardised milk yield, numbers of lactations, of days in milk, of days open, and calving interval) and milk quality traits (fat and protein contents, linear somatic cell count) of the time intervals corresponding to the treatments were obtained from National Milk Records. Farms ranged from 49 to 223 cows (109 on average), and all were followed by and were under the responsibility of the same veterinarian to avoid confounding veterinarian effects. Results showed an average of 0.42 and 0.34 treatment days/cow/month of antibacterials for systemic and intramammary use, respectively. The selection of the
Orally administered antimicrobials increase antimicrobial resistance (AMR) in *Escherichia coli* (*E. coli*) from pigs. *E. coli* can be reservoirs of resistance genes transferable to other pathogenic and zoonotic bacteria. To limit AMR levels and hence to protect the therapeutic effectiveness of drugs in the treatment of infections in animals and humans, it is important to identify risk factors relating to AMR in pigs receiving oral antimicrobial treatments. Therefore, the aim of this study was to review previous literature and to identify risk factors in addition to antimicrobial treatment for AMR occurrence in commensal *E. coli* in pigs receiving antimicrobial treatment. Relevant explorative studies were searched according to keyword combinations from electronic databases and assessed against eligibility criteria in 2014 to 2016. Thirteen articles (nine on observational, four on experimental studies) were finally selected as relevant. Association with AMR occurrence was shown for risk factors out of the main categories herd/farm management, treatment management and unmodifiable (e.g., geographical, climatical) aspects. It was reported that space allowance, production size/stage, cleanliness, entry of animals and humans into herds, dosage/frequency/route of administration, time-span between treatment and sampling date, herd size, distance to another farm, coldness and season all had an impact on AMR occurrence. Associations were shown by one to four studies per factor and differed in magnitude, direction and level of significance. The risk of bias was unclear in nearly half of the information of observational studies and in most of the information from experimental studies. Overall, several factors indicated potential to contribute to reducing the risk for AMR in *E. coli* from pigs. Further research on the effects of specific management practices and on relationships between the factors is needed to develop well-founded management advice.

**P12: What causes antimicrobial resistance besides antimicrobial treatment?**

Elke Burow, B.-A. Tenhagen and A. Käsbohrer
Unit Epidemiology, Zoonoses and Antimicrobial Resistance, Department Biological Safety, Federal Institute for Risk Assessment, Germany
elke.burow@bfr.bund.de

Within poultry production, antimicrobials are a therapeutic instrument to control bacterial diseases, safeguarding food safety and animal welfare. However, with regard to antibiotics in livestock there is scarce information about residues in poultry faeces. In this matrix, antibiotics may persist in high concentrations after treatment, thus producing eco-toxicological effects over the biotic community and environment, and potential recycling into the trophic chain, becoming a public health. Considering this, studies quantifying the impact of antibiotics from animal faeces as environmental contaminants are needed. A controlled treatment study was performed in broiler chickens in order to quantify chlortetracycline (CTC) and 4-epi-CTC concentrations in droppings after treatment with a CTC pharmaceutical formulation for this species. Sixty one-day old broiler chickens were kept with *ad libitum* access to water and non-medicated feed. At day 15, animals were allocated in two experimental groups:
A (48 birds) treated with a therapeutic dose of CTC 20% during seven days, and B (12 birds) not treated. On days 5, 8, 11, 15 and 18 post-treatment, corresponding to days 36, 39, 42, 46 and 49 of the chickens' lifespan, faeces were collected in plastic bags and stored at -80°C until LC-MS/MS analysis (API 4000; Sciex). For sample processing, 2±0.02 g of faeces were spiked with tetracycline (TC-d6) as internal standard (Toronto Research Chemicals). Analytes were extracted from the matrix with EDTA-McIlvaine buffer and acetonitrile. Concentrations of CTC and 4-epi-CTC in faeces from treated broilers were quantified using matrix-matched calibration curves with certified purity standards (Dr. Ehrenstorfer GmbH), while TC-d6 was used as internal standard. CTC plus 4-epi-CTC average concentrations, obtained each on the sampled days are shown in Table 1. In conclusion, high levels of antibiotics quantified in manure from treated birds demonstrate the need to assess the impact of animal production over antibiotics' pollution and re-entrance into the food chain.

**P14: In-house validation of analytical methodology for the determination of florfenicol and florfenicol amine in poultry faeces by liquid chromatography-tandem mass spectrometry and evaluation of concentrations after oral treatment**

**Javiera Cornejo**¹, R. Riquelme¹, E. Pokrant¹, K. Yevenes¹, A. Maddaleno², C. Araya² and B. San Martin²

¹Preventive Medicine Department, Faculty of Veterinary and Animal Sciences, University of Chile, Chile; ²Laboratory of Veterinary Pharmacology, Faculty of Veterinary and Animal Sciences, University of Chile, Chile

jacornej@uchile.cl

The use of antimicrobials in poultry production systems is an effective tool for the control of infectious diseases. However, significant percentage of antimicrobials is excreted as bioactive compounds. Therefore, antimicrobial residues may remain in this by-product used for animal feed and mainly applied as supplement to fertiliser. Despite its use, depletion studies of florfenicol (FF) and florfenicol amine (FFA) in faeces from treated animals are scarce. The objective of this study was in-house validation of an analytical methodology for the determination of FF and FFA and evaluation of concentrations of these analytes in treated broiler chickens' faeces. Eighty birds were kept with *ad libitum* access to water and non-medicated feed. At day 6, the birds were allocated into two groups: group A (64 birds), treated with a therapeutic dose of FF 10% during five days, and group B (12 birds), kept as a control. Between 5 and 40 days after treatment, faeces samples were collected. Previously, the analytical methodology was validated. Specificity, linearity, accuracy, and recovery were assessed according to parameters established by Commission Decision 2002/657/CE; the limit of detection (LOD) and limit of quantification (LOQ) were set according to VICH GL49 of the FDA. For sample processing, 2±0.02 g faeces were spiked with internal standard (chloramphenicol-d5; Sigma-Aldrich). Analytes were extracted with water and acetone HPLC-grade; for clean-up, dichloromethane was used. Detected concentrations by LC-MS/MS were calculated by the equation from the linear regression analysis of the matrix-matched calibration curves with a coefficient of determination ($r^2$) above 0.95. LOD was set at 50 µg/kg for both analytes based on signal noise ratio; an LOQ of 52.2 and 60.5 µg/kg for FF and FFA was calculated, respectively. For the linearity, the matrix calibration curves (50, 100, 200, 300, and 500 µg/kg) showed an $r^2$ above 0.98 and 0.97 for FF and FFA, respectively. Recovery ranged from 95.9 to 101.5% and precision showed a coefficient of variation (CV) from 9.6 to 25.4%. Average concentrations (FF plus FFA) from samples were 568.35 and 136.23 µg/kg for day 5 and 10 post-treatment, respectively. As of day 15 post-treatment, the concentrations for both analytes were lower than the LOD. In conclusion, high levels of FF persist in the poultry faeces until 10 days post-treatment proving the need to assess the impact of animal production on antibiotics' pollution and the re-entrance into the food chain.

### Table 1. CTC and 4-epi-CTC concentration at different times (days) in faeces after oral administration of 20% CTC (30 mg/kg) per 7 days.

<table>
<thead>
<tr>
<th>Time post-treatment (days)</th>
<th>Chicken’s lifespan (days)</th>
<th>CTC and 4-epi-CTC average concentration (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>36</td>
<td>665.82</td>
</tr>
<tr>
<td>8</td>
<td>39</td>
<td>368.17</td>
</tr>
<tr>
<td>11</td>
<td>42</td>
<td>363.24</td>
</tr>
<tr>
<td>15</td>
<td>46</td>
<td>183.81</td>
</tr>
<tr>
<td>18</td>
<td>49</td>
<td>95.63</td>
</tr>
</tbody>
</table>

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P15: Development and in-house validation of analytical methodology for the determination of tylosin in broiler chicken feathers by high-performance liquid chromatography with diode-array detection

Javiera Cornejo¹, C. Carvallo¹, E. Pokrant¹, A. Maddaleno², C. Araya² and B. San Martin²
¹Preventive Medicine Department, Faculty of Veterinary and Animal Sciences, University of Chile, Chile; ²Laboratory of Veterinary Pharmacology, Faculty of Veterinary and Animal Sciences, University of Chile, Chile
jacornej@uchile.cl

In poultry production, antimicrobials are widely used for the treatment of diseases of bacterial origin. There are few studies on the effects of antimicrobial residues derived from their use in animal production. However, they can remain in products and by-products of treated animals. The feathers, as feather meal, can be used in animal feed of food producing animals, re-entering antimicrobial residues into the food chain, thus representing a risk to public health. Accordingly, the quantification of antimicrobial residues in treated poultry feathers is needed. In this study, a simple and sensitive analytical methodology for the determination and quantification of tylosin in broiler chicken feathers by high-performance liquid chromatography with diode-array detection (HPLC-DAD) was developed and validated according to an in-house validation protocol. Antibiotic-free broiler feathers were fortified with certified standards at five concentrations (50, 100, 150, 200, and 250 µg/kg). For the extraction of the analyte from the matrix, 5±0.05 g feathers were weighed, and 10 ml phosphate buffer pH 2.5, 15 ml methanol and 15 ml HPLC-grade water were added. The supernatant was filtered on a glass wool column, followed by sample clean-up with aromatic sulfonic acid (SCX) solid phase extraction cartridges (SPE), conditioned with 5 ml methanol and 5 ml phosphate buffer pH 4.0. Samples were reconstituted with 300 µl water: acetonitrile (50:50) and transferred to glass vials. Instrumental analysis was performed by HPLC-DAD, with a constant mobile phase flow of 1ml/min, oven temperature of 50°C and a wavelength of 287 nm. A Chromolith Performance RP-18e (100 mm x 4.6 mm; Merck) was used as analytical column. The limit of detection (LOD) at 50 µg/kg and limit of quantification (LOQ) at 64.1 µg/kg were set according to VICHGL49 (FDA). Parameters of linearity, recovery and precision were calculated according to Commission Decision 657/2002/EC. For linearity, the determination coefficient obtained by the method above was 0.97. Recovery ranged from 93.9 to 109.1%. Repeatability of the method varied from 1.7 to 8%, while the reproducibility varied from 2.4 to 11.1%. According to these results, the methodology met the acceptance criteria for in-house validation parameters, showing that it is precise and reliable for the detection and quantification of this analyte in feathers. Acknowledgements. Founding: FONDECYT Initiation into Research 11140530.

P16: Understanding the culture of antimicrobial use behaviours in agriculture: a quantitative study of UK pig veterinary surgeons and farmers

Lucy A. Coyne
Institute of Infection and Global Health, University of Liverpool, UK
l.a.coyne@liverpool.ac.uk

The prudent use of antimicrobials is essential to reduce selection pressure and to preserve efficacy and availability for use by veterinary surgeons. In the UK, the greatest amount of single species antimicrobial products sold for use in farm animals are for pigs; the majority of these products are for administration through medicated feedstuffs. The aim of this study was to determine the drivers and attitudes around antibiotic prescribing decisions and behaviours in the UK pig industry. A quantitative cross-sectional questionnaire study was used on a representative sample of pig veterinary surgeons (n=61) and farmers (n=261) across England, Wales and Scotland. Non-clinical factors such as practitioner confidence in the diagnosis, advice from senior colleagues and the veterinary surgeon-client relationship were considered in the veterinary surgeons’ decision whether or not to prescribe an antimicrobial. Whereas, clinical factors such as the number of animals requiring treatment, the length of course and ease of administration determined which antimicrobial formulation and class practitioners prescribed. Questionnaire responses indicated that 100% of veterinary surgeons and 94% of farmers considered veterinary surgeons to be quite or very important in terms of responsibility for prudent use of antimicrobials, and 84% of veterinary surgeons and 94% of farmers valued the role of the farmer. Farmers cited their veterinary surgeon as the most frequent source of information on antimicrobials. Veterinary surgeons and farmers both showed a high level of confidence that their own antimicrobial use behaviours were responsible, however there was concern that the practices of others in pigs, other species and in human medicine may be less responsible. Policy measures such as benchmarking of antimicrobial use and a penalty system for high antimicrobial use farms were considered to be beneficial.

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in reducing the total antimicrobial use in UK pigs by veterinary surgeons and farmers. However, the impact of a ban on the use of in-feed antimicrobials in pigs divided opinion between the populations with the majority of veterinary surgeons considering this to be beneficial (51%) and the majority of farmers identifying this as a barrier (48%). Gaining in-depth insight and understanding into the influences behind prescribing decisions can identify behaviours associated with over or inappropriate use. Such studies have been used in human medicine to identify potential interventions and assess their efficacy on promoting prudent use. It is hoped that by understanding prescribing practices in veterinary medicine better, similar interventions may be developed to promote the judicious use of antimicrobials.

P17: Multidrug resistance of avian pathogenic Escherichia coli (APEC) isolated from diseased chicken flocks in the Mekong Delta region of Vietnam

Nguyen Van Cuong, N.T. Nhрог, J. Campbell and J. Carrique-Mas
Clinical Research Unit, Oxford University, Vietnam
cuongnv@oucrv.org

Avian pathogenic Escherichia coli (APEC) is mostly associated with extra-intestinal infections of chicken flocks, which result in severe economic losses [Maryvonne Dho-Moulin, 1999. Veterinary Research 30: 299-316]. In the Mekong Delta region of Vietnam, chicken farmers typically use high quantities of antimicrobials resulting in high levels of antimicrobial resistance (AMR) among commensal E. coli [Carrique-Mas et al., 2015. Public Health 62 Suppl. 1: 70-78; Nhрог et al., 2015. Applied and Environmental Microbiology 81: 812-820; Nhrog et al., 2016. Applied and Environmental Microbiology 82: 3727-3735]. The aim of this study was to characterise the AMR patterns and virulence genes among APEC and environmental E. coli isolates from farms with chickens with systemic E. coli infection (i.e., colibacillosis). A total of 28 flocks were investigated. Birds (n=56) were examined for gross post-mortem lesions and relevant tissues were used for invasive E. coli detection, while faecal boot swabs (n=28) were collected in order to isolate commensal (mostly enteric) E. coli. The species identity of E. coli isolates was confirmed by MALDI-TOF. The Kirby-Bauer disc diffusion test was used to determine the strains’ antimicrobial susceptibility against a panel of 10 antimicrobials: amoxicillin/clavulanic acid (30 µg), ceftriazone (30 µg), ceftazidime (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), doxycycline (30 g), neomycin (30 g), amoxicillin (10 µg), ampicillin (10 µg), norfloxacin (10 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg), and streptomycin (10 µg) (Oxoid, UK). In addition, strains were investigated for the presence of five predictor virulence genes: iron, ompT, hlyF, iss and iutA [Johnson et al., 2008. Journal of Clinical Microbiology 46: 3987-3996]. A total of 33 invasive E. coli from 33 birds in 21 flocks, as well as 130 commensal E. coli strains from 28 flocks were investigated. In general, over 90% of both types of strains were resistant against amoxicillin, tetracycline and ampicillin. Between 40-90% of the strains were resistance against gentamicin, norfloxacin, streptomycin, trimethoprim/sulfamethoxazole and nalidixic acid. Less than 20% isolates were resistant against amoxicillin/clavulanic acid and ceftazidime. The proportion of E. coli strains containing at least one virulence gene was significant higher in invasive vs. commensal E. coli (30% vs. 6.0%, P<0.001). More than 60% of both types of strains contained 4 to 5 virulence genes indicating high pathogenicity of these strains. Overall, the resistant proportion for each antimicrobial of invasive strains was greater than that of commensal strains (absolute differences ranging from 2.9-22.4%), with the exception of trimethoprim/sulfamethoxazole. The results suggest that contamination of the environment with virulent E. coli strains is likely to contribute to further spread of infection. High levels of AMR in invasive strains suggest that controlling APEC should not rely on the use of antimicrobials alone.

P18: Quantitative assessment of antimicrobial resistance in livestock during the course of a nationwide antimicrobial use reduction in the Netherlands

Alejandro Dorado-Garcia1,2, D.J. Mevius2,3, J.J.H. Jacobs1,4, I.M. van Geijlswijk4,5, J.W. Mouton4,6, J.A. Wagenaar2,4,6 and D.J.J. Heederik1,4
1Department of Environmental Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, the Netherlands; 2Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, the Netherlands; 3Wageningen Bio veterinary Research, Wageningen University & Research, the Netherlands; 4The Netherlands Veterinary Medicines Authority (SDa) Expert Panel, the Netherlands; 5Department of Pharmacy, Faculty of Veterinary Medicine, Utrecht University, the Netherlands; 6Department Medical Microbiology and Infectious Diseases, Erasmus Medical Centre, the Netherlands
adoradogarcia@uu.nl

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The objective of this study was to quantify associations between antimicrobial use and acquired resistance in commensal indicator E. coli over a maximum of 10 years (2004-2014) comprising sector-wide antimicrobial use reductions from 2009 in broilers, pigs, veal calves and dairy cattle. Predicted resistance prevalences were given for a hypothetical further decrease in antimicrobials use. Data annually reported in the Netherlands in the resistance surveillance programme were retrieved. Two multivariate random-effects logistic models by animal sector were used to link total and class-specific antimicrobial use (as defined daily dosages per animal per year, DDDA/Y) with probability of resistance to a panel of 10 antibiotics. Results: positive dose-response relationships (ORs) were obtained from all the models. Total antimicrobial use was more related to resistances than class-specific use. Associations were remarkably robust in pigs and veal calves. Resistances to long used antimicrobials (e.g., penicillins, tetracyclines) were, in relative terms, less influenced by drug use changes over time than resistances to newer or less prescribed antimicrobials (e.g., 3rd/4th generation cephalosporins, fluoroquinolones). In pigs and veal calves, prevalences to most common resistances were projected to decline ~3-9% during 2014-2016 if total antimicrobial use reduction reached 80%; projections for poultry and dairy cows were more modest. In conclusion, drug use history and co-/cross-resistance selection were revealed as key elements for perpetuation of resistance. Antimicrobial use-resistance associations differed in size and significance by animal species reflecting sector-specific drug prescription regimes and structural differences. Reduction in antimicrobials was indicated to decrease resistance with greater impact in the pig and veal calf sectors.

P19: Disc diffusion antimicrobial susceptibility outcomes for bovine mastitis streptococci beta-lactams reveal an urgent need for evidence based breakpoints

M. Melchior1,2, H. Mulder1 and Monique Driesse2
1MBM Veterinary Diagnostiek, the Netherlands; 2Boehringer Ingelheim Animal Health, the Netherlands
monique.driesse@boehringer-ingelheim.com

For the prevention of the spread of antimicrobial resistance, the availability of generally accepted evidence based antimicrobial susceptibility breakpoints is crucial. In Europe, EUCAST is the main institutional source for such breakpoints. However, for various important and frequently isolated veterinary pathogens these breakpoints are not available, even not for the most often used antimicrobials in the market. In the Netherlands, first choice antimicrobials for Streptococcus spp. mastitis infections are penicillin and cloxacillin antimicrobials. The breakpoint for penicillin is evaluated according to EUCAST criteria with a benzyl penicillin disc (1 unit), however, no criteria are available for the oxacillin (1 μg) disc, which is the disc of choice for evaluation of the cloxacillin susceptibility. Currently veterinary laboratories are evaluating the susceptibility of S. uberis and S. dysgalactiae with a tentative breakpoint from the CLSI criteria for veterinary staphylococci (CLSI M31-A3). Rationale for this is the generally accepted relevance of this breakpoint for Staphylococcus spp. from bovine mastitis infections. It is previously shown by our colleagues from the Milk Control Center in Flanders (Belgium) that the susceptibility distribution for S. uberis and S. dysgalactiae shows a typical bimodal pattern for oxacillin, indicative for a wild type susceptible and a resistant population [Supré et al., 2016. Abstract EuroResidue]. Data from our laboratory from January 2014 to July 2016 confirm that a considerable number of S. uberis and S. dysgalactiae strains are evaluated as not susceptible for oxacillin, and thus cloxacillin, with the tentative breakpoint. In our lab, we found for S. uberis 4.1% and 73.1% of strains are resistant for penicillin and oxacillin, respectively. For S. dysgalactiae 0.8% and 40.8% of strains are resistant for penicillin and oxacillin, respectively. The high prevalence of resistance for cloxacillin in Streptococcus spp. is of concern given the fact that cloxacillin is routinely used as first choice antimicrobial for most mastitis cases where Gram-positive infections are suspected. Already in 2010, Haenni et al. [Journal of Medical Microbiology 59: 993-995; Antimicrobial Agents and Chemotherapy 54: 1140-1145] published about the decreasing susceptibility of S. uberis for oxacillin and other beta-lactams, including a description of the underlying mechanisms. The resistance is caused by a mutation of the penicillin binding protein (PBP2), which is the target for efficacious therapeutic effect of beta-lactams. Compared to previous data from our laboratory the prevalence of oxacillin resistance seems to be still rising, which indicate the urgent need for co-operation between stakeholders for the establishment of evidence based breakpoints. Without official breakpoints labs cannot report Streptococcus spp. as resistant, thus supporting treatment with inappropriate antimicrobials and the increase of antimicrobial resistance.
Biofilms are sessile communities of microorganisms and are the most common life-style of bacteria in the real world. Biofilm formation is a multistep process encompassing the formation of an extracellular polysaccharide matrix and significant changes in gene expression, transferring microorganisms ultimately into a dormant stage. In the meanwhile, it is well accepted that biofilm formation occurs on all biological surfaces, including organ cavities among others the urinary bladder, the internal lung surface, the mammary gland, the endocardium and major blood vessels, as well as the skin, ear, eyes and the oral cavity. In an established biofilm, microorganism, including bacterial and fungal species, are almost entirely resistant to common therapeutic approach, due to their low metabolic activity, including the common targets (such as membrane, protein and DNA/RNA synthesis) of antimicrobial agents. Moreover, recent evidence is increasing that within a biofilm, mobile elements (plasmids) encoding resistance genes, are rapidly exchanged among individual microorganisms, and hence biofilms seem to play a major role in therapy resistance and the spread of resistant bacteria. It becomes therefor essential in clinical practice to prevent the formation of biofilms as a measure to maintain and improve the efficacy of antimicrobials. With the aim to assess the efficacy of natural antimicrobial substances, derived from medicinal plants, we stabilised an in vitro assay system using biofilm-positive and biofilm-negative type strains of *Staphylococcus pseudoepedermidis* (SPE- and SPE+), which is an emerging pathogen and a representative of the important group of staphylococci. SPE+ and SPE type strains were grown on plastic surfaces and biofilm formation quantifies op optical density measurements and staining, as well as re-plating efficiency. Moreover, confocal laser scanning microscopy (CLSM) was used to visualise biofilm architecture and to quantify the effects of the plant extracts on the biofilms. Finally, a targeted array of genes involved in the different stages of biofilm formation was established, and changes in the expression of the genes monitored by quantitative PCR. With this experimental approach, we demonstrated the unique efficacy of different garlic extracts, standardised for their active compound allicin against SPE+ biofilms. Moreover, the anti-biofilm properties of extracts from 10 different herbs used in the Traditional Chinese Medicine were compared. The obtained results show a very specific potency of individual extracts, and confirm that the established biofilm model is a valuable screening tool for testing of anti-biofilm agents targeting Gram-negative bacteria.

In conclusion, the data presented here confirm the potential of feed additives with anti-biofilm properties for use as a preventive measure against pathogenic biofilm formation in the gut. However, further studies are required to determine the long-term effects of these products and their potential for the treatment of established biofilms.

### P21: Antibiotics and a feed additive related to gut health (SANACORE®EN) have distinct effects on digestive microbial composition in post-weaning pigs

C. Soler¹,², Tim Goossens², A. Bermejo² and L. Fraile¹,³
¹Departament de Producció Animal, ETSEA, University de Lleida, Spain; ²Nutriad International N.V., Belgium; ³AgrotecnioCenter, Spain

t.goossens@nutriad.com

In the past, antibiotics have been used extensively to prevent intestinal disorder in post-weaning pigs. However, as a result of ongoing efforts to stimulate the prudent use of antibiotics, alternative strategies to control intestinal health have gained considerable attention over the past years. This includes the use of certain feed additives. However, the underlying mechanisms by which these products exert their effect on animal health remain to be further characterised. In that context, we explored the effects of a feed additive containing encapsulated butyrate and propionate, medium-chain fatty acids, and essential oils on gut microbial composition and compared them with those of antibiotics. 30 post-weaning piglets were selected from a production farm with recurrent problems of post-weaning colibacillosis and received a diet with Zn oxide (3,000 ppm) during the first week after weaning. After this first week, they were split in three experimental groups: a control group, a group that received antimicrobials (amoxicillin and colistin sulphate), and a group fed the feed additive (SANACORE®EN; Nutriad). Faecal samples were taken at day 0, 15 and 30 and analysed for microbial composition by massive sequencing of V1 and V2 hyper-variable regions of the 16S RNA gene. Sequencing data were quality filtered, analysed and compared using appropriate statistical methods. For all treatment groups and time points, *Bacteroidetes* and *Firmicutes* were found to be the most dominant phyla. Interestingly, the relative contribution of these phyla to the overall composition was similar for the control and additive-treated group, while the antibiotic-treated group had a more deviant profile. Additionally, in the control and in the additive-treated group, but not in the antibiotic-treated group, species diversity increased substantially over time. Finally, the observed differences in bacterial composition and diversity was reflected by significant differences in the predicted functional profile of the microbial populations under investigation. In conclusion, the
composition of the digestive microbiota in post-weaning piglets changes profoundly over time, and is dependent on the use of antibiotics or feed additives. The results suggest that these product types have a distinct mode of action, with an opposite effect on gut microbial diversity. Given the importance of this diversity to intestinal health, these data warrant further investigation to the health promoting potential of SANACORE®EN. Overall, these results show the potential of gut microbial analysis in paving the way to a better understanding of feed additives used as alternatives for antimicrobials during the post-weaning period.

P22: Effect of replacing in-feed antibiotic growth promotants with organic acid-based feed additives on growth performance, health, and gut microorganisms of weanling piglets

S. Long1, X. Piao1, Y. Wu2 and Yanning Han2
1State Key Laboratory of Animal Nutrition, China Agricultural University, China; 2Trouw Nutrition R&D, the Netherlands
yanming.han@trouwnutrition.com

The widespread use of antibiotic growth promotants (AGP) in food animal production has raised serious concerns about the spread of antimicrobial resistance. Although prohibited in the European Union (EU), AGP is still commonly used in the Chinese swine industry, for both disease prevention and feed efficiency enhancement. This experiment was conducted to evaluate two products based on the different types and combinations of organic acids on growth performance and intestinal health in weaned pigs, in comparison with in-feed AGP. 180 weaned piglets (Duroc × Landrace × Yorkshire, average weight of 8.63±1.65 kg) were randomly allocated to one of five dietary treatments with six replicate pens (6/pen) per treatment. The experiment was divided into phase 1 (day 0 to 14) and phase 2 (day 15 to 28). The basal diets were supplemented with 3,000 and 400 ppm of zinc oxide in phases 1 and 2, respectively. The five treatments included a basal diet (control); AGP (providing 10 ppm zinc bacitracin, 5 ppm of colistin sulphate, and 5 ppm of olaquindox in feed); Selacid GG (short- and medium chain fatty acids, 3 kg/ton; Selacid); Presan-FX (medium chain fatty acids, butyric acid and a phenolic compound; 2 kg/ton; Presan); and a combination of Selacid (3 kg/ton) and Presan (2 kg/ton). The results showed that Presan significantly improved the body weight (BW), average daily gain (ADG) and feed efficiency (FE) compared with the control in phases 1, 2 and the overall period, while AGP only improved ADG and FE in phase 2 and the overall FE. Selacid or Selacid + Presan significantly improved the FE in all phases. Furthermore, Selacid, Presan, or their combination significantly decreased (P<0.05) the incidence of diarrhoea in piglets. Interestingly, all feed supplements tended to reduce the faecal E. coli counts, while Presan + Selacid tended to reduce the total anaerobic bacterial counts. The faecal short chain fatty acid concentrations were not impacted by the diets. In conclusion, both Presan-FX or Selacid GG were able to replace AGP completely in improving the growth performance and reducing the diarrhoea problem of the weaned piglets.

P23: Antibiotics for surgical prophylaxis in small animal veterinary practice in Australia

Laura Y. Hardefeldt, K. Bailey and G.F. Browning
National Centre for Antibiotic Stewardship, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Australia
laura.hardefeldt@unimelb.edu.au

Antibiotics are widely used in Australian veterinary practices, however, there has been no investigation into the classes of antibiotics used, the appropriateness of drug doses and the duration of therapy for surgical prophylaxis. In addition, since the introduction of antimicrobial guidelines for a limited number of small animal conditions by the Australian Infectious Disease Advisory Panel (AIDAP) in 2013, there has been no investigation of compliance with these guidelines. This study investigates antibiotic use for surgical prophylaxis across small animal practice in Australia and assesses compliance with AIDAP guidelines for selected small animal conditions. An anonymous online survey was developed to evaluate surgical antimicrobial prophylaxis practices of Australian veterinarians. Respondents were self-selected, and were encouraged to respond through print and electronic media, social media, and through recruitment at a selection of major Australian veterinary conferences in 2016. The survey consisted of 5 sections. The initial section asked for demographic information such as year of graduation, number of veterinarians employed in the practice, location of practice (rural or metropolitan), type of practice (general, emergency, etc..), and gender. Sections 2, 3 and 4 required respondents to indicate the frequency with which they use antibiotics for specific surgical conditions for each of 3 categories of practice (small animal, bovine, equine). Dose, time of antibiotic initiation and duration of therapy were also requested. Practitioners were only required to complete those sections relevant to the species they
One of the measures to reduce use of antibiotics in animal production is to use specific feed and water additives for improving feed and water hygiene and for support of the mucosal barrier function in the gastrointestinal tract of the host. However, large scale studies reviewing the impact of this strategy are scarce. The objective of our study was to carry out a retrospective analysis of the potential contribution feed and water additives may have had in reducing antibiotic usage at broiler farms. The study was carried using data of 339 production cycles, 29 million broilers of 11 farms over a period of 5 years obtained from a provider in broiler farm management software and farm consultancy. In the first period (2010, period Control (C), at least 2 cycles), farms applied ‘common’ practices. In the second period, practices were optimised in feed programme, climate control and biosecurity (2010-2011, period Best Practice (BP), at least 3 cycles). During these first 2 review-periods some feed additives were already used but not water acidifiers. In the third period (2011-2015; best practice + additive period: BP+A), either an organic acid blend was applied to control microbiological quality of supplemented wheat (Fysal 2 kg/MT wheat; 8 of 11 farms) and/or a feed additive to support the mucosal barrier function (Presan, 1 kg/MT feed; 4 of 11 farms). All farms applied acidifiers in the drinking water (Selko pH, dose range 1 to 2 l/1000 l). FCR was significantly lower in the BP+A period compared to the C and BP period (1.60 versus 1.69 and 1.68, respectively). Mortality was better in the BP and BP+A period compared to the control period (resp. 2.4 and 2.6% versus 3.5%). Antibiotic usage was especially reduced in the BP+A period (34.1 mg/kg live broiler compared to 83.9 and 75.6 mg/kg in the C and BP period, a reduction of 59% compared to C). In combination with the effect of the management measures taken by the farmers, it can be concluded that the lowest use of antibiotics and the best performance in period BP+A coincided with the application of feed and water additives targeting feed and water hygiene and support of mucosal barrier function.

P25: Identification of blaCMY-2 and blaCTX-M-1 carrying Escherichia coli in slaughterhouse broilers and broiler meat in Finland – whole genome sequencing reveals high genetic diversity with multiple MLST types

Annamari Heikinheimo, S.-M. Latvio, M. Päiväranta and M. Fredriksson-Ahomaa
Department of Food Hygiene and Environmental Health, University of Helsinki, Finland
annamari.heikinheimo@helsinki.fi

Extended spectrum betalactamase/AmpC producing Escherichia coli (ESBL/AmpC E. coli) causes increasingly human infections worldwide, evidenced by global emergence of highly pathogenic clone of blaCTX-M-15 carrying E. coli ST131. Increasing carbapenem and colistin resistance in these bacteria further complicates treatment of these infections. Poultry is considered as a reservoir for ESBL/AmpC E. coli in many countries, also in Finland. However, the occurrence of ESBL/AmpC E. coli in Finnish broilers is low, maybe partly due to the Finnish concept of raising broilers without antibiotics. The aim of the study was to assess the occurrence and genetic diversity of ESBL/AmpC E. coli in Finnish broilers and broiler meat. Altogether, 520 broiler caecal swabs were enriched in Mueller-Hinton (1 ml) with cefotaxime (1 μg/ml) at 37°C for 20-24 h and 85 broiler meat retail samples (25 g) were enriched in buffered peptone water (225 ml) at 37°C for 18-22 h. Samples were cultivated on MacConkey agar with cefotaxime (1 μg/ml) and incubated at 44°C for 18-22 h. Typical colonies were identified as E. coli with Gram-staining, API20E and Oxidase test. Disc diffusion was used to define susceptibility profiles. EUCAST epidemiological cut-off values were used to determine resistance. A collection of ESBL/AmpC E. coli isolates (n=10; 5 from caeca, 5 from meat) were further subjected to DNA purification and whole-
After the antibiotic growth promoter (AGP) ban, an increased interest for alternative to antibiotics occurred. This study aims to check if a copper-zeolite compound included at low dose could impact
broiler microflora composition similarly to AGP. 105 one-day old chickens were allocated to 3 treatments for 21 days: a control group (CG), a group fed CG with 6 g/t of copper-zeolite B-SAFE (CZ), and a group fed CG with zinc bacitracin at 30 mg/l in drinking water (ZB). At the end of the trial, 12 chickens per treatment were randomly selected weighed and slaughtered. Their small intestine was carefully sampled from gizzard to caeca, frozen and then stored at -80°C. They were then subsequently analysed for morphology (weight, length and diameter of the duodenum, jejenum and ileum subsections) and microbiota (whole small intestine DNA was extracted and 16S rRNA amplification and sequencing performed). The microbiota was analysed and microbial population dispersion was visualised using subsystem technology. Ecology diversity was observed and taxonomic identification performed [Le Gall-David et al., PLoS ONE, under review]. Birds from CZ and ZB had higher live weight than CG animals (809 and 852 vs. 762 g, respectively ($P<0.05$)). CZ and ZB had increased small intestine length and weight vs. CG ($P<0.05$). Jejunum was the small intestine subsection the most impacted by the treatments. Jejunum diameters of CG were significantly smaller than the ones of CZ and ZB. Jejunum and ileum lengths were improved in CZ group. CZ, ZB and CG had 3 different bacterial populations. The predominant phylum in each group was *Firmicutes* accounting for 90% of all sequences; however, it represented 99% for the CZ group. *Firmicutes* were dominated by the *Lactobacillus* genus. While CZ samples were enriched in lactobacilli compared to CG, ZB showed a *Lactobacillus* reduction. *Proteobacteria* (*Shigella* and *Escherichia*) were mostly present in ZB samples. ZB treatment resulted in the depletion of *Lactobacillus johnsonii*, absence of *Streptococcus* group *bovis* and development of enterobacteria, *Clostridium bifermantans*-like and *Enterococcus faecium* groups. CZ animals showed a reduced complexity of their microbiota with an increased and exclusive presence of *Lactobacillus johnsonii* and *reuteri*. The use of CZ at low dose and ZB at subtherapeutic level clearly influenced gut morphology and microbiota of broiler chickens. Microbiota composition modification between CZ and ZB indicate a different mode of action of the 2 products on the microflora, opening need feed additive alternative opportunities to AGP replacement.

**P28: Responsible use of fluoroquinolones in farm animals; a mandatory prerequisite**

L. Klostermann, **Anno de Jong**, B. Stephan, S. Tennagels, J.R. Vazquez, B. Martin, C. Christensen and Bernhard Stahl
Bayer Animal Health GmbH, Germany
anno.jong@bayer.com

Effective antimicrobial drugs, including innovative classes of compounds such as fluoroquinolones, should be available in veterinary medicine in order to safeguard animal health and well-being, and to provide healthy animal products for the consumer. Reducing the availability of therapeutic medicines to the veterinarian will have a significant detrimental impact on animal health and may in turn increase the risk of pathogen transmission to humans, thereby ultimately impairing public health. Every use of antibacterial products, both in human and veterinary medicine, presents the possibility for selection of resistant bacteria that can be transferred from animals to humans, from humans to humans, from animals to animals, and also from humans to animals. Furthermore, bacteria can be transferred from animals to humans via the food chain, the degree of which and the associated risks for the consumer continue to be a source of controversy in the scientific community. To minimise the risk of resistance selection during veterinary therapeutic use and to safeguard the future utility of the fluoroquinolone class of antibacterials in veterinary and human medicine, Bayer Animal Health is committed to promoting the prudent use of Baytril® (enrofloxacin) in farm animals as outlined in the ‘Principles for the Responsible Use of Fluoroquinolones in Veterinary Medicine’, first published in 1998 and latest revised in 2014. Specifically, fluoroquinolones should be administered on prescription-only and under the supervision of a veterinarian. Only authorised fluoroquinolones should be used, in strict accordance with the terms of their marketing authorisations. A diagnosis should, whenever possible, be supported by bacterial culture and susceptibility testing. Records of all antimicrobial usage in farm animals should be kept by the veterinarian or the farmer. Fluoroquinolones should not be used as performance enhancer/growth promoter, in aquaculture, as in-feed medication, for viral or trivial bacterial infections or for routine prophylaxis. Monitoring the susceptibility status of major target animal and zoonotic bacterial pathogens to fluoroquinolones should be mandatory for all companies marketing this class of antimicrobials. Adherence to the above guidelines is regarded essential for ensuring therapeutic efficacy while at the same time minimising selection of resistance in animal pathogens and safeguarding the utility of the fluoroquinolones for treating infectious diseases in humans and animals.
P29: Promoting of prudent use of antimicrobials in food-producing animals – experience from Finland

H. Helin-Sollevaara1, S. Nykäsenoja1, K. Kivilahti-Mäntylä2, L. Suojala3 and Liisa Kaartinen1
1Finnish Food Safety Authority (EVIRA), Finland; 2Finnish Medicines Agency (Fimea), Finland;
3Finnish Central Union of Agricultural Producers and Forest Owners MTK, Finland
liisa.kaartinen@evira.fi

Overall consumption of antimicrobials for food-producing animals in Finland is among the lowest in the European countries. For decades, our aim has been to reduce the need of antimicrobials instead of reducing their use. The focus has been on the control of zoonoses and animal diseases and herd health programmes. Also, making profit on sales of medicines by veterinarians has been banned and antimicrobials for systemic treatment has been subject to veterinary prescription since 1949. Systematic resistance monitoring has been carried out in Salmonella species since 1983 and FINRES-Vet programme covering resistance monitoring in major zoonotic and indicator bacteria was initiated in 2002. The resistance in zoonotic bacteria and in many animal pathogens is still moderate or low. For example, despite the peak in resistance in 2014 among Campylobacter jejuni from broilers, the resistance levels have remained favourable [EFSA Report 2016]. Sales of antimicrobials has been published annually since 1995. Total sales of antimicrobials for food-producing animals in Finland is low (ESVAC data). Population corrected sales for 2010-2014 has been approximately 22 mg/PCU. Beta-lactamase sensitive penicillins were the most used antimicrobials (40 % in 2014), followed by tetracyclines (23%) and sulphonamides (20%). Over half of the antimicrobials were given as individual treatment, while the proportion of group treatment was 42%. The legislation emphasises the role of the veterinarian; making a clinical diagnosis and microbiological analyses are a prerequisite for an antimicrobial treatment especially in group treatments and recurrent infections. Restrictions are set for the use of human critically important antimicrobials (HCIA). Indication-based recommendations for the use of antimicrobials in animals have been in place since 1996. The latest revision was published in May 2016. Veterinarians are obliged to take into account the official recommendations and guidelines on the use of antimicrobials when making the treatment decision. The national antimicrobial policy is strongly supported by Finnish farmers’ organisation MTK. Co-operation between all sectors is necessary to ensure continuous vigilance in prudent use, training and dissemination of information as well as measures to increase biosecurity and disease controls on farms. The national target is to maintain this low-level consumption also in the future although the structure of animal production is changing.

P30: In vitro evaluation of synergetic effects between commercial mixtures of essential oils and antibiotics on strains of Escherichia coli, Clostridium perfringens and Pasteurella multocida

Sylvain Kerros
Phytosynthese, France
sylvain.kerros@phytosynthese.com

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. It became crucial to find new drugs in order to maintain breeding production performance and plant extracts are now proven as an efficient alternative. This study has been done to evaluate the MIC of different commercial blends of essential oils alone, different antibiotics alone and then the investigation of synergism between these plant extracts and drugs. The protocol used was a well microplate dilution method; MIC was evaluated by spectrophotometry. Each result has been confirmed by an agar diffusion test. This study was conducted against three different bacteria. Escherichia coli from pig; Clostridium perfringens and Pasteurella multocida from poultry. 6 different blends of essential oils were tested (M1: 100,000 ppm aromatic aldehydes; M2: 50,000 ppm aromatic aldehydes and 55,000 ppm phenols; M3: 45,000 ppm aromatic aldehydes, 12,500 ppm phenols, 3,000 ppm terpenic alcohols and 1,500 ppm diallyl sulfides; M4: 20,000 ppm monoterpenes, 10,000 ppm phenols and 100,000 ppm cyclic ethers; M5: 20,000 ppm monoterpenes and 115,000 ppm cyclic ethers; M6: 110,000 ppm aromatic aldehydes, 15,000 ppm phenols and 3,500 ppm diallyl sulfides). Antibiotics of different families were chosen (polymixins: colistin sulphate salt; quinolones: oxolinic acid; aminosides: neomycin trisulphate salt hydate; pleuromutilins: tiamulin fumarate and tetracyclins: oxytetracycline HCl). An example of results shows that the MIC of colistin alone against E. coli is 0.5 mg/l. The MIC of M6 alone against E. coli is reached at a dilution of 1/4096. The MIC of M6 combined with colistin at the dose of MIC/2 (0.25 mg/l) is this time reached at the dilution of 1/16384. The effective dilution corresponding to the MIC of M6 when combined with a half effective dose of colistin was quartered demonstrating a strong synergetic effect. The results of the conducted experiments show that
several mixtures of essential oils showed in vitro synergisms with antibiotics, generally with an improvement of 2 dilutions. It suggests that their combinations could minimise antibiotic application concentrations and consequently reduce any adverse impact such as resistance. However, this study needs to be further investigated in order to establish an exhaustive list of ‘triplets’ bacteria/antibiotic/essential oil showing synergetic effects [Adwan & Mhanna, 2009. Asian Pacific Journal of Tropical Medicine 2: 46-51; Silva & Fernandes Jr., 2010. Journal of Venomous Animals and Toxins including Tropical Diseases 16: 402-413; Zago et al., 2009. Revista Brasileira de Farmacognosia 19: 828-833].

P31: Antimicrobial susceptibility of Escherichia coli and Salmonella isolates from healthy pigs in Australia: results of a pilot national survey

Amanda Kidsley1, S. Abraham2, J.M. Bell1, P. Mitchell3 and D. Trott1
1Australian Centre for Antimicrobial Resistance Ecology, University of Adelaide, Australia; 2Murdoch University, Australia; 3Australian Pork Limited, Australia
amanda.kidsley@adelaide.edu.au

The use of antimicrobials in food-producing animals is deemed to be important for animal health, welfare and efficient production. In Australia, use of antimicrobials in livestock production is heavily regulated, with critically important drugs highly restricted. For example, Australia is the only country that has legal measures in place to exclude the use of fluoroquinolones and gentamicin in food-producing animals. Additionally, by international comparison, label constraints for the use of third generation cephalosporins in Australian livestock are very strict. While a national antimicrobial resistance surveillance programme focused on animals in Australia has only just commenced, a number of one off surveys conducted in recent years have confirmed a low public health risk in the food animal sector in relation to resistance to critically important drugs such as fluoroquinolones. The aim of this pilot study was to investigate the prevalence of antimicrobial resistance among Escherichia coli and Salmonella spp. isolated from the gut of Australian finisher pigs at slaughter and characterise any isolates resistant to critically important drugs. E. coli and Salmonella spp. were isolated from caecal samples collected from healthy finisher pigs at slaughter in Australian pigs (2015). Isolates were identified using MALDI-TOF analysis. Minimum inhibitory concentrations were determined by broth microdilution using commercially prepared dry-form panels (Sensititre™ CMV3AGNF NARMS; Trek Diagnostic Systems, Thermofisher Scientific), and interpreted using CLSI VET01S (2015) guidelines or epidemiological cut-off values (EUCAST) where no interpretative criteria were available. E. coli (n=203) showed the highest levels of resistance to amoxicillin (60.1%), tetracycline (68.5%), chloramphenicol (47.3%) and trimethoprim/sulfamethoxazole (34%). In comparison, Salmonella spp. (n=69) also showed the highest levels of resistance to amoxicillin and tetracycline, albeit at much lower percentages (20.3% and 26.1%, respectively), and much lower levels of resistance to trimethoprim/sulfamethoxazole (11.6%) and chloramphenicol (7.2%). Low levels of resistance were observed in both species to amoxicillin/clavulanate (E. coli 9.4%; Salmonella 2.9%) and gentamicin (E. coli 0.5%; Salmonella 2.9%). No ceftiofur resistance was observed in either E. coli or Salmonella spp. Two E. coli had ciprofloxacin MICs of 8 mg/L which is above the wild-type ECOFF. Six isolates of interest are currently undergoing whole genome sequencing analysis to identify phylogenetic group, serogroup, multilocus sequence type and virulence/antimicrobial resistance genes. Overall, low levels of resistance were detected to critically important antimicrobials. The two E. coli
Antimicrobial resistance is a phenomenon of great concern worldwide because of its consequences on both human and animal health, and negative economic impact. For these reasons it is important to monitor resistance in zoonotic strains, such as food-borne Salmonella. In Chile, there are currently no programmes for monitoring resistance in food-producing animals. The objective of this study was to determine the prevalence and susceptibility to clinically important antibiotics in Salmonella spp. strains, isolated from mesenteric lymphatic nodes of swines intended for human consumption. Six slaughtering plants were sampled. Thirty samples from different pig mesenteric lymph nodes (no more than 10 samples per lot), with a total of 180 samples were taken. The isolation was performed according to ISO 6579. All the isolated strains were analysed by the minimal inhibitory concentration technique (MIC) described by the Clinical and Laboratory Standards Institute [CLSI, 2012] for the following antibiotics: ceftazidime, nalidixic acid, tetracycline, ampicillin, amoxicillin + clavulanic acid, chloramphenicol, sulfizoxazole, sulfamethoxazol + trimethoprim, streptomycin, gentamicin, trimethoprim, and erythromycin. E. coli ATCC 25922 was used as quality control. 33 Salmonella strains were isolated from 180 mesenteric lymph nodes of pigs, indicating a prevalence of 18.3%. For these isolated strains, the percentages of resistance were: ceftazidime, 0%; nalidixic acid, 54.5%; tetracycline, 72.7%; ampicillin, 0%; amoxicillin + clavulanic acid, 0%; chloramphenicol, 3%; ciprofloxacin, 0%; sulfizoxazole, 63.6%; sulfamethoxazole + trimethoprim, 3%; streptomycin, 100%; gentamicin, 12%; trimethoprim, 3%; and erythromycin, 0%. 12 strains were multi-resistant (resistance to 3 or more antibiotics) and one strain showed resistance to 8 antibiotics simultaneously, the resistance profile of this strain was: nalidixicacid/tetracycline/chloramphenicol/ sulfizoxazole/ sulfamethoxazole + trimethoprim/streptomycin/ gentamicin/trimethoprim. It is important to note the high percentage of resistance to quinolone nalidixic acid, which exceeded 50%. In conclusion, the results show the importance of establishing an integrated AMR surveillance system within the framework of responsible use of antibiotics stewardship in pig production in Chile. This would allow monitoring the trends of antibiotics susceptibility, especially in the case of quinolones and fluoroquinolones, tetracyclines and aminoglycosides. All these drugs are used in the treatment of human patients with complicated salmonellosis infections.
food production. Accordingly, most economies take actions to carry out a surveillance system of antimicrobial resistance only in the human health area, and only in the catalogued as ‘Advanced economies’ these actions are also extended to the animal health area.

P34: Comparison between yeast administration at different timing on health status of weaned pigs in response to Escherichia coli F4ac infection

Diana Luise1, D. Priori1, V. Motta1, E. Auclair2, P. Bosi1 and P. Trevisi1
1Department of Agricultural and Food Science, University of Bologna, Italy; 2Phileo-Lesaffre Feed Additives, France
diana.luise2@unibo.it

In pigs, the multitude of stressors related with the weaning exacerbates the overgrowth of enteropathogens that are directly involved in the occurrence of the post-weaning diarrhoea (PWD). To reduce the use of antibiotic therapy for PWD, the prophylactic administration of live yeast seems to be effective. This study aims to disclose the application of Saccharomyces cerevisiae CNCM I-4407 (Sc), supplied at different patterns, to contain the detrimental effect of enterotoxigenic Escherichia coli F4ac (ETEC) in weaned pigs. Sixty piglets weaned at 24 days of age (d0) were allotted to one of following groups for 21 days: control (CO), CO+colistin, 1 g/kg feed (AB), CO+5x10^10 cfu Sc/kg feed, from d0 to d21 (PR), CO+5x10^10 cfu Sc/kg feed from d7 (infection with ETEC) to d11 (CM). After orally inoculation with ETEC (d7), 5 pigs/group were slaughtered 24 h later. Pigs were sampled for blood and for the jejunum mucosa (d8). The faecal consistency was daily determined by visual appraisal (1=hard, 5=watery). The growth performances did not differ between groups, but the CO group showed higher mortality than the other groups. Colistin protects the pigs against ETEC infection completely, while Sc supplementation compared with the CO group reduced the severity of diarrhoea until 48 h after infection (P=0.04). Furthermore, the blood metabolomic profile, showed that 24 h after infection yeast reduced the sphingomyelin-ceramide and decadienyl-L-carnitine concentrations (P=0.02), which play a key role in inflammation, confirming a positive Sc-host interaction in reducing the pathogenicity of ETEC. On the other hand, PR and AB diets down-regulated several genes directly associated with inflammation, while the CM group did not completely prevent the activation of genes involved in mucosal inflammation. In summary, the prophylactic use of Sc reduces the detrimental effect of ETEC infection; otherwise, a brief administration of Sc, from the day of infection, slightly affects the negative effect of ETEC, even if some positive signals have been observed. The competitive strategy offers the perspective to develop a new generation of probiotics, already effective shortly after the infection, for a most targeted use against the pathogen in the post-antibiotic era.

P35: FDA’s regulatory approach to preserving the safety and effectiveness of medically important antimicrobials used in food-producing animals

Ron A. Miller1, R. Singh1, H.C. Harbottle1, S.A. Piñeiro1, S.S. Yan1, J.M. Gilbert1 and W.T. Flynn2
1Division of Human Food Safety, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, U.S. Food and Drug Administration, USA; 2Office of the Director, Center for Veterinary Medicine, U.S. Food and Drug Administration, USA
ron.miller@fda.hhs.gov

Medically important antimicrobials (MAs) are used in veterinary medicine to treat, control, and prevent diseases in food-producing animals. In addition, some MAs are currently approved for production purposes (e.g., increased rate of weight gain or improved feed efficiency). All antimicrobial uses carry a risk of antimicrobial resistance (AMR) development; however, production uses may particularly be a contributing factor in the rise of AMR due to their frequent administration to entire herds or flocks at low doses and for prolonged durations. To address these concerns, the FDA has improved its post-approval risk management options through urging sponsors to convert their production claims to therapeutic claims under veterinary oversight, and by requiring sponsors to submit annual reports of antimicrobial drugs they sell or distribute for use in food-producing animals by total active drug ingredient and by animal species. Further, prior to approval of new animal drugs, the FDA has mitigation strategies available to reduce the potential risk of foodborne illness caused by AMR bacteria that are attributable to an animal-derived food commodity. These commonly include making a proposed product prescription-only or under a veterinary feed directive (VFD), extra-label use (ELU) restrictions, restricting the extent of use by limiting the number of animals that can be targeted for treatment, and post-approval monitoring for resistance development. FDA thinks using MAs judiciously is critical to both minimise the selection and dissemination of AMR bacteria from food-producing animals and to help preserve their effectiveness.
P36: A comparison of clinical and economic outcomes when metaphylactically administering either a novel DNA immunostimulant or tilmicosin to beef calves at medium – high risk of developing bovine respiratory disease in the feedlot

Jason Nickell1, D. Keil1, T. Settje1, K. Rogers2 and D. Miles2
1Bayer Animal Health, USA; 2Veterinary Research and Consulting Service, LLC, USA
jason.nickell@bayer.com

Bovine respiratory disease (BRD) is a major source of morbidity and production loss in the beef and dairy industries. The objective of this study was to evaluate the efficacy of a novel DNA immunostimulant (Zelnate®), labelled to aid in the treatment of BRD, versus the currently approved antibiotic tilmicosin (Micotil®) when administered for the control of BRD in feedlot cattle. The inclusion criteria consisted of healthy cattle who were categorised as being of medium to high risk for the development of BRD by the Investigator and the participating feedlot. On day one, 2,004 calves were enrolled into the study population (tilmicosin, n=1002; DNA Immunostimulant, n=1002). All calves were followed for 56 days. On day 57, calves remaining on the study were weighed and subsequently eligible to return to commerce. Descriptively, across both treatment groups, BRD morbidity, BRD repulls, BRD chronicity, overall BRD mortality, and BRD case-fatality estimates were observed to be 10.7, 18.3, 29.4, 0.6, and 3.3%, respectively. Overall, the raw BRD morbidity estimates for tilmicosin (7.65%) and the DNA immunostimulant (13.84%) were evaluated for non-inferiority based upon a 10% margin of difference (determined a priori) of having no clinical significance. The inferential statistical analysis performed on the difference (-6.19) observed a 95% confidence interval of -8.91 to -3.47%. Therefore, the BRD incidence of the DNA immunostimulant was observed to be non-inferior to tilmicosin (lower bound within 10% margin of difference). The time to first BRD diagnosis was significantly less for the DNA Immunostimulant (22.6 days) compared to tilmicosin (28.1 days). No significant differences (P>0.05) between the treatment groups were observed in the remaining study outcome variables: BRD repulls (tilmicosin=17.9%, DNA Immunostimulant=11.1%), BRD chronicity (tilmicosin=27.9%, DNA immunostimulant=29.1%), overall BRD mortality (tilmicosin=0.44%, DNA immunostimulant=0.50%), BRD case-fatality (tilmicosin=3.95%, DNA immunostimulant=2.99%), average daily gain (tilmicosin=2.96 lbs/day, DNA immunostimulant=2.91 lbs/day), dry-matter intake (tilmicosin=12.96 lbs/day, DNA immunostimulant =12.81 lbs/day), and feed:gain (tilmicosin=4.50, DNA immunostimulant=4.55). In this study, the DNA immunostimulant was shown to be a viable non-antibiotic option for metaphylaxis in classes of cattle like that reflected in the current study population. No adverse events were observed in this study.

P37: Development of a new bioluminescent murine model of bacterial wound infections

Abiodun D. Oggunyi1, Z. Kopecki2, E.E. Hickey1, M. Khazandi1, A.J. Cowin2, S.W. Page3 and D.J. Trott1
1Australian Centre for Antimicrobial Resistance Ecology, School of Animal and Veterinary Sciences, The University of Adelaide, Australia; 2Future Industries Institute, The University of South Australia, Australia; 3Advanced Veterinary Therapeutics, Australia
david.oggunyi@adelaide.edu.au

Skin wounds, such as skin blisters and burned skin, provide an ideal environment for infection by bacteria that are either resident on the skin surface or from the environment. Alarmingly, infection of skin wounds by pathogenic bacteria that are resistant to multiple classes of antimicrobials account for massive morbidity and mortality in both animals and humans worldwide, of which coagulase-positive Staphylococcus spp. is a leading cause. However, animal models for detailed investigation of disease progression in a scenario that mimics skin infections in animals and humans are few and often involve using separate cohorts of animals at each stage of the disease process. Here, we have developed and optimised a bioluminescent (light-emitting) model of bacterial infection of partial-thickness scald wounds in mice by topical infection with a recombinant luciferase-expressing Staphylococcus aureus strain Xen29. Mice were infected with Xen29 two days post-scald wounding after which separate groups were treated twice daily with either a 2% topical mupirocin cream or PBS control, over a 7-day period. We obtained consistent and reproducible bacterial burden data from individually-infected mice by daily quantification of photon intensity on a Xenogen IVIS Lumina XRMS Series III live animal biophotonic imaging system, with concomitant significant reduction in photon intensities in mupirocin-treated mice. Post-mortem analyses show the bacterial burden of wounds correlated strongly with the total flux obtained from the bioluminescent signals of Xen29. We propose that this new bioluminescent model of bacterial wound infection has the potential to replace laborious, time consuming and costly microbiology techniques associated with harvesting, plating and colony counting of bacteria derived from infected mice. The model will also allow an assessment of the dynamics of multiple bacterial infection of the skin.
Foodborne diseases are a widespread and growing public health and economic concern worldwide, and developing effective methods for reducing or eradicating microbial spoilage and foodborne pathogens as alternatives to using chemicals or antibiotics is important for the food industry and of high global priority. We have evaluated the efficacy of a HOCl-containing water sanitisation product (Electro-Chemically Activated Solution 4 (ECAS4), synthesised with the patented ECAS4 technology) in extending the shelf life of the Southern Australia King George Whiting (KGW) and Tasmanian Atlantic Salmon (TAS) fillets. This was carried out by washing fillets with ECAS4 solution containing chlorine level of either 45 ppm (15%) or 150 ppm (50%) and enumeration of the bacterial species on selective and non-selective media, followed by identification of pure isolates by 16S rRNA gene sequence analysis. We found that ECAS4 at either concentration significantly reduced total bacterial load and specific spoilage organisms on KGW and TAS fillets throughout storage and significantly extended the shelf life of the KGW and TAS fillets by 2 and 4 days, respectively. The two-day difference between the extended shelf lives of ECAS4-treated KGW and TAS reflects the divergent microbiota of the two fish populations, with the KGW having a higher microbial population compared to TAS. The significant increase in shelf life and quality of fillets was corroborated by raw and cooked sensory evaluation. These outcomes suggest that the use of ECAS4 sanitisation is highly likely to have a profound impact on the overall food industry, and should translate into health and economic benefit through reduction and/or elimination of food spoilage bacteria without the use of antibiotics, thereby reducing potential for antimicrobial resistance development. It will also fortify engagement with industry and other stakeholders, leading to paradigm shifts in policy implementation.

P39: Antibiogram, virulence traits, and genetic relatedness of methicillin-resistant Staphylococcus aureus isolated from bovine mastitic milk in Korea

Department of Veterinary Microbiology, College of Veterinary Medicine, Seoul National University,
Republic of Korea
magic007@snu.ac.kr

Staphylococcus aureus is a major etiological pathogen for bovine mastitis, foodborne illness, and various clinical infections. Methicillin-resistant S. aureus (MRSA) has been isolated from bovine mastitic milk, and the presence of MRSA in milk is a major public health concern. Here, we investigated the frequency of MRSA isolation from mastitic raw milk in Korea and characterised the patterns of antimicrobial resistance, virulence, and genotypes of these MRSA. 1,222 raw milk samples were collected from 47 dairy farms in Gyeonggi province from 2011 to 2012. Of these samples, 643 were determined as mastitic milk based on somatic cell counts (SCCs) of more than 200,000 SCC/ml, and 165 S. aureus (25.7%) were isolated from these samples. Of them, 23 S. aureus (13.9%) collected from five different farms were confirmed as MRSA by detection of the mecA gene. Disc diffusion and MIC tests for antibiotic resistance revealed that all MRSA isolates had resistance to four or more antimicrobials. Moreover, all MRSA had staphylococcal enterotoxin (SE) genes, and two clusters of SE genes were identified: seg-sei-sek-sem-sen-seo (20 isolates, 87%) and sed-seg-sei-sej-sem-sen-seo (3 isolates, 13%). Each MRSA-positive farm had only one spa-SCCmec type. Nine MRSA isolates (39.1%) with the t324-IvA genotype, which is related to community acquired MRSA, were isolated from three dairy farms. Additional genotypes of t148-IvA and t002-II were detected and related to human MRSA strains. Most MRSA isolates had distinct PFGE subtypes, indicating that they were not the same clones. Only two isolates collected from the same farm during different years had an identical PFGE type, indicating persistence of the clone at this farm. Taken together, these findings may indicate an increased virulence and risk of MRSA strains in dairy farms. Therefore, an efficient surveillance and control programme is needed to prevent the transmission of MRSA from animals to humans.
P40: Comparison of oregano essential oil with antibiotic growth promoters on the performance of broilers

Qingyun Peng1,2, J. Li3, Z. Li3, Z. Duan2, Y. Lao2 and Y. Wu1
1School of Life Sciences, Sun Yat-sen University, China; 2Kemin Industries (Zhuhai) Co., Ltd., China; 3School of Environmental and Biological Engineering, Liaoning Shihua University, China
qingyun.peng@kemin.com

Non-antibiotic animal husbandry is a trend in the world. Many investigations have been conducted to search for natural alternatives with similar beneficial effects to antibiotic growth promoters (AGPs) for using in the animal industry [Huyghebaert et al., 2011. The Veterinary Journal 187: 182-188]. Phytogenic feed additives, such as oregano essential oil (OEO), are natural and residue free and thus received increasing attention as potential alternatives for AGPs [Windisch et al., 2008. Journal of Animal Science 86: E140-E148]. One of the key questions of using OEO as an alternative of AGPs is whether OEO has similar growth promoting effect comparable to that of AGPs. Orsential™ Dry was developed by Kemin Industries using the OEO extracted from proprietary clonal lines of oregano by Kemin. This study was conducted to compare the effects of Orsential Dry with common AGPs on the growth performance, carcass traits and immune organ index of broilers during a 42-day production period. 500 one-day old Arbor Acres broilers were randomly distributed into 5 dietary treatments (5 replicates per treatment and 20 birds per replicate). The dietary treatments included a basal diet (control), the basal diet supplemented with AGPs (virginiamycin, enramycin, or bacitracin methylene disalicylate (BMD)), and the basal diet supplemented with Orsential Dry (300 mg/kg). The results showed that supplementation of Orsential Dry significantly improved (P<0.05) the feed conversion ratio (FCR) of broilers on day 21. Broilers fed with Orsential Dry showed higher (P<0.05) average daily feed intake (ADFI) compared with the broilers fed with the other diets. All AGPs had no significant effect on the growth performance of broilers compared with the control group. Orsential Dry supplementation improved dressing percentage, half-eviscerated rate, and eviscerated rate compared with the control group (P<0.05). In addition, the broilers fed with Orsential Dry had the lowest abdominal fat percentage (P<0.05) among all the groups. All AGPs had only numerical improvement of the carcass traits of broilers. All treatments had significant impact on the thymus index. Moreover, birds fed with Orsential Dry had higher thymus index compared with those fed with AGPs. The addition of virginiamycin and Orsential Dry to the basal diet significantly increased (P<0.05) the bursa index versus control group and enramycin; Orsential Dry had the highest bursa index. In conclusion, supplementation of Orsential Dry had a positive effect on growth performance, carcass traits and immune response of broilers. Therefore, Orsential Dry could be a feasible alternative to AGPs, such as virginiamycin, enramycin and BMD.

P41: Antimicrobial resistance prevalence in bacteria isolated from harbour (Phoca vitulina) and grey (Halichoerus grypus) seal pups stranded in the Netherlands: a first screening

Ana Rubio-García1, J. van Zeijl2, S. Rosema3, S. Garcia Cobos4, A.W. Friedrich3, J.A. Wagenaar4 and J.W. Rossen3
1Sealcentre, the Netherlands; 2Department of Medical Microbiology, Ixore Center for Infectious Diseases, the Netherlands; 3Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, the Netherlands; 4Faculty of Veterinary Medicine, Utrecht University, the Netherlands
ana@zeethondencreche.nl

The use of antimicrobials in humans and animals leads to the selection of resistance genes. Most antimicrobials used in animals belong to the same classes as the ones used for humans. Similar resistance genes found in humans are also found in pristine ecosystems without any record of antimicrobial contamination. The Netherlands is a good example where human populations in coastal areas continue to increase. Therefore, coastal ecosystems could be considered important reservoirs or sentinels for infectious organisms and antimicrobial resistance (AMR). Since many marine mammal species share the coastal environment with humans and consume the same food, they also may serve as sentinels for ocean and human health. Little is known on the antimicrobial susceptibility patterns in free ranging and/or stranded marine mammals, more specifically in harbour and grey seals in the Netherlands. In this study, we revealed the prevalence of AMR in bacteria isolated from the rectum of harbour and grey seal pups and weaners admitted for rehabilitation at the Sealcentre in Pieterburen, the Netherlands. Rectal swabs for bacterial culture were collected during health assessment from 100 harbour seals during summer 2015 and 50 grey seals during winter 2015-2016, including pups and weaners. All animals had stranded alive along the Dutch coast and islands and were transported to the Sealcentre Pieterburen. Within 48 h after collecting the swabs were streaked onto different agar plates
to screen for methicillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum beta-lactamase (ESBL) and beta-lactamase-hyperproducing Gram-negative bacteria, and vancomycin resistant enterococci (VRE). ESBL producing *E. coli* were isolated from 4 harbour seal pups (4%) and 1 grey seal pup (2%), but no MRSA and VRE were detected. Subsequently, the whole genome of the four isolates from the harbour seals was sequenced on the MiSeq and the genomes were compared to genomes obtained from human isolates by a core genome multi-locus sequence typing (cgMLST) approach. Isolates from seals differ only in 40 of 2,764 analysed genes from human *E. coli* isolates. In addition, the presence of resistance genes was determined. *E. coli* isolates of this study contained genes causing resistance against aminoglycosides, beta-lactams, macrolides-lincosamide-streptogramin, sulphonamides, tetracyclines and trimethoprim. In conclusion, prevalence of MRSA, VRE and ESBL producing *E. coli* in harbour and grey seal pups is low. *E. coli* isolates found in them are closely related to those found in human and contain resistance genes also found in human isolates.

**P42: Bacillus subtilis** PB6 – a potential alternative for antibiotic growth promoters (AGPs)

**Prakash Chandra Saini**, S. Jayaraman, S. Sankaran and R. Chanthirasekaran
Kemin Industries South Asia Pvt. Ltd., India
prakash.saini@kemin.com

Antibiotic growth promoters (AGPs) have been used in poultry diets since 1950s for promoting growth by reducing the occurrence of necrotic enteritis (NE). There are increasing concerns of AGPs-related antibiotic resistance, and residues in meat and egg. A good AGPs’ alternative should alter the gut microflora positively by reducing *Clostridium perfringens* and altering the intestine morphology for better absorption of nutrients and anti-inflammatory action. *Bacillus subtilis* PB6, a proprietary strain of Kemin, is well-documented for prevention of NE and improving poultry performance. This paper illustrates the use of *B. subtilis* PB6 as a potential alternative to AGPs. In the first scientific study, NE was experimentally induced in the broilers via oral inoculation of mixed strains of *Eimeria* species and *C. perfringens* (108 cfu/ml). The birds were analysed for weight gain, mortality, feed conversion ratio (FCR), and villus histomorphometry. The supplementation of *B. subtilis* PB6 reduced the FCR (<0.05) and intestinal *C. perfringens* counts significantly (P<0.05) compared with the infected control group. It was also observed that *B. subtilis* PB6 improved villi length by 10.88 and 30.46% (P<0.05) compared with uninfected and infected control groups, respectively including villi length to crypt depth ratio. The in vivo anti-inflammatory capacity of *B. subtilis* PB6 was performed using a mouse model of acute, TNBS (2,4,6-trinitrobenzene sulfonic acid) induced inflammation. Performance was compared with the drug prednisolone, and was based on blinded macroscopic and histological scores and blood inflammatory markers like serum amyloid A and interleukin-6. Results showed that *B. subtilis* PB6 was able to reduce both blood inflammatory markers and lesion scores significantly (P<0.05) compared to the challenged control. The impact of *B. subtilis* PB6 on improving weight gain, FCR, and European efficiency factor of broilers in comparison with AGPs, BMD and avilamycin has been studied. The group supplemented with *B. subtilis* PB6 or BMD had higher (P<0.05) body weight compared to all other treatment groups whereas *B. subtilis* PB6 showed a significantly better FCR compared to the other groups with highest European efficiency factor. The combined data from all the three studies indicates that *B. subtilis* PB6 (CiOStat™) can be used as a potential alternative to AGPs.

**P43: Responsible use of antibiotics in pig production: an alternative approach to reduce mass metaphylaxis**

**Annalisa Scollo**1,2, F. Gottardo2 and C. Mazzoni1
1Suivet snc, Italy; 2MAPS Department, University of Padova, Italy
scollo@suivet.it

Porcine respiratory complex disease (PRCD) is due to a combination of infectious agents affecting the pigs’ health and resulting in reduced performance and a huge economic impact. The usual PRDC intervention is metaphylaxis, or rather antibiotic administration to the entire batch in critical phases during the growing cycle (i.e., mixing, moving, seroconversion). The efficacy of metaphylaxis to reduce pigs’ health risks and the subsequent benefit on growth performance are well known. However, the use of mass routine treatments is facing intense public scrutiny for antibiotic resistance and residuals in meat, so it should be abandoned. Aim of the present study was to assess feasibility and economic sustainability of the individual injectable approach (IT) in weaners compared to the mass metaphylaxis (MT). The study lasted 50 days from weaning and involved 650 weaner pigs (4-weeks old; 7.2 kg live weight) coming from a farrowing site with a documented history of respiratory problems. Piglets were allotted in 7 rooms with a central corridor: on the left side the animals received tilmicosin (Pulmotil ac,
Elanco; 20 mg/kg bw) in water for 7 days (MG=323 pigs), whereas on the right side (IG=318 pigs) only sick animals received injectable tulathromycin (Draxxin 100, Zoetis; 2.5 mg/kg bw once). The protocol for the individual treatment was based on two thresholds: (i) if more than 20% of pen-mates required the individual treatment, then all the pen was injected; and (ii) if more than 15% of the pens was totally treated, then mass oral treatment was administered. During the study, all adjunctive treatments (type, route, volume, symptomatology), mortality, culling rate and weight gain (weighing at 7, 29 and 50 days) were recorded for each pen. The results showed no difference in daily gain, mortality and culling rate ($P<0.05$) between the MT and IGT groups. The use of antibiotics was reduced by 26.42% in the IT compared to the MT group (3,719 vs. 5,054.6 g/group; 11.69 vs. 15.64 g/head; $P<0.05$), and this reduction implicated the decrease of costs for antibiotics (0.31 €/weaner). In conclusion, the present approach to the antibiotic use in weaners meets the needs of both public opinion (reduction of treatments) and farmers (reduction of costs). However, the success of the IT approach is related to the key role of the stockman in the prompt identification of sick animals. This underlies the relevant importance to have motivated and well-trained personnel at the farm.

P44: A national near real-time antimicrobial surveillance in companion animals in the UK – a health informatics approach

David Singleton
Epidemiology and Population Health, Institute of Infection and Global Health, University of Liverpool, UK
d.a.singleton@liv.ac.uk

The Small Animal Veterinary Surveillance Network (SAVSNET), a collaborative project between the University of Liverpool and The British Small Animal Veterinary Association (BSAVA), uses a ‘health informatics’ approach to collect and analyse large volumes of electronic records from veterinary practices (>1.1 million consults) and diagnostic laboratories (>2 million submissions). At consultation, a range of anonymised health data including prescription (items charged for during a consultation) are captured in real-time. Antibacterial-prescribing consultations were identified (22/11/2013–31/12/2015) across 428 veterinary sites comprising 694,818 canine consultations (310,907 dogs) and 265,259 feline consultations (148,934 cats). Proportions and confidence intervals (95%) were calculated using robust standard errors to allow for clustering within individual veterinary sites. For dogs and cats, at least one antibacterial was prescribed in 19.9% (19.2-20.6) and 18.4% (17.7-19.1) of all consultations, respectively. Practitioner-determined syndromic badging (gastroenteritis; respiratory; pruritus; kidney disease; trauma; tumour; other unwell; vaccination; other healthy or post-operative) revealed that in dogs, pruritus contained the highest proportion of antibacterial-prescription (51.3%, 50.0-52.7), compared with 33.7% (31.8-35.7) in cats. Trauma contained the highest proportion in cats (54.5%, 53.1-56.0), contrasting with dogs (27.9%, 26.5-29.3). Antibacterials were specifically authorised for use in dogs at a proportion of 84.1% (82.7-85.6) of prescriptions, and in cats, 83.9% (82.4-85.5), with human-authorised antibacterial prescription at proportions of 11.1% (10.0-12.3) and 5.5% (5.0-6.0) respectively. The most commonly prescribed canine antibacterial was clavulanic-acid-potentiated-amoxicillin (28.3%, 26.9-29.7), the majority (80.7%, 79.2-82.2) being prescribed for oral administration. In cats, most commonly prescribed antibacterial was cefovecin (36.8%, 34.4-39.2), all of which was authorised for systemic (subcutaneous) administration. For dogs, metronidazole was the most common human-authorised antibacterial (40.1% of human-authorised antibacterials, 35.3-45.0); in cats it was an ocular product containing neomycin sulphate and polymyxin B sulphate (27.8%, 25.1-30.1). ‘Highest Priority Critically Important Antimicrobials’ (WHO, 2011), including fluoroquinolones, 3rd generation cephalosporins and macrolides, represented 5.6% (4.7-6.4) of canine prescriptions (of which 89.5% (86.7-92.3) were veterinary-authorised), and 38.9% (36.1-41.7) of feline prescriptions (of which 99.0% (98.8-99.2%) were veterinary-authorised). These analyses describe a novel real-time antibacterial surveillance system utilising previously unavailable data from a diverse population of independent veterinary practices. In addition to research outputs, SAVSNET has developed a unique ‘practice portal’ which enables participating practitioners to compare their antibacterial prescribing habits with their peers. It is hoped that such a ‘health informatics’ approach will provide a new, exciting opportunity to both describe antibacterial usage in practice, but also influence practitioner behaviour to promote responsible use of antibacterials.
The spore-forming *Bacillus subtilis* PB6 (ATCC PTA-6737), a probiotic strain isolated from the gastrointestinal tract of healthy chicken, has been shown to assist in maintaining a healthy gut microbiome by inhibiting *Clostridium perfringens*, the causative agent of necrotic enteritis. The current study evaluated the effect of *B. subtilis* PB6 on performance and gut health of broiler chickens. A total of 480 one-day old male Ross 308 broilers with an initial average body weight of 43.6 g were randomly allocated to two treatments with 8 replicate pens per treatment. The two treatments were (i) negative control without additives, and (ii) negative control + 500 g/ton CLOSTAT® (Kemin Europa NV, Belgium). Performance parameters, including body weight, average daily weight gain, feed intake and weight adjusted feed conversion ratio (adjusted for the same mean body weight, WAFCR) were measured per pen during starter (0-9 days, grower (9-25 days) and finisher (25-39 days) period and during the entire period (0-39 days). Mortality was recorded daily and the European poultry efficiency factor (EPEF) was calculated. At the end of the trial (day 39), 1 bird per pen was euthanised and the abdominal fat weight (relative to the total body weight) was recorded. In addition, gut health was assessed using the standard ‘Kemin gut health scoring system’. Parameters scored were ballooning, muscle tone, thin or fragile intestine, abnormal gut content, inflammation, sloughed off mucosa, and presence of undigested feed. The use of the probiotic significantly improved gut health score, compared to the control group (P<0.05). In addition, the WAFCR was significantly lower during the finisher (1.82 vs. 1.90) and the total period (1.59 vs. 1.64). A numerical improvement (P>0.05) in final body weight (2,135 g vs. 2,119 g), mortality (4.6% vs. 6.3%), relative abdominal fat weight (1.18% vs. 1.42%), and EPEF (328 vs. 311) was also recorded. In conclusion, the probiotic *B. subtilis* PB6 strain can significantly improve performance and intestinal health parameters when fed to broiler chickens and could be used as an alternative to in-feed antibiotics.

**P46: CLOSTAT™ – a direct-fed microbial containing Bacillus subtilis PB6 for the replacement of antibiotic growth promoter in animal feed**

**BoonFei Tan, P.-S. Chan, A. Chua and E. Schoeters**

Kemin Animal Nutrition and Health, Asia Pacific, Singapore
boonfei.tan@kemin.com

The livestock industry, over the last few decades, has benefited tremendously from the use of antibiotics as antimicrobial growth promoter (AGP). The unregulated use of antibiotics in food animals has been linked to the emergence and spread of antibiotic resistant bacteria, and ultimately led to the loss of effectiveness of many antibiotics in controlling animal and human pathogens. Many countries over the years have either started or have planned to ban the use of antibiotics used as AGP in feed. In response, the livestock industry has for many years been seeking for effective antibiotic alternatives that control diseases while promoting animal performance. Kemin Industries responded to this call with a patented probiotic: CLOSTAT™, which is effective in inhibiting the *in vitro* and *in vivo* growth of *Clostridium perfringens*, the causative agent in clinical and subclinical necrotic enteritis in poultry. In vitro studies have also shown that CLOSTAT™ is effective against other pathogenic bacteria including *Salmonella enteritidis* and *Escherichia coli*. The aim of this research was to investigate the effect of replacing antibiotic in broiler feed with CLOSTAT™ on animal performance. In one experiment, 64,000 day-old Ross 308 chicks were divided equally into two groups receiving a diet containing either 10 ppm enramycin (control) or 500 g/ton CLOSTAT™ (treatment). The experiment was conducted with all chicks receiving diet and water ad libitum plus 60 ppm of salinomycin from day 0 until day 45. The second experiment was to compare the efficacy of CLOSTAT™ to zinc bacitracin on broiler performance. Replicated trials were conducted in which 17,000 to 19,000 day-old chicks were fed with diet containing either 1.35 kg/ton zinc bacitracin (control) or 250 g/ton CLOSTAT™ (treatment), from day 0 until day 21. From day 22 until 35, zinc bacitracin was administered at 500 g/ton, while the dosage of CLOSTAT™ was maintained. Measurements including feed intake and body weight gain of control and treatment groups in the two experiments were collected during the trials. At the end of both trials, all birds in both control and treatment groups had comparable indexes in livability, mortality and feed conversion rate, with birds receiving CLOSTAT™ in the first trial having numerical improvement in performance index and average body weight. Together, our results here showed that CLOSTAT™ can be safely used in broiler feed as AGP to replace some antibiotics that are used to control *C. perfringens*. 

**P45: Effect of a Bacillus subtilis probiotic on performance and gut health of broiler chickens**

**Natasja Smeets, S. de Smet and F. Nuyens**

Kemin Europa NV, Belgium
natasja.smeets@kemin.com

The spore-forming *Bacillus subtilis* PB6 (ATCC PTA-6737), a probiotic strain isolated from the gastrointestinal tract of healthy chicken, has been shown to assist in maintaining a healthy gut microbiome by inhibiting *Clostridium perfringens*, the causative agent of necrotic enteritis. The current study evaluated the effect of *B. subtilis* PB6 on performance and gut health of broiler chickens. A total of 480 one-day old male Ross 308 broilers with an initial average body weight of 43.6 g were randomly allocated to two treatments with 8 replicate pens per treatment. The two treatments were (i) negative control without additives, and (ii) negative control + 500 g/ton CLOSTAT® (Kemin Europa NV, Belgium). Performance parameters, including body weight, average daily weight gain, feed intake and weight adjusted feed conversion ratio (adjusted for the same mean body weight, WAFCR) were calculated. At the end of the trial (day 39), 1 bird per pen was euthanised and the abdominal fat weight (relative to the total body weight) was recorded. In addition, gut health was assessed using the standard ‘Kemin gut health scoring system’. Parameters scored were ballooning, muscle tone, thin or fragile intestine, abnormal gut content, inflammation, sloughed off mucosa, and presence of undigested feed. The use of the probiotic significantly improved gut health score, compared to the control group (4.6% vs. 3.9%). A numerical improvement (P>0.05) in final body weight (2,135 g vs. 2,119 g), mortality (4.6% vs. 6.3%), relative abdominal fat weight (1.18% vs. 1.42%), and EPEF (328 vs. 311) was also recorded. In conclusion, the probiotic *B. subtilis* PB6 strain can significantly improve performance and intestinal health parameters when fed to broiler chickens and could be used as an alternative to in-feed antibiotics.
P47: Antibiotic growth promoter replacement by a synergistic butyrate based product in piglets

Valentine Van Hamme¹, A. Eto² and L.B. Costa³
¹Impextraco NV, Belgium; ²Impextraco Latin America, Brazil; ³Pontifícia Universidade Católica do Paraná (PUCPR), Brazil
valentine@impextraco.com

Since 2006, antibiotic growth promoters (AGP) are banned in the EU. Questions are raising about the effectiveness of alternatives to replace AGPs. Widely used alternatives in EU are products based on butyric acid (C4). C4 is a short chain fatty acid with a biological role, consistently present in the intestinal ecosystem, as it is naturally produced by fermentation by the intestinal microbiota. Butyrate is seen as an AGP alternative as it plays a major role in promoting gut health by stimulating the intestinal barrier, modulation of the immune system, balancing the intestinal flora and improving digestion and absorption of nutrients, leading to enhanced performance. The aim of this trial was to evaluate the effect on performance of a butyrate based product comparing to the use of a traditional AGP, lincomycin, in piglets. As positive effects of lincomycin on average daily gain and feed conversion ratio in swine have already been reported. A trial was carried out during 35 days at the Swine Research Unit of PUCPR, Brazil. 48 weaned piglets were divided into 3 groups of 8 replicates and were housed in nursery pens with slatted floor. 2 maize- and soybean meal-based diets were formulated according to animal’s age: prestarter (1 to 14 days) and starter feed (15 to 35 days). Treatments consisted of a negative control group with no antibiotics nor feed additives (C), a group treated with lincomycin (L) at 0.05% in prestarter and starter feed and a group supplemented with a calcium butyrate based synergistic product (B) (Butifour® NF) at 0.15% in prestarter and 0.075% in starter feed. Body weight (BW) and feed consumption were controlled weekly to provide data on feed conversion ratio (FCR). Additionally, the incidence of diarrhoea was verified daily, and a score was attributed according to Vassalo et al., 1997. Collected data were analysed by a mixed linear model, except of fecal score data. These were analysed by Dunn test with Bonferroni adjustment. All statements of difference were made considering P≤0.05. At the end of the evaluation, B showed a numerically higher body weight compared to L and C. B and L improved FCR and diarrhoea score significantly compared to C. In this trial, a synergistic butyrate based supplementation of piglet feed was regarded to be a suitable alternative for the use of an AGP, lincomycin.

P48: Medium chain fatty acids to reduce antibiotic resistance

Ellen Van Meenen, W. Naeyaert, M. De Laet, J. Krijnen, G. De Clercq and K. Lannoo
Nuscience, Belgium
ellen.van.meenen@nusciencegroup.com

Antibiotics are an integral part of industrialised livestock production. The indiscriminate use of antibiotics in animal agriculture has been subjected to a critical scrutiny by governments but also by consumers. Consumers worldwide increasingly want antibiotic-free, healthy food, preserving for as long as possible the ever-diminishing arsenal of antimicrobials effective in humans. How can we responsibly use antibiotics in animals without compromising food safety and human health, as well as animal health, welfare and productivity? If we want farming to remain profitable there will be a big need for healthy food, preserving for as long as possible the digestive and absorptive capacities of the intestines. Next to promoting gut health, MCFA have a positive influence on the immunity of the animal. White blood cells (neutrophils) remain more active making the animal also more resistant against pathogens, the outcome of disease is altered and intestinal and systemic colonisation is reduced, as shown in scientific trials. The combination of these antibacterial actions will result in a beneficial microbial ecosystem and thus a higher villus/crypt ratio favouring the digestive and absorptive capacities of the intestines. Next to promoting gut health, MCFA have a positive influence on the immunity of the animal. Since 2006, antibiotic growth promotors (AGP) are banned in the EU. Questions are raising about the effectiveness of alternatives to replace AGPs. Widely used alternatives in EU are products based on butyric acid (C4). C4 is a short chain fatty acid with a biological role, consistently present in the intestinal ecosystem, as it is naturally produced by fermentation by the intestinal microbiota. Butyrate is seen as an AGP alternative as it plays a major role in promoting gut health by stimulating the intestinal barrier, modulation of the immune system, balancing the intestinal flora and improving digestion and absorption of nutrients, leading to enhanced performance. The aim of this trial was to evaluate the effect on performance of a butyrate based product comparing to the use of a traditional AGP, lincomycin, in piglets. As positive effects of lincomycin on average daily gain and feed conversion ratio in swine have already been reported. A trial was carried out during 35 days at the Swine Research Unit of PUCPR, Brazil. 48 weaned piglets were divided into 3 groups of 8 replicates and were housed in nursery pens with slatted floor. 2 maize- and soybean meal-based diets were formulated according to animal’s age: prestarter (1 to 14 days) and starter feed (15 to 35 days). Treatments consisted of a negative control group with no antibiotics nor feed additives (C), a group treated with lincomycin (L) at 0.05% in prestarter and starter feed and a group supplemented with a calcium butyrate based synergistic product (B) (Butifour® NF) at 0.15% in prestarter and 0.075% in starter feed. Body weight (BW) and feed consumption were controlled weekly to provide data on feed conversion ratio (FCR). Additionally, the incidence of diarrhoea was verified daily, and a score was attributed according to Vassalo et al., 1997. Collected data were analysed by a mixed linear model, except of fecal score data. These were analysed by Dunn test with Bonferroni adjustment. All statements of difference were made considering P≤0.05. At the end of the evaluation, B showed a numerically higher body weight compared to L and C. B and L improved FCR and diarrhoea score significantly compared to C. In this trial, a synergistic butyrate based supplementation of piglet feed was regarded to be a suitable alternative for the use of an AGP, lincomycin.
non-digestive disorders. Reducing the use of antibiotics has a great impact on the occurrence of antibiotic resistance. To reduce the impact on technical performance the need for natural alternatives is high. MCFAs have proven worldwide to be a reliable partner in taking the hurdle towards producing animals on a cost effective way without using too much antibiotics.

**P49: Canadian dairy producer perceptions towards antibiotic treatment and prevention of mastitis and on-farm record quality**


1University of Guelph, Canada; 2Ontario Ministry of Agriculture, Food, and Rural Affairs, Canada; 3Public Health Agency of Canada, Canada

mwatters@uoguelph.ca

The majority of dairy-cattle antibiotic use in Canada is due to mastitis treatment and prevention [Young *et al.*, 2010. Preventive Veterinary Medicine 94: 43-53]. Given public health concerns over antibiotics in production animals, the objectives of our study were to understand producer perceptions surrounding antibiotic use for the treatment and prevention of mastitis and to assess the quality of on-farm antibiotic use records. A survey was utilised to determine producer perceptions and on-farm visits were completed to acquire 12-months of treatment records. 101 Ontario dairy producers responded to the survey. Average lactating herd size of survey respondents was 80±75 cows (min. 15; max. 500) with 47%, 42%, and 11% of herds being pipeline, parlor, and automatic milking systems, respectively. Table 1 represents select producer responses. 52% of producers indicated they use antibiotics in ≥75% of mastitis cases. Based on preliminary analyses, larger herds were more likely to indicate that antibiotics were their first treatment option for mastitis, as were herds very concerned about public perception, veterinarian perception, and government regulations leading to changes in regulating on-farm antibiotic use. Interestingly, herds which were very concerned about human medical associations demanding a change to antibiotic regulation were less likely to indicate antibiotics were their first treatment option. To date, records from 77 herds have been evaluated representing 8,316 treatment events; 2 of these herds lacked legible records and 7 indicated they had no treatment records. 33% of the records were computer-based while 67% were hand written. The most common missing information was dosage, route of administration, milk and meat withhold, and initialling the treatment; 37%, 41%, 41%, 38%, and 41%, respectively. These results will be beneficial in understanding how producers utilise antibiotics and in determining potential areas for education and correction to improve food safety.

**Table 1. Producer responses towards antibiotic treatment/prevention of mastitis.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you utilise veterinary consult for antibiotic treatment of clinical mastitis?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Do you consider bacteria type when administering antibiotics?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
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<tr>
<td>Do you consider effectiveness when selecting antibiotics the majority of the time?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Do you consider antibiotic resistance when selecting antibiotics the majority of the time?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Do you consider antibiotics your first treatment option for mastitis?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

treatment option for mastitis, as were herds very concerned about public perception, veterinarian perception, and government regulations leading to changes in regulating on-farm antibiotic use. Interestingly, herds which were very concerned about human medical associations demanding a change to antibiotic regulation were less likely to indicate antibiotics were their first treatment option. To date, records from 77 herds have been evaluated representing 8,316 treatment events; 2 of these herds lacked legible records and 7 indicated they had no treatment records. 33% of the records were computer-based while 67% were hand written. The most common missing information was dosage, route of administration, milk and meat withhold, and initialling the treatment; 37%, 41%, 41%, 38%, and 41%, respectively. These results will be beneficial in understanding how producers utilise antibiotics and in determining potential areas for education and correction to improve food safety.

**P50: Bactericidal activity of a novel antisense peptide-peptide nucleic acid against the cytidine monophosphate kinase of *Staphylococcus aureus***

**Jang Won Yoon**, **H.T. Lee**, **D. Han**, **J.B. Lee** and **Y.H. Park**

1College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Republic of Korea; 2College of Veterinary Medicine, Seoul National University, Republic of Korea

jwyy706@kangwon.ac.kr

*Staphylococcus aureus* is an important zoonotic bacterial pathogen that has been frequently associated with food poisoning and nosocomial infections in humans as well as mastitis in dairy cows. Since the microorganism can easily acquire resistance to several antimicrobial drugs, novel antibiotics and/or non-
antibiotic based anti-infective strategies need to be developed for the intervention of notorious methicillin- or multidrug-resistant S. aureus. Here we report a novel bactericidal peptide nucleic acid (PNA) that can antisense the cytidine monophosphate kinase, a putative essential gene product in S. aureus. Based on the genome sequence of S. aureus N315, a set of PNA conjugates with a bacterial penetration peptide (KFF)3K-L- was synthesised to antisense the seven potentially essential genes (cmk, deoD, ligA, smpB, glmU, pyrH, and ftsA) and further evaluated for their antibacterial properties in vitro as well as in vivo. Our experiments demonstrated that two peptide conjugated-PNAs (P-PNAs) targeting either cmk or deoD genes, Pjyh-cmk1 and Pjyh-deoD1, had the strongest growth inhibitory effects against S. aureus ATCC 29740 (a bovine mastitic milk isolate) in a dose-dependent manner. In vivo application of Pjyh-cmk1 resulted in the significant reduction of bacterial loads in the intra-peritoneally infected mice with a sublethal dose of S. aureus. Moreover, Pjyh-cmk1 could dramatically increase the survival rate of the mice lactated after intra-mammary infection. Taken together, our characterisation of Pjyh-cmk1 demonstrated the bactericidal activity against S. aureus as well as in vivo effectiveness. This is another demonstration implying possible application of the antisense P-PNA as an alternative anti-infective agent.

P51: Development of an enzyme-linked-receptor assay based on the carboxy-terminal of penicillin-binding protein BlaR for the detection of β-lactams in tissues of food-producing animals

G. Cheng, J. Peng, L. Huang, Y. Wang, H. Hao, D. Peng and Zonghui Yuan
National Reference Laboratory of Veterinary Drug Residues (HZAU) and MOA Key Laboratory for the Detection of Veterinary Drug Residues in Foods, Huazhong Agricultural University, China
yuan5802@mail.hzau.edu.cn

β-lactam antibiotics are widely used in food-producing animals. Illegal use and abuse of such drugs may result in residues that cause allergy in consumers and select antimicrobial resistance. The accurate, sensitive and high-throughput methods for monitoring β-lactams in animal-derived foods are reasonably needed. In contrast to the time-consuming and poor detectability of microbiological method and the narrow specificity of immunoassay, the receptor assay can detect both penicillins and cephalosporins with more sensitivity and higher accuracy. However, the commonly used radioactive receptor assay, CHARM, is harmful to analysts’ health and requires expensive equipment. The aim of this study is to develop an easy, time-saving and environmentally friendly enzyme-linked-receptor assay (ELRA) for the detection and determination of β-lactams in animal-derived foods. The gene of the carboxy-terminal of penicillin-recognising protein BlaR (BlaR-CTD) from Bacillus licheniformis ATCC 14580 was cloned and heterogeneously expressed in Escherichia coli BL21 (DE3). The recombinant BlaR-CTD protein was purified by Ni2+-affinity chromatography and immobilised on the inside surface of each well of a 96-well microtitre plate. The horseradish peroxidase (HRP)-labelled ampicillin was synthesised by EDC/NHS-crosslinking. The spiked samples were prepared by phosphate-buffered saline extraction. The ELRA was established based on directly competitive inhibition of HRP-ampicillin binding to the immobilised BlaR-CTD by β-lactams in samples. BlaR-CTD with molecular size of 26 kDa was successfully expressed as a His-tag fusion protein and approximately 20 mg of 95% pure BlaR-CTD was obtained from 1 l of cells. The IC50 values for 15 β-lactams including benzylpenicillin, ampicillin, amoxicillin, dicloxacillin, oxacillin, nafcillin, cefapirin, cefoperazone, cefalotin, cefazolin, ceftiquinome, ceftriaxone, cefotaxime, cefalexin, cefotiolur, and desfuroycetiotiolur were ranging from 0.18 to 170.81 μg/l, exhibiting >1 % cross reactivity with ceftiquinome. The standard curve of ceftiquinome calibration was y=-0.5462x +0.6546 with good linearity of R=0.995 at concentrations ranging from 0.5 to 8 μg/l. The limits of detection in milk, beef, and chicken muscles with ceftiquinome matrix calibration were 2.1, 30.7, and 31.1 μg/kg, respectively. The recoveries of β-lactams in milk, beef, and chicken muscles were in the range of 53% to 128%, with the inter-assay variability below 30 %. In conclusion, this study firstly established a rapid, simple and accurate semiquantitative method for simultaneous determination of 15 β-lactams in edible tissues, among which 11 β-lactams could be detected below EU maximum residue limits.
P52: Utilising glycoside hydrolases to degrade bacterial biofilms and increase the efficacy of antibiotics

D. Fleming and Kendra P. Rumbaugh
Department of Surgery and the Texas Tech University Health Sciences Center Burn Center of Research Excellence, Texas Tech University Health Sciences Center, USA
kendra.rumbaugh@ttuhsc.edu

Soft tissue infections, including burns, diabetic chronic wounds, abscesses and necrotising fasciitis, are a major source of morbidity and mortality. The bacteria that cause these infections are often biofilm-associated and resistant to today’s strongest antibiotics. The ability of pathogens to persist within a biofilm can make them up to 1000-fold more tolerant to antimicrobials. Thus, therapies that act by breaking down the biofilm and dispersing bacteria into their planktonic form may enhance the ability of the host immune system, or conventional therapeutics, to clear the infection. Glycoside hydrolases (GHs) are natural enzymes, produced by many organisms, which hydrolyse the glycosidic linkages between two or more carbohydrates, including the polysaccharides that comprise the biofilm matrix. Our hypothesis is that GHs can be used topically to degrade wound biofilms and significantly improve the efficacy of conventional antibiotics. To measure the efficacy of GH treatment on biofilms in vitro, bacterial biofilms were grown in an artificial wound media and their biomass and percentage of cells dispersed was assessed before and after treatment with two common GHs, cellulase and α-amylase. A mouse chronic wound infection model was used to test the efficacy of GHs in vivo. Treatment of biofilms with solutions of GHs resulted in significant reductions in biomass and dispersion of bacterial cells. Additionally, GH treatment of biofilms grown in artificial wound media resulted in the complete dissolution of biofilm and an increase in the efficacy of antibiotic treatments. However, GHs applied topically to murine chronic wounds were not as effective in enhancing the efficacy of antibiotics. In conclusion, the advantages of using GHs to attack biofilms are: the wide variety of GHs commercially available at low cost; GHs have yet to be exploited for therapeutic purposes; GHs have the potential to dramatically improve the efficacy of a wide range of existing antimicrobials (e.g., antibiotics, phage and immune cells); and GHs are potentially broad spectrum. We saw that GH therapy significantly reduced biofilm biomass, leaving bacteria more susceptible to conventional antimicrobials. However, for unknown reasons, GHs were less efficacious in vivo and current studies are focused on optimising biodelivery methods for in vivo application.
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