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Introduction

Welcome to the proceedings from the 7th International Conference on Colonic Spirochaetal Infections in Animals and Humans

The organizing committee would like to welcome delegates to the 7th International Conference on Colonic Spirochaetal Infections in Animals and Humans. We hope this conference will promote the exchange of scientific knowledge and, based on the discussions, progress will be made in the research on intestinal spirochaetes, their role in diseases in different target species and on the diagnostic procedures used for spirochaete identification.

The scientific program is organized into five main themes covering new understanding of Brachyspira species (session 1), intestinal spirochaetosis in poultry and wild birds (session 2), role of treponemes in cattle and humans (session 3), re-emergence of swine dysentery / control of swine dysentery (session 4), and harmonization of diagnostic procedures and susceptibility testing (round-table discussion).

We are honoured to have as invited speakers outstanding scientists in the research of spirochaetes, including, in order of appearance in the scientific program, David Smith, David Burch, Désirée Jansson, Stuart Carter, Nandita Mirajkar, Roberto Guedes, Pedro Rubio Nistal and Maxime Mahu. Moreover, we are very grateful to Claes Fellström, who kindly accepted to chair the round-table discussion on harmonization of diagnostic procedures and susceptibility testing. We also wish to express our thanks to Jill Thomson and Stefan Schwarz who accepted to introduce to the topics discussed during the round-table discussion.

Elanco Animal Health is proud to be the principal sponsor of this conference on spirochaetal infections. At these conferences current knowledge is present and shared, thus bringing forth important information useful to swine, poultry and cattle producers around the world.

Elanco Animal Health is also pleased, that through its sponsorship and involvement in organization, these conferences will facilitate future co-operations among researchers and possible co-operative efforts with Elanco Animal Health.

We are looking forward to an interesting, inspiring exchange of ideas and wish you a pleasant stay in Hannover.

The local Organizing Committee

Judith Rohde
Institute for Microbiology
University of Veterinary Medicine, Hannover, Germany

Michael Wendt
Clinic for Swine and Small Ruminants
University of Veterinary Medicine, Hannover, Germany

Peter Valentin-Weigand
Head of Institute for Microbiology
University of Veterinary Medicine, Hannover, Germany

Ulrich Klein
Global Technical Services Manager
Elanco Animal Health, Basel, Switzerland
Proceedings of the 7th International Conference on Colonic Spirochaetal Infections in Animals and Humans

6th-7th October 2016
Hannover, Germany

The Scientific Committee of the conference in alphabetical order:

Nick Evans, Liverpool University, United Kingdom
David Hampson, Murdoch University, Australia
Désirée Jansson, National Veterinary Institute (SVA), Sweden
David Smith, Heriot-Watt University, Scotland
Jill Thomson, Scottish Agriculture College, United Kingdom

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Program

Wednesday, 5th October, 2016

18:00 – 20:00  Unofficial welcome dinner buffet at the conference hotel

Thursday, 6th October, 2016

08:00  Registration
08:30  Opening comments
08:45  Session 1: New Understanding of *Brachyspira* species
11:15  Session 2: Intestinal Spirochaetes in Poultry and Wild Birds
12:40  Lunch
14:15  Session 3: Role of Treponemes in Cattle and Humans
16:30  Poster walk-around
17:30  Wrap-up of the day
19:30  Conference dinner

Friday, 7th October, 2016

08:30  Session 4: Re-emergence of Swine Dysentery, Control of Swine Dysentery
12:10  Poster walk-around
13:15  Lunch
14:45  “Claes Fellström” poster prize
15:00  Round-table Discussion: Harmonization of Diagnostic Procedures and Susceptibility Testing
17:15  Wrap-up and farewell
18:30  unofficial social program
Scientific Program
Thursday, 6th October, 2016

08:30  Peter Valentin-Weigand (Institute for Microbiology, University of Veterinary Medicine Hannover, Hannover, Germany)
Opening comments

Session 1: New Understanding of Brachyspira species
Chair: David Smith (Heriot-Watt University, Edinburgh, Scotland)

08:45 - 09:30  David Smith (Heriot-Watt University, Edinburgh, Scotland)
T01 Keynote: WHY does molecular understanding of intestinal spirochaetes matter and WHAT might systematic molecular investigation reveal?

09:30 - 09:45  Roderick M Card, Emma Stubberfield, Jon Rogers, Javier Nunez, Richard Ellis, Ben Strugnell, Chris Teale, Susanna Williamson, Muna F Anjum
T02 Exploring the molecular basis for antimicrobial resistance in Brachyspira hyodysenteriae using whole genome sequencing

09:45 - 10:00  Roderick M. Card, Eric R. Burrough, Richard Ellis, Javier Nunez-Garcia, Judith Rohde, Jill Thomson, Alexander W. Tucker, Lucy Weinert
T03 Whole genome sequencing of weakly haemolytic Brachyspira genetically identified as Brachyspira hyodysenteriae

10:00 - 10:30  Coffee

10:30 - 10:45  Jessica Joerling, Karen Schlez, Stefanie Barth, Judith Rohde, Jörg Heuser, Christa Ewers, Werner Herbst
T04 Determining Brachyspira hyodysenteriae sequence types and possible relations to virulence genes and antimicrobial susceptibility

10:45 - 11:00  Cole B. Enns, John C.S. Harding, Matthew E. Loewen
T05 Characterization of the electrogenic secretory response in porcine colon following in vivo challenge with Brachyspira hyodysenteriae and “Brachyspira hampsonii”

11:00 - 11:15  Macarena P. Quintana-Hayashi, Nazanin Navabi, Maxime Mahu, Nele De Pauw, Filip Boyen, Harvey R. Fernandez, An Martel, Freddy Haesebrouk, Frank Pasmans, Sara Lindén
T06 Immune factors found in the colon during Brachyspira hyodysenteriae infection induce increased mucin transport and production in a mucus secreting, polarized in vitro colonic mucosal surface

Session 2: Intestinal Spirochaetes in Poultry and Wild Birds
Chair: Désirée Jansson (National Veterinary Institute (SVA), Uppsala, Sweden)

11:15 - 11:35  David Burch (Octagon Services Ltd, Old Windsor, Berkshire, UK)
T07 Keynote: Intestinal spirochaetes in poultry

11:35 - 11:55  Désirée Jansson (National Veterinary Institute (SVA), Uppsala, Sweden)
T08 Keynote: Brachyspira spp. in free-living wild birds

11:55 - 12:10  Discussion of keynotes
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<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Speakers</th>
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<tr>
<td>12:10 - 12:25</td>
<td>T09</td>
<td>Histopathological intestinal changes and shifts in intestinal microbiota in experimentally induced dysbacteriosis in chickens</td>
<td>Naomi de Bruijn, Anneke Feberwee, Jos van der Vossen, Martien Caspers, Gert Jan Boelm, Wil J.M. Landman</td>
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<td>12:40 – 14:15</td>
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<td>Lunch</td>
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**Session 3: Role of Treponemes in Cattle and Humans**  
Chair: Nickolas J. Evans (Department of Infection Biology, University of Liverpool, Liverpool, UK)

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<tr>
<td>14:15 - 15:00</td>
<td>T11</td>
<td>Keynote: Attempting to control the spread of digital dermatitis treponemes in farm and wildlife species</td>
<td>Stuart Carter (Institute of Infection and Global Health, University of Liverpool, Liverpool, UK)</td>
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<tr>
<td>15:00 - 15:15</td>
<td>T12</td>
<td>Digital dermatitis treponemes: different pathology, different host species, but same bacteria?</td>
<td>Simon R. Clegg, Stuart D. Carter, Nickolas J. Evans</td>
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<tr>
<td>15:15 - 15:45</td>
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<td>Coffee</td>
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<tr>
<td>15:45 - 16:00</td>
<td>T13</td>
<td>Identifying infection reservoirs of digital dermatitis in dairy cattle</td>
<td>Jennifer Bell, Stuart D. Carter, Roger Blowey, Simon R. Clegg, Richard Murray, Nicholas J. Evans</td>
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<tr>
<td>16:00 - 16:15</td>
<td>T14</td>
<td>Molecular diagnosis of human intestinal spirochaetosis: A six years’ experience</td>
<td>Pablo Rojas, Judith Kikhney, Annett Petrich, Julia Schulze, Ulf B. Göbel, Janina Masseck, Michael Vieth, Annette Moter</td>
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<td>16:15 - 16:30</td>
<td>T15</td>
<td>Whole genome sequencing of isolates from human colonic spirochaetosis</td>
<td>Kaisa Thorell, Linn Inganäs, Annette Backhans, Lars Agreus, Anna Andreasson, Lars Engstrand</td>
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<tr>
<td>16:30</td>
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<td>Poster walk-around</td>
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<tr>
<td>17:30</td>
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<td>Wrap-up of the day</td>
<td>Ulrich Klein (Elanco Animal Health, Basel, Switzerland)</td>
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**Scientific Program**  
**Friday, 7th October, 2016**

### Session 4: Re-emergence of Swine Dysentery, Control of Swine Dysentery

**Chair:** David Hampson (School of Veterinary and Life Sciences, Murdoch University, Perth, Australia)

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<tr>
<th>Time</th>
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<tr>
<td>8:30 - 8:55</td>
<td><strong>Nandita Mirajkar and Connie Gebhart</strong> (Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, USA)</td>
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<tr>
<td><strong>T16</strong></td>
<td><strong>Keynote:</strong> Re-emergence of swine dysentery in North America</td>
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<tr>
<td>8:55 - 9:05</td>
<td><strong>Roberto Guedes</strong> (Veterinary School, Universidade Federal de Minas Gerais, Belo Horizonte/MG, Brazil)</td>
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<tr>
<td><strong>T17</strong></td>
<td><strong>Keynote:</strong> Swine dysentery in South America and genetic characterization of Brazilian <em>Brachyspira hyodysenteriae</em> isolates</td>
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<td>9:05 - 9:15</td>
<td><strong>Pedro Rubio Nistal</strong> (Department of Animal Health, University of Leon, Leon, Spain)</td>
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<tr>
<td><strong>T18</strong></td>
<td><strong>Keynote:</strong> Is there an emergence of swine dysentery in Spain?</td>
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<td>9:15 - 9:30</td>
<td>Discussion of keynotes</td>
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<td>9:30 - 9:45</td>
<td><strong>Tom La, Nyree D. Phillips, David J. Hampson</strong></td>
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<td><strong>T19</strong></td>
<td><strong>An investigation into the occurrence of agents of swine dysentery in Australian pig herds</strong></td>
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<td>9:45 - 10:00</td>
<td><strong>Francesca Romana Massacci, Lucilla Cucco, Silvio De Luca, Michele Tentellini, Giovanni Pezzotti, Chiara Francesca Magistrali</strong></td>
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<tr>
<td><strong>T20</strong></td>
<td><strong>A longitudinal study on the epidemiology of <em>Brachyspira hyodysenteriae</em> infection in fattening pigs</strong></td>
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<td>10:00 - 10:15</td>
<td><strong>Eric Burrough, Bailey Arruda, Paul Plummer</strong></td>
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<td><strong>T21</strong></td>
<td><strong>Comparison of the luminal and mucosa-associated microbiota in the spiral colon of pigs with and without swine dysentery</strong></td>
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<td>10:15 - 10:45</td>
<td>Coffee</td>
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<td>10:45 - 11:00</td>
<td><strong>Matheus O. Costa, Champika Fernando, Roman Nosach, John C.S. Harding, Janet E.Hill</strong></td>
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<td><strong>T22</strong></td>
<td><strong>Development of an in vitro swine colon culture protocol as a model for “<em>B. hampsonii</em>” infection</strong></td>
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<td>11:00 - 11:15</td>
<td><strong>John Harding, Courtney EK, Roman Nosach, Jason Perez, Champika Fernando, Yanyun Huang, Janet Hill</strong></td>
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<td><strong>T23</strong></td>
<td><strong>Optimization of a murine challenge model for &quot;<em>Brachyspira hampsonii</em>&quot;</strong></td>
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<td>11:15 - 12:00</td>
<td><strong>Maxime Mahu</strong> (Department of Pathology, Bacteriology and Avian Diseases, Ghent University, Ghent, Belgium)</td>
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<td><strong>T24</strong></td>
<td><strong>Keynote:</strong> Administration of an avirulent strain of <em>Brachyspira hyodysenteriae</em> partially protects pigs against challenge with a virulent <em>B. hyodysenteriae</em> strain</td>
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<td>12:00 - 12:10</td>
<td><strong>M. Pesciaroli, L. Cucco, L. Curcio, F.R. Massacci, C. Maresca, M. Tentellini, G. Pezzotti, E. Scoccia, C.F. Magistrali</strong></td>
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<tr>
<td><strong>T25</strong></td>
<td><strong>Effect of coated calcium butyrate on the course of swine dysentery: an experimental study</strong></td>
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<td>Poster walk-around</td>
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</table>
13:15 - 14:45  Lunch

14:45  “Claes Fellström” poster prize

15:00  Round-table Discussion: Harmonization of Diagnostic Procedures and Susceptibility Testing

Moderation: Claes Fellström
Podium: Jill Thomson; David Hampson; Eric Burrough; Ana Carjaival; Maxime Mahu; Roberto Guedes; John Harding

15:00 - 15:10  Jill Thomson (Scottish Agricultural College, Veterinary Science Division Edinburgh, Penicuik, UK)
Introduction

15:10 - 15:40  Discussion

15:40 - 16:10  Coffee

16:10 - 16:30  Stefan Schwarz (Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany; CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing)
Introduction

16:30 - 17:00  Discussion

17:15 - 17:45  Michael Wendt (Clinic for Swine and Small Ruminants, University of Veterinary Medicine Hannover, Hannover, Germany)
Wrap-up and farewell
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Posters

Session 1

P01 Yoshikazu Adachi and Midori Kozawa The differences of colony formation between *Brachyspira pilosicoli* and *Brachyspira aalborgi*

P02 Shō Ogata, Ken Shimizu, Susumu Tominaga and Kuniaki Nakanishi Immunohistochemistry of mucins in human intestinal spirochetosis

P03 Werner Herbst, Jessica Joerling, Annegret Reiher, Stefanie Barth and Christa Ewers Genetic and phenotypic studies on the hemolysins of different *Brachyspira spp.*

P04 Ewart Sheldon, Roderick M. Card, Emma Stubberfield, Gemma Kay, Susanna Williamson, Nabil-Fareed Alikhan, Muna F. Anjum and Mark J. Pallen Whole-genome sequencing to investigate pleuromutilin resistance and the epidemiology of *Brachyspira hyodysenteriae*

P05 Brandon A. Keith, John Harding and Matthew E. Loewen Characterization of tlyA and tlyC from "Brachyspira hampsonii"

Session 2

P06 Elise Bertet and Jean Le Guennec Looking back over five years of *Brachyspira* PCR result monitoring in French laying hens (2010-2014)

P07 M. Harms, V. Schmidt, T. Heydel, J. Hauptmann, R. Bergmann, C. Ahlers, J. Rohde and C. Baums Differentiation of *Brachyspira spp.* isolated from laying hens by PCR-based methods and MALDI-TOF-MS

P08 Jade Passey, Martin J. Woodward, Jorge Gutierrez and Roberto M. La Ragione Lactobacilli probiotics as a potential control strategy for avian intestinal spirochaetosis

Session 3

P09 Kerry Newbrook, Gareth J. Staton, Simon R. Clegg, Richard J. Birtles, Stuart D. Carter, Nicholas J. Evans *Treponema ruminis* sp. nov., a spirochaete isolated from the bovine rumen.

Session 4

P10 Lorena Álvarez, Marta García, Ana Carvajal, Joan Marco, Núria Aloy, Ana M. Pérez de Rozas, Sergio López-Soria, Ignacio Badiola-Sáiz, and Pedro Rubio Evaluation of an infection model of swine dysentery

P11 Anna Carlertz, Claes Fellström, Magdalena Jacobson, Maria Lindberg, Therese Råsbäck, Helle Ericsson Unnerstad and Annette Backhans Occurrence and epidemiology of *Brachyspira hyodysenteriae* in Swedish pig herds

P12 Judith Rohde, Petra Grünig and Kerstin Habighorst-Blome *Brachyspira suanatina* and “*Brachyspira hampsonii*” in German pigs


P14 Anna Borgström, Simone Scherrer and Max M. Wittenbrink *Brachyspira* diagnosis - faster, cheaper and more sensitive

P15 Magdalena Jacobson, Annette Backhans and Claes Fellström Elimination of *Brachyspira spp.* during the establishment of the new Swedish University Research Centre pig herd

P16 Ruwini Kulathunga and Joseph E. Rubin Antimicrobial susceptibility of Western Canadian *Brachyspira* isolates

P17 Constanze Kirchgässner, Max M. Wittenbrink and Sarah Schmitt Challenges of antimicrobial susceptibility testing of *Brachyspira hyodysenteriae*

P18 Friederike Zeeh, Joachim Frey, Niels Grützner, Silvio De Luca, Pamela Nicholson, Vincent Perreten and Heiko Nathues Detection of *Brachyspira hyodysenteriae* in three different parts of the intestine from slaughtered pigs in Switzerland

P19 Lorena Álvarez, Rubén Miranda, Joan Marco, Pedro Rubio and Ana Carvajal Antimicrobial resistance trends among *Brachyspira hyodysenteriae* isolates recovered from swine dysentery outbreaks in Spain (2011-2016)

Abstracts
Session 1:

New Understanding of *Brachyspira* species
**Introduction and Objectives**

The past decade or so has seen a transformation in molecular biology disciplines through the rapid advance and adoption of high-throughput and high-resolution methods for gaining systematic molecular understanding of organisms. Broadly, these methods may be termed “omics” and rely on advanced technologies such as sequencing and mass spectrometry. Whilst these have been revolutionary technologies, data analyses have lagged behind and biological contextualisation and interpretation has often been a secondary consideration. Notably, animal pathogens have received relatively little attention [1,2].

The objectives of this contribution are to summarise some key recent developments in Spirochaete molecular biology; to present some applications of ‘omics and to speculate how these approaches might be applied to advance understanding of intestinal spirochaetes and associated infectious states.

**Methodological considerations**

A survey of intestinal spirochaete (principally *Brachyspira* species) scientific literature of recent years and of publicly available ‘omics resources has revealed relatively little activity in these areas. This has been despite the ongoing advances in cost-effective, high-throughput methodologies for systematic molecular characterisation particularly in the areas of genome and transcriptome sequencing and – to a lesser extent – proteomics and metabolomics. ‘Oomics methodologies are increasingly widely used and offer effective means for detailed characterisation of fastidious and host-associated micro-organisms. Some recent ‘omics-based investigations have targeted spirochaetes, mainly *Borrelia*, *Leptospira* or *Treponema*. Nonetheless, these – and many other studies – provide examples of preparative and analytical approaches that could be adopted for ‘omics characterisation of intestinal spirochaetes. Whilst the technologies that enable ‘omics have advanced rapidly, there has been a lag phase towards the development and application of computational and bioinformatics tools that facilitate of interpretation for clinical and biological scientists.

**Current status and options**

To date, molecular investigations of intestinal spirochaetes have covered a spectrum of purposes including: development and utilisation of molecular typing approaches (MLVA & MLST); vaccine candidate identification; characterisation of plasmid and phage carriage, composition and role; definition of surface antigen heterogeneity within and between strains; and, characteristics of antibiotic resistance. Added to these, proteomics (MALDI mass spectrometry) has been assessed as a means for identification of isolates and comparative genomics has been applied in some instances. All provide important advances in their own right although fully systematic investigations have not been reported to date.

Given the significant impact of intestinal spirochaetes – *Brachyspira* in particular – as pathogens (as well as their relative amenability for use in addressing fundamental biological aspects of spirochaetes) it is perhaps an as yet missed opportunity that so few ‘omics resources and applications have become available for these organisms. Central among these resources are genomes and – as of mid-2016 – only 34 genome sequences representing 8 named *Brachyspira* species were present in public repositories. Of these, only one species (*B. hyodysenteriae*) was represented by more than 10 genomes. Although relatively well-resourced compared to many other livestock pathogens, this relative paucity of information constrains potential analyses of much wider significance and productivity. Furthermore, as seven of these species are represented by few genomes, most of the resource relates to individual isolates rather than representatives of strains or species. Supplementation of the systematic molecular resource for intestinal spirochaetes would provide major benefits crucial towards biological characterisation and rationale design of interventions.

The value of systematic molecular (‘omics) studies of numerous pathogenic and non-pathogenic bacteria is well-documented in many studies. A range of approaches can be applied and tailored dependent upon the purpose – a non-exhaustive summary is as below:
Genomic analyses with substantial numbers of isolates from disparate origins (e.g. different countries, production systems, host species) provides a highly effective means for identifying both common and variable features among strains and species. This may be useful not only for (re)designing rapid, reliable and accurate molecular diagnostic and typing systems but also for selection and prioritisation of potential vaccine candidates, for instance.

Functional aspects can begin to be defined through transcriptomics and/or proteomics which measure expression of genes (as transcripts) and proteins, respectively. As well as adding a physiologically relevant perspective, these approaches may, for instance, define inter-strain differences which may only become apparent at level of expression. These methods can also provide significant insight into determinants of adaptation, fitness and pathogenicity. For instance, assessment of sub-cellular compartments such as surface or exported proteins, offers a route towards identification of factors involved in host interaction. These methods remain most suitable for in vitro investigations although they are increasingly applicable to infection in vivo with suitably planned and executed experimentation.

Phenomenal advances have been made in the application of high-throughput functional appraisal. These systems allow genome-wide screening of bacteria to identify genes and their products that confer a function of interest. For example, there is an increasing number of examples where variations on this approach have been taken to identify bacterial genes necessary for colonisation and pathogenicity in a range of host species. These methods require capability to genetically manipulate the target organism and for a relevant in vitro or in vivo infection system. As well as providing comprehensive coverage of genome function, this approach may allow fast-track identification of intervention targets. Although the technological, analytical and computational aspects are crucial in the approaches briefly summarised here, the gaining of biologically meaningful knowledge of fundamental and applied value is paramount.

Summary and Perspective

The molecular biological investigations of intestinal spirochaetes carried out to date have provided important advances in understanding which have advanced basic understanding of these organisms as well as offering utility. This work offers a platform for further systematic investigations of these organisms in the laboratory and in their ecological niche. Recent advances in ‘omics can be adopted for these goals, however, a significant requirement is the prioritisation of objectives and clarity of purpose and benefit. What should the priorities centre on: Improving genome population biology? Defining pathogenicity determinants and vaccine candidates? Identifying host-range and pathotype-determining factors? Determining host responses and disease resistance mechanisms? Focussing on our shared interests in intestinal spirochaetes, we should address the challenge: “What, to you, are the important issues that molecular understanding can bring?” and “How do we achieve this?”.

References


Exploring the molecular basis for antimicrobial resistance in *Brachyspira hyodysenteriae* using whole genome sequencing

Roderick M Card¹, Emma Stubberfield¹, Jon Rogers², Javier Nunez¹, Richard Ellis¹, Ben Strugnell³, Chris Teale⁴, Susanna Williamson², Muna F Anjum¹

¹Animal and Plant Health Agency (APHA), Weybridge, Addlestone, UK; ²APHA Bury St Edmunds, Bury St Edmunds, UK; ³Farm Post Mortems Ltd., Bishop Auckland, UK; ⁴APHA Shrewsbury, Shrewsbury, UK

Introduction and Objectives

*Brachyspira hyodysenteriae* is the causative agent of swine dysentery, a disease of significant economic and animal health importance. Resistance to tiamulin and other antibiotics presents a significant risk to the successful treatment and control of swine dysentery. Although single nucleotide polymorphisms (SNPs) have been associated with resistance to tiamulin and other antibiotics there remains an incomplete understanding of the mechanisms of antibiotic resistance in this pathogen. In England the diversity of *B. hyodysenteriae* isolates is poorly defined.

Objectives

3. Investigate associations between antibiotic resistance phenotype and genotype.

Materials and Methods

Thirty-four *B. hyodysenteriae* isolates from the APHA culture collection were tested for susceptibility to six antibiotics by broth dilution (VetMIC Brachy; SVA, Sweden). The whole genome sequence (WGS) of each isolate was determined with the Illumina MiSeq. Each WGS was mapped to the reference genome WA1, core genome SNPs extracted using SeqFinder¹ and a phylogenetic tree generated. Published antibiotic resistance SNPs were identified in the WGS and compared to susceptibility data.

To screen for tiamulin resistant mutants in vitro, 19 isolates were cultured at 10⁸ CFU on plates containing inhibitory concentrations of tiamulin. Mutant isolates growing on these plates were recovered, tested for antibiotic susceptibility and sequenced. SNPs unique to the mutant isolate were identified by comparison of WGS to the parent isolate.

Results

A phylogenetic tree constructed using core genome SNPs showed considerable overall diversity in the isolates and also identified closely related strains on different farms. At one farm, two episodes of swine dysentery were separated by one year and the same clone was found to be responsible for both episodes. The first disease episode was treated with tiamulin and isolates from cases a year later had acquired resistance to tiamulin due to a G2032A mutation in the 23S rRNA gene, a mutation known to confer resistance to pleuromutilins. For the remaining tiamulin-resistant isolates studied, however, there was no clear association with known resistance mutations.

Importantly, all isolates that exceeded wild-type breakpoints² for doxycycline, lincomycin, tylosin and tylvalosin had mutations at SNPs known to confer resistance to these antibiotics; at the position associated with doxycycline resistance we identified a new polymorphism in three isolates.

From the in vitro screen, 16 mutants with decreased susceptibility to tiamulin were recovered from 6 field isolates. SNPs at positions associated with tiamulin resistance in 23S rRNA or L3 protein were identified in 11 mutants, of which 9 had new polymorphisms. One mutant had a SNP that caused an amino acid substitution in EF-G, that may account for decreased susceptibility. In 4 mutants no SNP was uncovered and these are being examined further.

Discussion

WGS uncovered considerable diversity in English *B. hyodysenteriae* and revealed the development of tiamulin resistance on farm following antibiotic administration. Decreased susceptibility to doxycycline, lincomycin, tylosin and tylvalosin could be predicted from the WGS. This work has expanded the repertoire of SNPs associated with tiamulin resistance thereby giving a broader understanding of this pathogen’s mechanisms of antibiotic resistance.

References

Whole genome sequencing of weakly haemolytic *Brachyspira* genetically identified as *B. hyodysenteriae*

Roderick Card¹, Eric R. Burrough², Richard Ellis¹, Javier Nunez-Garcia¹, Judith Rohde³, Jill Thomson⁴, Alexander W. Tucker⁵, Lucy Weinert⁵

¹ Animal and Plant Health Agency, Weybridge, UK, ² Iowa State University, Ames, Iowa, USA, ³ Institute for Microbiology, Department of Infectious Diseases, University of Veterinary Medicine, Hannover, Germany, ⁴ Scottish Agricultural College Veterinary Services, Bush Estate, Penicuik, Midlothian, Scotland, UK., ⁵ Dept. Vet. Med., University of Cambridge, UK.

**Introduction and Objectives**

*Brachyspira hyodysenteriae* (Bh) is described as a strongly haemolytic (sh) *Brachyspira* species and, along with other sh species, is recognized as a cause of swine dysentery (SD), a disease of significant economic importance. Within the pig industry, the requirement for breeding companies to supply pigs that are free from swine dysentery, as part of their health assurance policies, requires reliable detection methods. Currently this involves bacterial culture plus phenotypic characterisation and/or a number of molecular-based methods.

Recently *Brachyspira* spp. isolates have been recovered from high health pig holdings without SD that were identified as *B. hyodysenteriae* by PCR and partialnox-gene sequencing but on culture were weakly haemolytic (wh) and identified phenotypically as *Brachyspira intermedia*. Whole genome sequencing (WGS) of such whBh isolates was initiated to elucidate their speciation and to characterise their diversity and relationship with shBh isolates.

**Materials and Methods**

*Bacterial isolates* A total of nine *B. hyodysenteriae* isolates were investigated: Four whBh isolates from German pigs without SD; three shBh isolates from German cases of SD and two shBh Spanish isolates from pigs with SD.

The WGS of each isolate was determined using the illumina MiSeq. MLST profiles and nox gene sequences were extracted from the data. Isolate speciation was by blastn of the nox gene and by analysis of the WGS with Kraken [1]. Each WGS was mapped to the reference genome WA1, the core genome SNPs extracted using SeqFinder [2] and a phylogenetic tree generated. The core and accessory genome of our isolates were also compared to published genomes by rapid large-scale prokaryote pan genome analysis (Roary) [3].

**Results**

*Speciation* All isolates were identified as *B. hyodysenteriae* by Kraken and blast of nox gene. MLST and core genome phylogeny Three epidemiologically related German whBh isolates had identical (new) MLST profiles and clustered together in the phylogenetic tree. The shBh isolates had different ST from whBh isolates. A fourth epidemiologically unrelated whBh isolate had a different (new) ST but clustered closer with the other three whBh isolates than with any other of the *B. hyodysenteriae* isolates.

*Roary* The Roary analysis showed differences in the accessory genome between shBh and the three epidemiological related whBh isolates. Differences, although visually less clear cut, were still apparent when the additional unrelated whBh isolate was included in this analysis. Sequences uniquely present in the accessory genome of the shBh or whBh groups were identified.

**Discussion**

Weakly haemolytic *Brachyspira* isolates from healthy German pigs were identified as *B. hyodysenteriae* using a whole genome approach. Based on MLST and core genome phylogeny they were distinct from other concurrent sh *B. hyodysenteriae* as well as from other published sequences. These whBh isolates are indistinguishable from shBh isolates in currently available molecular tests and pose a serious complication for pathogen surveillance in pig trade. Strong haemolysis is considered to be an important determinant of virulence among *Brachyspira* spp. and a predictor of association with SD. Future studies may shed light on the basis for reduced haemolysis, and virulence has to be assessed in challenge studies. Genome wide association studies may support the design of more sensitive and specific diagnostics tests. Until that time our findings support the combined use of molecular and haemolysis testing for comprehensive surveillance purposes in SD negative herds.

**References**

Determining *Brachyspira hyodysenteriae* sequence types and possible relations to virulence genes and antimicrobial susceptibility

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Introduction and Objectives

Swine dysentery (SD) is an economically important diarrheal disease in pigs caused by *Brachyspira (B.) hyodysenteriae*. As phylogenetic studies of *B. hyodysenteriae* are very scarce, we aimed to elucidate the genetic diversity of this pathogen and decipher possible relations between multilocus sequence types (STs), virulence-associated genes (VAGs) and antimicrobial susceptibility.

Materials and Methods

*B. hyodysenteriae* isolates collected between 2003 and 2015 from diarrheic pigs from Hesse and Northern Germany (n=106) were used for MLST analysis (1, 2). VAGs coding for hemolysins (*tlyA, tlyB, tlyC, hly, hlyA, 1870, yplQ*), outer membrane proteins (*bhlp16, bhlp17.6, bhlp29.7, bhmp39f, bhmp39h*) and iron acquisition factors (*ftnA, bitC*) were screened by PCR (3). Antimicrobial susceptibility was determined by a microdilution assay (4).

Results

MLST grouped the majority of our isolates into ST52 (n=47), ST8 (n=14) and the novel ST112 (n=29). Another 16 isolates belonged to the new STs ST113-118, ST120-123, and ST131. According to published breakpoints, 35.9% of the strains were resistant to tiamulin and to valnemulin (5). Resistant strains were significantly associated with ST52 and ST112, while susceptible strains mostly belonged to ST8. While hemolysin, ferritin and OMP gene *bhlp29.7* were regularly detected, all isolates were negative for *bhlp17.6*. *Bhlp16* was present in 58.5% of the isolates, but absent in ST116, ST122, and ST131 and rarely detected in ST52 isolates (14.9%).

Discussion

Our data indicate that a few dominant STs are prevailing in Germany, while the presence of new STs suggests an ongoing diversification of the pathogen. The MLST website (http://pubmlst.org/brachyspira/) lists 138 STs and 344 *B. hyodysenteriae* isolates (april 2016). Here, ST8 and ST52 are not present among 164 non-European isolates, but among single isolates from European countries, suggesting a spatial distribution of genotypes. While VAGs were rather conserved among STs, antimicrobial resistance followed the phylogeny of the strains. This may be due to the underlying resistance mechanism (mutations in ribosomal proteins) and provides valuable information regarding the worldwide use of valnemulin and tiamulin for SD therapy.

References

Introduction and Objectives
The mechanism by which *Brachyspira hyodysenteriae* and "*Brachyspira hampsonii*" (clade 2) cause mucohaemorrhagic colitis and diarrhea is currently unknown. Argenzio et al. demonstrated that *B. hyodysenteriae* infection abolished the absorptive capacity of the porcine colon resulting in malabsorptive diarrhea (1). Characterization of Cl- secretion and the effect on Cl- channel gene expression in the colonic mucosa has not been assessed in *B. hyodysenteriae* or "*B. hampsonii*" infected animals. Excessive Cl- secretion in the colon can cause the onset of diarrhea and be a potential pathophysiological mechanism.

The purpose of the present study is to characterize the electrogenic Cl- secretory response in the porcine colon and to determine whether there is a change in Cl- channel gene transcripts and inflammatory cytokine profiles in *B. hyodysenteriae* and "*B. hampsonii*" (clade 2) diseased pigs.

Materials and Methods

Electrogenic Secretory Ussing Chamber Studies
Ussing chamber studies were conducted on the proximal, apex and distal segments of the porcine colon after pigs were experimentally inoculated and then euthanized at peak clinical signs. Secretion was activated via isoproterenol (adrenergic agonist), carbachol (cholinergic agonist) and forskolin / 3-isobutyl-1-methylxanthine (IBMX) to assess the diseases effect on cAMP and calcium-activated short-circuit current (*Isc*). *Isc* is a measurement of ionic movement described as the current needed to stop the movement of ions. Additionally, inhibition of Na-K-Cl co-transporter 1 (NKCC1) by bumetanide, was used to determine its effect on secretion. These studies were performed on control, *Brachyspira hyodysenteriae*, and "*Brachyspira hampsonii*" (clade 2) infected samples.

qPCR Analysis of Inflammatory Cytokine, Ion Channel and transporter mRNA Expression
Fold changes of ion channels and transporters as well as inflammatory cytokines were compared between infected and control samples through the use of RT-qPCR in the three segments of the porcine colon.

Results
Activation of secretion via isoproterenol (adrenergic agonist), carbachol (cholinergic agonist) and forskolin / 3-isobutyl-1-methylxanthine (IBMX) demonstrated a significantly decreased change in short-circuit current (*Isc*) in infected pigs in all three sections of colon compared to control. Tissue resistances did not account for this difference, rather, it was attributed to a decrease in Cl- secretion as indicated by a significant decrease in bumetanide inhibitable short-circuit current in infected animals. RT-qPCR was conducted to determine if a decrease in gene transcript was responsible for the loss of Cl- secretory function. The major chloride transport proteins of the epithelium, were compared between healthy and diseased animals in all three sections of colon. mRNA expression for these channels as well as inflammatory cytokines were found to be elevated in the infected samples compared to controls.

Discussion
Significantly decreased change in short-circuit current (*Isc*) paired with elevated channel and inflammatory cytokine gene transcripts suggests that these two *Brachyspira* species are able to disrupt ion transport by three possible mechanisms. The decrease in Cl- secretion may be attributed to *Brachyspira*s ability to directly inhibit ion channel function. Furthermore, it is also possible that these bacteria reduce translation of channel mRNA to protein or inhibit protein trafficking to the epithelial surface.

References
Introduction and Objectives

*Brachyspira hyodysenteriae* colonizes the pig colon causing swine dysentery (SD). The pig colon is covered by a mucus layer composed by large glycoproteins called mucins. *B. hyodysenteriae* infection causes changes in the mucin environment characterized by a disorganized mucus structure and a massive mucus induction with *de novo* expression of MUC5AC and increased production of MUC2 in the colon (1).

The aims of the present study were to determine how factors from the immune system change in the pig colon during SD and identify which of these changes are important for mucin production.

Materials and Methods

Colon specimens from experimentally inoculated pigs with *B. hyodysenteriae* (n=5) and control pigs (n=6) were obtained in order to determine the gene expression of factors from the immune system during infection. RNA was isolated from the pig colon tissue samples using Trizol. Total RNA (5 µg) was DNase treated and cDNA was synthesized to be used in a real-time PCR reaction.

Polarized mucus producing in *vitro* mucosal surfaces developed from HT29 MTX-E12 colonic cells (2) were stimulated with IL-1β (0.25 ng/ml), IL-6 (15 ng/ml), IL-8 (4 ng/ml), IL-17A (2 ng/ml) and neutrophil elastase (NE, 0.2 µg/ml), individually and combined, with and without *B. hyodysenteriae* infection. The production and transport speed of newly synthesized mucin was assessed after incubating the treated in *vitro* mucosal surfaces with an azide-modified galactosamine (GalNAz) for two hours. GalNAz was metabolically incorporated into the newly synthesized mucin O-glycans to determine its intensity and location by immunofluorescence. The percent of goblet cells was assessed through PAS/Alcian blue staining, and immunofluorescence staining of MUC2 and MUC5AC.

Results

Quantification of the mRNA levels of several factors from the immune system by qPCR revealed increased expression levels of NE in the colon tissue of *B. hyodysenteriae* infected pigs, a nine-fold increase in the mRNA levels of IL-17A, and upregulation of IL-1β, IL-6 and IL-8 in at least two out of five pigs. The results showed that the combined effect of NE and *B. hyodysenteriae* infection increased the total number of mucin containing goblet cells in the in *vitro* mucosal surface, when compared to NE treatment without infection (P<0.05). This increment in the number of goblet cells occurred regardless of the cytokine environment. The combined effect of *B. hyodysenteriae* infection and NE also increased the mucin production and transport speed from golgi (around the nucleus) towards the secretory vesicles at the apical surface of goblet cells in the in *vitro* mucosal surfaces by 50% when compared to NE treatment without infection (P<0.0001), and by 30% when compared to the infected untreated control (P<0.0001).

Discussion

The results from the present study show that several factors from the immune system are differentially expressed in the pig colon during *B. hyodysenteriae* infection; furthermore we determined one of these factors to be associated with mucin production in *vitro*.

Treatment of the in *vitro* mucosal surfaces with the upregulated factors from the immune system showed that the combined effect of *B. hyodysenteriae* infection and NE treatment increased the mucin production and total number of mucin containing goblet regardless of the cytokine environment.

Overall, our data suggests a synergistic effect between bacterial factors and NE in mucus induction during *B. hyodysenteriae* infection. Thus, the local increase in expression of NE during *B. hyodysenteriae* infection is likely to affect the mucin production and translocation in the pig colon.

References

Session 2:

Intestinal Spirochaetes in Poultry and Wild Birds
Intestinal spirochaetes in poultry

David Burch

Introduction

Reports on the findings that spirochaetes, now recognised as *Brachyspira* spp, were causing typhlocolitis and diarrhoea with subsequent affects on egg production in chicken flocks, started to appear in Europe in 1986 & 1987, in the Netherlands and the UK, respectively (1, 2). Subsequently, the infections in hens were reported as being caused by *B. pilosicoli*, *B. intermedia*, and a variety of other species, such as *B. innocens*, *B. murdochii*, *B. alvinipulli*, *B. pulli*, even *B. hyodysenteriae*, normally associated with pigs, sometimes as single infections but often as mixed ones. There have been reports of *Brachyspira* spp infections in other avian domesticated species, such as the turkey (3), goose (4) and duck (5).

This initial work was taken up in particular by the Perth University Team that really explored the incidence of infections in layer and breeder flocks, their epidemiology, pathology, possible contributory factors and also its treatment. As this work was going on, the technology to improve diagnosis and strain differentiation was exploding from microscopy, culture and metabolic profile to genetic markers for PCR work and even MALDI-TOF classification.

Strain pathogenicity has also been an important feature to determine which strains are likely to cause a disease effect and depress egg production or growth. *Brachyspira pilosicoli*, *B. intermedia*, *B. alvinipulli* and *B. hyodysenteriae* are considered likely to be pathogenic and *B. innocens* and *B. murdochii* non-pathogenic. However, infection with pathogenic strains did not necessarily mean disease on a flock basis, although individually infected hens may have been affected.

Incidence of infections in chicken flocks

A number of studies have been carried out in Australia, Europe (NL, UK, It), Asia (Malaysia), Colombia and the USA, looking at the incidence of infections, mainly in layer flocks but also in breeders. Commonly spirochaetes can be found in 50-80% of flocks investigated but the pathogenic strains usually in about 25 to 50% of flocks.

In a survey (6) in the UK it was noted that free-range flocks seemed to develop infection earlier, soon after point of lay (22 weeks), in comparison with caged flocks at about 36 weeks of age. So the age of flock tested can have a significant effect on incidence. A study in the US (7) showed that 86% of flocks were infected with potentially pathogenic strains but flocks of over 40 weeks of age were tested.

The type of housing and other factors are also considered important in the transmission of infection and its impact. Free-range hens can go out into ranges, which may have been contaminated previously or may be contaminated by other means, as there is no real means of biosecurity. The hens like to drink from potentially contaminated puddles hence the spread and impact of infection can be quicker and higher. Older caged flocks with deep pit waste disposal also appeared to be at a higher risk and it is thought that contamination by flies of hen feed may be an important infection route. It is often difficult to control flies in these circumstances and also in open-sided sheds as found in hot climates like Asia. Rodents are also considered potential carriers and transmitters of infection (8).

The transmission and infection rate of a flock is considered important. Even if a flock is infected but few birds are affected then it is unlikely to have a major impact on egg production. It is only when a substantial percentage of birds (>20%) are affected that production anomalies start to show. For example in some deep pit sheds 40% of the droppings can be abnormal but in free-range units 80% of the droppings can be affected and consequently lower egg production and increased mortality are reported. In some cage systems, where the droppings are removed from the house and potential contact is less, abnormal caecal droppings can be as low as 1% and production unaffected.

Broiler breeder flocks seem to have a lower infection rate than layers (9) and it is uncommon to find it in broiler flocks or in replacement pullet flocks. This may be due to a number of factors such as improved hygiene or management or the use of anticoccidial drugs, such as the ionophores, which do have activity against *Brachyspira* spp.

Effects on production

In a UK study (10), an untreated layer flock with *B. pilosicoli* showed a 6% reduction in egg production
and an increase in mortality of 8.8% in comparison with the breed standard over a complete laying period to 72 weeks of age. A number of authors reported drops in egg production of 5-13%, especially in free-range hens and onset would occur around peak production time. These infections are chronic in nature and the mortality increase is considered a result of this. Broiler breeder flocks can be similarly affected and may affect broiler flock performance subsequently (11).

Treatment and control of infections

A number of antibiotics have been used to treat and prevent the infection with *Brachyspira* spp including ronidazole, lincomycin and tiamulin. Trials have shown the efficacy of these products both in artificial infection studies and in field trials (10, 11).

The minimal inhibitory concentrations (MICs) of a number of antibiotics were reported (12) against 20 *B. intermedia* isolates.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC 50</th>
<th>MIC 90</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tilmicosin</td>
<td>0.5</td>
<td>2.0</td>
<td>0.125-≥128</td>
</tr>
<tr>
<td>Tylosin</td>
<td>1.0</td>
<td>2.0</td>
<td>0.5-≥128</td>
</tr>
<tr>
<td>Tiamulin</td>
<td>0.125</td>
<td>0.5</td>
<td>0.031-2.0</td>
</tr>
<tr>
<td>Valnemulin</td>
<td>0.063</td>
<td>0.25</td>
<td>≤0.016-0.25</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.25</td>
<td>2.0</td>
<td>0.063-2.0</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>0.5</td>
<td>1.0</td>
<td>0.125-8.0</td>
</tr>
</tbody>
</table>

There was very little resistance development in comparison with *B. hyodysenteriae* from pigs.

Tiamulin was tested against 25 isolates of avian *B. pilosicoli, B. intermedia* and *B. innocens* (13).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC 50</th>
<th>MIC 90</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. pilosicoli</em></td>
<td>0.062</td>
<td>0.25</td>
<td>0.0075-4.0</td>
</tr>
<tr>
<td><em>B. intermedia</em></td>
<td>0.125</td>
<td>0.5</td>
<td>0.015-8.0</td>
</tr>
<tr>
<td><em>B. innocens</em></td>
<td>0.062</td>
<td>2.0</td>
<td>0.015-8.0</td>
</tr>
</tbody>
</table>

Similar susceptibility ranges for each species were found.

Following the original extensive use of antibiotics to control the infection, especially in free-range systems in the UK, many practices are trying to reduce antibiotic use in poultry and have explored different methods of control. The inclusion of organic acids, mixtures of probiotics and gut immunity stimulants have been successfully employed.

Discussion

*Spirochaetosis* appears to be well spread around the world. The type and age of flock is important in recovering *Brachyspira* spp and the severity of the disease. It can cause a reduction in egg production and an increase in mortality, depending on the prevalence of the disease in the flock. Flies and rodents may be helpful in transmitting the infection. Treatment with antibiotics is usually very effective but subsequent breakdown is common, especially if hygiene is not improved. Alternative methods of control are being evaluated and appear to be quite successful.

References

Introduction and Objectives
There is mounting evidence of a Brachyspira carrier state in some free-living wild bird species, but more information is needed on host range and transmission capabilities to livestock and poultry. Studies from Australia, Sweden, Spain and Canada have shown high isolation rates (9–78%) from various wild anseriform bird species (ducks and geese). Moreover, available studies show that ducks and geese may harbour a wide range of Brachyspira spp. and genotypes, including several potential pathogens. No domestic species is yet known to host such a broad range of intestinal spirochaetes as ducks. Similar data from non-anseriform species is rudimentary.

Possible transmission between wild birds and domestic animals
Two recent examples suggest that Brachyspira spp. may not only be transmitted from waterfowl to pigs, but also that some strains may cause dysentery-like disease in pigs. “Brachyspira suanatina” was isolated from pigs and migratory mallards (Anas platyrhynchos) in northern Europe (1), and transmission between the two host species was experimentally proven (2). “Brachyspira hampsonii” was isolated from pigs and waterfowl (Lesser snow goose, Chen caerulescens caerulescens; Greylag goose, Anser anser; and mallard) in North America and Europe (3, 4). These results thus suggest that intestinal spirochaetes may occasionally be transmitted from the wild avifauna to domestic animals, and allowed to adapt to new hosts. Wild anseriform birds must therefore be considered as a possible source of Brachyspira.

Southern Atlantic island and Antarctica
Another recent study (5) described findings of intestinal spirochaetes in 10 out of 205 (5%) samples originating from 3 out of 11 avian species on the Falkland Islands, southern Atlantic island and the Antarctic Peninsula. Isolates orginated from three kelp geese (Chloephaga hybrida subsp. malvinarum), snowy sheathbills (Chionis albus), and three brown skua (Stercorarius antarcticus subsp. lonnbergi). Interestingly, the two latter wader species are both scavenging and could be expected to represent a variety of ecological niches including the intestines of mammals and other birds, the immediate environment and water. It is unknown if these birds become colonized locally or at other sites where they migrate during the austral winter season. However, a livestock, poultry or human source is highly unlikely.

Below, we present some previously unpublished culture results from free-living wild birds in northern Europe.

Materials and Methods
Between 2004 and 2010 cloacal swabs and faecal samples were collected from a variety of free-living wild birds. Samples originated from dead birds submitted for diagnostic necropsy to the National Veterinary Institute (SVA) of from migratory birds caught and examined at Ottenby Bird Station on the island of Öland in south-eastern Sweden. A variety of diagnoses were found in necropsied birds, but none displayed signs or lesions suggesting spirochaetal enteric disease. All migratory birds were in good condition and appeared healthy.

The samples were cultured and phenotyped by routine diagnostic methods. Identification as intestinal spirochaetes relied on phase contrast microscopy.

Results
Table 1. Intestinal spirochaete culture results (no of positive/total) from free-living wild avian hosts. (Previously published results from ducks, corvid birds and southern Atlantic/Antarctic birds not included).

<table>
<thead>
<tr>
<th>Species</th>
<th>Latin name</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common buzzard</td>
<td>Buteo buteo</td>
<td>0/1</td>
</tr>
<tr>
<td>Eurasian sparrowhawk</td>
<td>Accipiter nisus</td>
<td>0/1</td>
</tr>
<tr>
<td>Willow Ptarmigan</td>
<td>Lagopus lagopus</td>
<td>0/11</td>
</tr>
<tr>
<td>Common crane</td>
<td>Grus grus</td>
<td>0/2</td>
</tr>
<tr>
<td>Eurasian coot</td>
<td>Fulica atra</td>
<td>1/1</td>
</tr>
<tr>
<td>Black-tailed godwit</td>
<td>Limosa limosa</td>
<td>0/9</td>
</tr>
</tbody>
</table>
Eight out of 121 bird were culture positive for intestinal spirochaetes; waders (Eurasian coot and Ruff) and passerines (Blackbird and Song thrush).

All eight isolates were weakly haemolytic, and indole-spot and hippurate test negative. Several were slow-growing.

**Discussion**

Even though the number of sampled birds and species is small and represents a single geographical region the results confirm previous studies that have shown that free-living wild birds may be colonized by spirochaetes. Interestingly some families and species seem to have a very complex spirochaetal flora while others are not or only rarely colonized.

Clearly, more research needs to be performed to assess risks of transmission associated with birds to domestic animals. In the meantime, biosecurity measures in pig herds and on poultry farms should be strictly adhered to.

**References**

Histopathological intestinal changes and shifts in intestinal microbiota in experimentally induced dysbacteriosis in chickens

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Introduction

Dysbacteriosis was induced in brown commercial layers by exposing them to one of four different treatments. The aim was to study whether histopathological intestinal abnormalities and shifts in intestinal microbiota occurred, and whether the histopathological and microbiota changes found were unique for each of the respective treatments.

Materials and Methods

Five experimental groups each consisting of nine commercial Lohmann brown laying hens of 18 weeks of age were made and housed on negative pressure isolators. A non-treated group acted as negative control. The other groups were treated as follows: provision of feed with anomalous composition during five consecutive days (group I), administration via drinking water of 50 mg/kg body weight ampicillin during 5 consecutive days (group II), oral administration of a NetB and β2 toxin producing Clostridium perfringens type A strain as a single dose of 10^6 cfu after 36 hours of feed withdrawal (group III) and oral administration of 10^4-8 cfu of a virulent avian Brachyspira intermedia strain 1380 during 5 consecutive days. The latter three treatments were chosen as they have been associated with intestinal disorders previously (1,2,3,4). Haematoxylin and eosin-stained tissue samples of intestinal wall (duodenum, jejunum, ileum and cecum) were collected for histopathology. Besides samples of intestinal wall as described, intestinal content and droppings were collected to characterize the gut microbiota by mass pyrosequencing using the Roche 454 GLX system. Samples were obtained during post-mortem examination 24 hours after completion of the respective treatments and also during the recovery phase. At each time point three birds per group were examined.

Results

Although histopathological changes were generally mild, they often showed typical features for each treatment. Histopathological changes were absent or minimal in the negative control group. Villus atrophy with mild enteritis characterized by infiltrations of macrophages, gastrointestinal lymphoid tissue (GALT) activation and crypt hyperplasia was present in the caeca of all chickens inoculated with B. intermedia. Furthermore, these lesions were less consistently present in the ileum, jejunum and duodenum. Histological changes in the group inoculated with NetB and β2 toxin producing C. perfringens type A strain were most prominent in the jejunum and the ileum. Multifocal to diffuse mild infiltrates of heterophils and macrophages were present within the lamina propria, accompanied by villus atrophy. Crypt hyperplasia as regenerative hallmark was found in a few animals during the recovery phase. In chickens provided with feed with anomalous composition the jejunum was predominantly affected showing increased numbers of intraepithelial lymphocytes, sometimes accompanied by duodenal and jejunal villus atrophy and crypt hyperplasia during the recovery phase. Animals that were given ampicillin, sometimes showed increased intraepithelial lymphocytes and GALT activation in the jejunum and duodenum. Mass pyrosequencing showed a shift in microbiota in the treated birds compared to the negative control birds. However, shifted microbiota patterns could not be related unambiguously to the respective treatments. Exception was the group inoculated with B. intermedia in which increased numbers of Corynebacterium spp. were found in the ileum, caecum and droppings.

Conclusion

Although in general the histopathological changes were mild, they often showed typical features for each treatment. Shifted microbiota patterns could not be related unambiguously to the respective treatments. Exception was the group inoculated with B. intermedia. In this group increased numbers of Corynebacterium spp. were found in samples of ileum, cecum and droppings.

References

Introduction and Objectives

Introduction:

Avian intestinal spirochaetosis (AIS) caused by Brachyspira spp., and notably Brachyspira pilosicoli, is common in layer flocks and reportedly of increasing incidence in broilers and broiler breeders in the UK. Disease manifests as diarrhoea, delayed onset of lay, reduced egg weights, faecal staining of eggshells. Treatment with Denagard® Tiamulin has been used to protect against AIS in commercial layers, but to date there has been no definitive study validating efficacy. Here, we used a poultry model of B. pilosicoli infection of layers to compare the impact of three doses (58.7 ppm, 113 ppm and 225 ppm) of Denagard® Tiamulin.

Objective:

To compare the impact of three doses (58.7 ppm, 113 ppm and 225 ppm) of Denagard® Tiamulin in laying hens.

Materials and Methods

Prior to in vivo studies MICs for the test Brachyspira strain were pre-determined in vitro. For the in vivo studies, a poultry model of B. pilosicoli infection of layers (Mappley et al., 2013) was used to compare the impact of three doses of Denagard® Tiamulin. Briefly, four groups of thirty 17 week old commercial pre-lay birds were all challenged with B. pilosicoli strain B2904 (a UK strain isolated from a case of AIS) with three oral doses two days apart. All birds were colonised within 2 days after the final oral challenge and mild onset of clinical signs observed thereafter. A fifth group that was unchallenged and untreated was also included for comparison as healthy birds. Four groups of thirty 17 week old commercial pre-lay birds were all challenged with B. pilosicoli with three oral doses two days apart. A fifth group that was unchallenged and untreated was also included for comparison. Five days after the final Brachyspira challenge, three groups were dosed with Denagard® Tiamulin in the drinking water for 5 days.

Results

Weight gain, body condition and diarrhoea in birds infected with B. pilosicoli were improved and shedding of Brachyspira reduced significantly (p=0.001) following treatment with Denagard® Tiamulin, irrespective of the treatment dose. The level and duration of colonisation of tissues of birds infected with B. pilosicoli was also reduced. We observed that the ileum, caeca, colon, liver and spleen were colonised and that treatment with Denagard® Tiamulin resulted in significant reduction in the numbers of Brachyspira found in each of these sites and a dramatic reduction in faecal shedding (p<0.001). Although the number of eggs produced per bird and the level of eggshell staining appeared unaffected, egg weights of treated birds were greater than those of untreated birds for a period of approximately two weeks following treatment (Woodward et al., 2015).

Discussion

Brachyspira pilosicoli has been identified as one of three anaerobic spirochaetes that are considered to be pathogenic in poultry and responsible for the disease AIS, the other two being Brachyspira intermedia and Brachyspira alvinipulli. AIS is a significant global economic burden for the poultry industry and thus effective treatments are urgently required. This study aimed to investigate the efficacy of different doses of Denagard® Tiamulin as a treatment for AIS.

In summary, this study demonstrated that Denagard® Tiamulin at three doses was effective at reducing B. pilosicoli infection in laying hens. Whilst the symptoms induced in the positive control were mild, the treatment with Denagard® Tiamulin clearly reduced the burden of infection in deep tissues, reduced shedding, improved weight gain and improved egg weight. These parameters are of economic importance to producers and suggest Denagard® Tiamulin is a viable treatment of AIS.

References

Session 3:
Role of Treponemes in Cattle and Humans
Introduction

Digital dermatitis (DD) is a major disease of dairy cattle which presents as severely inflamed lesions on the rear feet. The lesions are extremely painful, and are associated with significant lameness making DD an important animal health concern and one of the most important infectious diseases of cattle (1).

The infectious nature of the disease is apparent and it is now widely accepted that the primary infection in each host species are novel spirochetal (treponemal) bacteria. These are found in large numbers in bovine DD cases, often deep within foot lesions. Cloning of bacterial 16S rRNA genes indicated 5 phylotypes of treponemes in BDD lesions from Germany. This has led to the isolation and characterisation of three of these five treponeme groups from lesions in USA and UK. Furthermore, an infection model recently used pure cultures of treponemes applied to the bovine host feet to elicit DD lesions.

The currently applied treatments for cattle DD (primarily toxic footbaths or topical antibiotics) are relatively ineffective and the DD lesions recur; there is no protective immunity. Hence, it is imperative that we better understand transmission of treponeme infections and the pathological and clinical outcomes. This will be central to developing effective intervention strategies.

Our previous studies have been based on culturing and isolating treponemes from lesions which has been very successful and allowed phenotypic and genotypic analyses, so that speciation of the DD treponemes is now possible. The key finding of this work is that the same treponemes are present in all DD lesions and that they are rarely found elsewhere in cattle, apart from some detection in faeces and parts of the GI tract in some animals. Unfortunately, the antibiotics which are effective against these bacteria in vitro cannot be used in dairy cattle, due to milk withhold. Consequently, another approach to control of DD spread within and between farms is required.

The problem has been recently compounded by the spread of DD into sheep, as contagious ovine digital dermatitis (CODD), in the British Isles. The lesions are even more severe than in cattle, often resulting in complete loss of the hoof shell. Again we have identified the same treponemes as in cattle DD. Very recently, we have identified DD lesions in the feet of goats in the UK with a severe inflammatory outcome and acute lameness. Once more, the DD-associated treponemes (all 3 culturable phylogroups) are present in the lesions and not on healthy feet. We have also demonstrated that the transmission of these organisms to wildlife can occur and they are associated with DD-like lesions in elk from the US. Within farms, we have been detecting further opportunistic DD treponeme infections in a range of other cattle foot and skin lesions, making them very difficult to treat. These include toe necrosis, white line disease, sole ulcers, pressure sores and ischaemic teat necrosis. Just a few months ago, we demonstrated the DD treponemes in skin lesions in farmed pigs in the UK.

Hence, the challenge is to not only devise a way to control DD in cattle but to come up with a protocol which can be used to manage the disease in other species, stop the spread between species and deal with the effects of infection in other tissues. Our aim is to actually block disease by preventing infection with DD treponemes.

Aims and Objectives

Develop an effective vaccine against treponeme antigens to prevent initiation of DD and other DD treponeme associated lesions in farm and wildlife species.

Materials and Methods

Approach

Use reverse vaccinology technologies to make a DD vaccine based on immunisation against treponeme infection. Generate DD treponeme genomes and use bioinformatics to select putative vaccine candidates. Express as recombinants in vitro and test for immunogenicity and DD lesion prevention in cattle and sheep.
DD treponemes

We have an archive of 120 treponeme isolates from DD lesions and GI tract samples; the majority were generated at Liverpool. Genomes of representatives of the 3 culturable DD treponeme phylogroups were generated by Roche 454 sequencing. An MLST scheme for the archive has been recently developed (2).

Bioinformatics

In silico analysis utilised SignalP to identify likely surface expressed proteins TM-BETA & PRED-TMB to predict surface topology.

Production of recombinant treponeme proteins.

Genes for selected proteins were cloned into an entry vector (pENTR), transferred into an expression vector (pDEST) and expressed in Escherichia coli (BL21 DE3), eventually in large scale cultures. Recombinant proteins were purified by metal affinity chromatography and refolded to native structures as assessed by SDS-PAGE and CD spectroscopy.

Antigenicity of the recombinant treponeme proteins (RTPs).

Sera from cattle with and without DD lesions were screened for reactivity to recombinant treponeme proteins by ELISA and Western blotting.

Function of recombinant treponeme proteins

Bio-assays were developed to assess the ability of recombinant treponeme proteins to bind to cattle connective tissue molecules as this would be important in pathogenesis.

Results

Recombinant proteins

To date 45 RTPs have been cloned, expressed purified and successfully refolded. These have then been assessed for biofunctions such as antigenicity and evidence of virulence potential.

Function of recombinant treponeme proteins

Bio-assays were developed to assess the ability of recombinant treponeme proteins to bind to cattle connective tissue molecules as this would be important in pathogenesis. These assays highlighted those RTPs likely to attach well to host tissues and hence be potential key vaccine targets.

Antigenicity

Many of the RTPs were not detected as antigenic in naturally occurring disease. Most were undetectable by seropositive sera in ELISA and Western blotting.

Immunogenicity studies.

Currently, we are testing a short list of recombinant treponeme proteins (selected from the above antigenicity and bioassays) in calves to identify any generating strong immunity. Any successful proteins will then be taken forward into efficacy trials.

Discussion

From our in silico analyses, over 70 surface proteins were identified as potential vaccine targets. The majority of these were successfully expressed and refolded and several tested for immunogenicity in DD cattle with high levels of existing antibodies to DD treponeme proteins; most were undetectable by seropositive sera in ELISA and Western blotting. Hence, they may be novel antigens not normally seen by the cows’ immune response and are possibly able to generate protective immunity. If successful, this vaccine would need to be used in conjunction with other measures (eg biosecurity) to control this major endemic disease and prevent further spread of infection.

References

Introduction and Objectives

Digital dermatitis (DD) is a severe disease considered to be caused by bacteria of the genus Treponema. These fastidious, anaerobic bacteria have been shown to cause severe lameness, of varying severity in cattle, sheep and goats, and are associated with skin lesions in pigs (1, 2). These diseases lead to substantial economical losses and animal welfare issues among the affected species. Additionally, these bacteria have been reported to cause severe lameness in wild elk in North America: the first report of DD in wildlife (3). In addition, treponemes are considered to cause human periodontitis, which, like all spirochetal diseases, is generally difficult to treat.

The 16S rRNA genes of these bacteria have been sequenced and found to be similar, or in some cases identical across the different hosts, raising suggestions that the same bacteria can infect multiple hosts, and readily transmit between hosts. The cultivable DD associated spirochete 16S rRNA gene sequences consist of three phylogroups, Treponema medium, Treponema phagedenis and Treponema pedis (4).

This study used a multi locus sequence typing (MLST) approach in an attempt to ascertain if the same bacteria can cause lesions in all the species mentioned above, or if they are different subspecies affecting the different hosts.

Materials and Methods

Pure isolates of treponemes were obtained from the different host species as described previously (4). DNA was extracted from the pure cultures and isolates typed into the three different cultivable phylogroups using the 16S rRNA gene sequence (4). The MLST scheme used amplification and sequencing of seven housekeeping genes (adK, gdh, glpK, rplB, groEL, recA, and pyrG) from over 120 isolates from all different species.

These sequences were aligned and analysed using phylogenetic methods.

Results

In total, the 121 different bacterial isolates consisted of 34 T. medium phylogroup treponemes, 70 from the T. phagedensis phylogroup, and 17 from the T. pedis phylogroup. In total, 11, 35 and 7, sequence types (STs) were seen.

There was no separation of sequences by host, or by geography, and 13 of the farms in the study had different STs circulating among the animals. Conversely, some STs were geographically limited, found only circulating on a single farm, commonly on sheep farms, whereas others were much more geographically diverse. Small levels of diversity were seen within each of the MLST loci tested, and high levels of recombination within phylogroup was also observed. Interestingly, T. pedis appears to be evolving more quickly than the T. medium or T. phagedenis DD treponeme phylogroups by forming two unique ST complexes.

Human isolates whilst similar genetically demonstrated distinct STs to animal isolates.

Discussion

The recent expansion of the host range of DD treponemes to include goats, sheep and elk makes this study both valuable and timely. This study shows that similar, or in some cases, the same DD treponemes with identical STs can infect multiple hosts, including cattle and sheep, causing lameness in both species. In addition, it appears that the same treponeme sequence types can circulate around a farm, infecting multiple animals, indicating that on farm biosecurity is important for disease control.

High levels of gene similarity between isolates from the same phylogroups including human treponemes indicate isolates should be referred to by phylogroup, as for example, ‘T. phagedenis phylogroup’ excluding the ‘–like’ suffix used in previous studies.

References

Identifying infection reservoirs of digital dermatitis in dairy cattle

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Introduction and Objectives
Bacteria belonging to the Treponema genus are generally considered the primary causative agents involved in digital dermatitis (DD); an infectious ulcerative lesion that typically develops on skin of the hind feet of dairy cattle. DD has a large impact both economically and with regards to welfare in the dairy industry. A polytreponemal aetiology has been described with treponemes from three phylogroups (T. medium-like, T. phagedenis-like and T. pedis) consistently identified and isolated from DD lesions in the UK and USA (1,2). There is currently little known about the infection reservoirs of DD associated treponemes and how the disease is transmitted, making effective prevention and control strategies difficult. This study aimed to identify infection reservoirs of DD by conducting a sampling survey of host tissue and the dairy farm environment as well as studies to determine the survival of DD associated treponemes under different conditions.

Materials and Methods

Survey of the dairy cow and dairy farm environment for the detection of DD treponemes
Over a two year period tissue samples (gingiva, rectal-anal junction (RAJ), DD lesions and healthy foot tissue) and farm environment samples (faeces, mucin casts, feed, water, crush and parlour floor foot prints and foot trimming equipment) were collected. Samples underwent culturing, DNA extraction and PCR assays specific for detection of the three DD treponeme phylogroups and the Treponema genus.

DD treponeme survival in different host and environmental conditions
DD treponemes underwent survival studies using microcosm methodology to determine their survival in bovine faeces and different bedding types used in the dairy industry (3). DD treponemes were incubated for one week, on each day microcosms were subcultured into selective medium. DD treponeme growth and survival was monitored by phase contrast microscopy. DD treponeme survival in different temperatures was investigated by incubation of DD treponemes in six different temperatures (4°C, 12°C, 20°C, 37°C, 45°C and 60°C). On select days, over three weeks, cultures were subcultured and incubated under normal conditions. DD treponeme survival was monitored using phase contrast microscopy. DD treponeme survival in pHs 4.5-9 were investigated using an adapted microplate methodology (4).

Results

Survey of the dairy cow and dairy farm environment for the detection of DD treponemes
DD treponemes were detected in gingival tissue, lesions and the RAJ using PCR assays. DD treponeme positive healthy feet were confirmed as potential early lesions with immunohistochemistry. DD treponemes were successfully isolated from some of the tissues. DD treponemes were also detected on foot trimming equipment and foot prints from the parlour and crush floors. However, DD treponemes have thus far not been successfully detected in faeces, mucin casts, food or water.

DD treponeme survival in different host and environmental conditions
DD treponemes were able to survive in faeces for a maximum of 2 days depending on phylogroup. Faeces appeared to have an inhibitory effect on one of the phylogroups compared to the control. DD treponemes appeared to survive and remain viable for at least 4 days at all temperatures except 45 and 60°C. DD treponemes are also able to grow and survive in pHs 4.5-7. Survival studies for pH7.5-9 and bedding are still ongoing at time of writing.

Discussion
The results from this study will help to provide knowledge of how transmission of DD occurs and inform on prevention strategies to aid in the eradication of DD on farm.

References
Molecular diagnosis of human intestinal spirochaetosis: A six years experience

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Introduction and Objectives

Human intestinal spirochetosis (HIS) is characterized by colonization of the colonic mucosa of the human distal intestinal tract by Brachyspira spp.. Microbiological diagnosis of HIS is hampered by the fastidious nature and slow growth of Brachyspira spp. in culture. In clinical practice, HIS is diagnosed histopathologically without identification of the spirochetes. We previously developed a fluorescence in situ hybridization (FISH) probe to detect and identify Brachyspira spp. in histological gut biopsies (1).

Materials and Methods

In the past six years we collected 133 intestinal biopsy samples from 75 patients suspected or diagnosed by histopathology with HIS. We investigated the samples by FISH and PCR amplification, followed by 16S rRNA gene sequencing.

Results

We identified B. aalborgi/B. ibaraki, B. pilosicoli, and one patient with a mixed infection. One case could be assigned to the ‘B. hominis’ cluster and three cases to the B. aalborgi-like cluster, supporting the presence of this separate Brachyspira subgroup. In most cases the bright FISH signal allowed rapid localization of Brachyspira spp. already at 400x magnification.

Discussion

In summary, FISH in combination with molecular identification by 16S rRNA gene sequencing proved to be a valuable addition to histopathology. It provided definite diagnosis of HIS and allowed insights in phylogeny and distribution of Brachyspira spp.. Based on our results we recommend further prospective investigation of the HIS risk groups children and patients with adenoma, hyperplastic polyps, ulcerative colitis or HIV.

References

Whole genome sequencing of isolates from human colonic spirochetosis.

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Introduction and Objectives

*Brachyspira aalborgi* and *Brachyspira pilosicoli* are associated with colonic spirochaetosis in humans, a condition with unclear clinical relevance despite striking histopathological appearance (1). In a randomized population based study performed in Stockholm, Sweden, 745 healthy individuals underwent colonoscopy with biopsy sampling of the terminal ileum and four additional sites of the colon. Out of these individuals, 17 (2.3%) presented with colonic spirochaetosis, associated with an increased number of lymphoid follicles and infiltration of eosinophils in the colon mucosa (2).

Materials and Methods

Sampling and culturing

Spirochetal bacteria were selectively isolated from frozen biopsies from individuals with the above mentioned histopathological changes. Isolates were further subcultured on FAA plates at 37 °C, and characterized as previously described (3). Obtained isolates were whole genome sequenced. The *B. aalborgi* type strain 513A and the Swedish clinical isolate W1 were included in the whole genome sequencing for comparison. Quality trimmed and filtered reads were used for de novo assembly. Following open reading frame prediction and annotation, phylogenetic, and comparative and functional genomic analysis were performed. Species designation was performed by 16S rRNA gene phylogeny.

Results

Spirochetal isolates could be retrieved from 14 of the 17 biopsied individuals. As previously described, the isolates showed a considerable variation in phenotype, and by PCR, one isolate was characterized as *B. pilosicoli* (3). Whole genome sequencing showed that all genomes were approximately 2,6 Mb in size with between 2293 and 2559 predicted coding sequences. The core genome size among the 16 isolates was 1560 genes and the pan genome 4076 genes. If the *B. pilosicoli* isolate was included the core genome shrunk to 455 genes while the pan-genome increased to 6435 genes. Phylogenetic analysis designated thirteen of the isolates to the *B. aalborgi* cluster, and one clustered with *B. pilosicoli*. When including previously described *B. aalborgi* sequences into a phylogenic tree, it was revealed that all but one of the obtained *B. aalborgi* isolates could be grouped into the previously described cluster 1 (4,5). However, this cluster consisted of 4 sub-clusters of isolates that are deviating from each other. One isolate was divergent from the *B. aalborgi* - lineage and was most similar to a 16S rDNA sequence derived from colon biopsy of a Dutch patient with colonic spirochetosis (4).

Discussion

Previous studies on *Brachyspira* genomics have shown an extensive heterogeneity on whole genome level, both in genomic size and genetic content. This could also be confirmed in the *B. aalborgi* genomes. The genome of the strain designated as *B. pilosicoli* had higher number of transposable genetic elements making the assembly of this strain more difficult. Phylogeny analysis supports previous observations of sub-clusters within the *B. aalborgi* and *B. pilosicoli* lineages. However, none of the isolates belonged to the previously described clusters 2 (“*B. hominis*”) or 3 (4,5), which may indicate that these are in fact uncultivable spirochetes. More thorough analysis is needed to verify if any of the sub-clusters represent separate species. Further virulence factor and functional genomic analysis will be performed to outline the level of pathogenic potential in these bacteria and if there is any correlation to clinical manifestations in the carriers.

References

2. Walker et al. 2015, Human Pathology, 46, 277-283
4. Westerman et al. 2012. PLOS One, 7, 12, e52281
Session 4:

Re-emergence of Swine Dysentery, Control of Swine Dysentery
Re-emergence of swine dysentery in North America

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Background

Swine dysentery is a severe mucohemorrhagic diarrheal disease affecting grower-finisher pigs. In addition to the adverse impact on the health and welfare of pigs, its negative impact on productivity has led to significant economic losses to pork producing countries worldwide. In the 1970s, the etiological agent of swine dysentery was identified to be Brachyspira hyodysenteriae, which at the time was the only known strongly beta-hemolytic Brachyspira species. Recent advances in microbiology and diagnostics have led to the detection of considerable genetic diversity within the Brachyspira genus. A novel strongly beta-hemolytic spirochete, Brachyspira suanatina, was isolated from swine dysentery-like cases in Sweden and Denmark in 2003 (1). In contrast to the continued presence of B. hyodysenteriae-induced swine dysentery in other countries, clinical disease was rarely reported in North America after the early 1990s, likely due to changes in industry structure and operations. After almost two decades of quiescence, outbreaks of bloody diarrhea in commercial swine herds in the late 2000s signaled the re-emergence of swine dysentery in North America. The detection of re-emergent B. hyodysenteriae was accompanied by the unexpected discovery of another novel strongly beta-hemolytic spirochete provisionally designated “Brachyspira hampsonii” (2). Both B. hyodysenteriae and B. hampsonii were isolated from typical swine dysentery cases. Preliminary characterization of the novel B. hampsonii led to the identification of two diverse genetic groups (I and II) (2). Swine dysentery has also been reproduced experimentally in pigs by oral inoculation of B. hampsonii, thus fulfilling Koch’s postulates of disease causation for this newly discovered pathogen (3). Since the initial identification of B. hampsonii in North American pigs, it has also been detected in European pigs (4) and in migratory birds (5).

Diagnostic trends

Rapid DNA-based diagnostic methods such as PCR must be used with caution for diagnosing swine dysentery as they have limited sensitivity for detection of Brachyspira species in feces. Selective culture remains the most sensitive method of detecting Brachyspira species, although the sensitivity can be affected by the conditions of sample procurement, transport, and time to culture. Strongly beta-hemolytic species can be differentiated from weakly beta-hemolytic species by the presence of the characteristic ‘ring phenomenon’. However since the discovery of B. suanatina and B. hampsonii, culture alone is insufficient to identify the etiological agents of swine dysentery, and must be supplemented by other genotypic or phenotypic approaches. These speciation methods include species-specific PCRs, sequence analysis of the NADH oxidase (nox) gene, in situ hybridization, biochemical tests and most recently, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) (6). Currently, selective culture followed by MALDI-TOF or PCR can identify B. hyodysenteriae and B. hampsonii, and in the case of any atypical isolates, speciation can be achieved by sequence analysis of the nox gene.

Epidemiology

B. hyodysenteriae is reported across Europe, Asia, North America and Australia, while B. suanatina is limited to Northern European countries. In addition to its presence as a pathogen in North America, B. hampsonii has been reported in Europe; however, the clinical significance in the latter geographical region is unknown. Molecular tools such as multi-locus sequence typing have been used to characterize the North American genotypes of B. hyodysenteriae (7) and B. hampsonii (8) and to understand the re-emergence of swine dysentery. Both pathogens were found to be heterogeneous on a population level with evidence of clonality in closed herds. Comparison of post re-emergence B. hyodysenteriae isolates with pre re-emergence isolates from the US showed close genotypic relatedness, and in contrast, showed less relatedness to isolates from other countries. This supports the hypothesis that B. hyodysenteriae persisted in the US over the last two decades without causing typical swine dysentery. Thus the current re-emergence is likely due to a resurgence of clinical signs (caused either by B. hyodysenteriae itself or by the newly discovered B. hampsonii) and/or increased awareness of the disease (7). Evaluation of B. hampsonii from various global epidemiological sources led to the identification of four diverse genetic groups (including the two previously identified...
groups), that formed a single cluster when compared to other *Brachyspira* species (8). Genetic group I, isolated from pigs in Europe and North America, appears to be more widespread globally. Within North America, genetic groups I and II appear to be more frequently isolated in the US and Canada, respectively. Finally, *B. hampsonii* was found to be more genetically diverse than *B. hyodysenteriae* (8).

**Antimicrobial susceptibility**

Due to lack of effective and commercial vaccines for swine dysentery, antimicrobial usage plays a central role in the control of the disease. Reports of multidrug resistant strains of *B. hyodysenteriae* in Europe have complicated swine dysentery control efforts over the last decade. The unexpected re-emergence of swine dysentery in North America was hypothesized to be due to decreased antimicrobial susceptibility. However both pathogens, particularly *B. hampsonii*, were found to have high susceptibility to most commonly used antimicrobials (9). In general, US origin *B. hyodysenteriae* isolates were more susceptible to evaluated antimicrobials than isolates originating from other countries. However, a few isolates with reduced susceptibility to multiple antimicrobials were identified. These isolates were found to be associated with the genotype, stage of production and production system from which they originated (9).

**Recent developments and future directions**

The recent re-emergence of swine dysentery emphasized the need for genomic information for the pathogens *B. hyodysenteriae* and *B. hampsonii* originating from North America. Since type strain B-78T (ATCC 27164^T^), isolated from the US in the 1970s, was found to be closely related to re-emergent *B. hyodysenteriae*, the complete genome of strain B-78T was sequenced (10). The 3.1-Mb genome comprises a 3.06-Mb chromosome (G+C content: 27.07%), a 45-kb plasmid (G+C content: 22.77%) and also includes a prophage. Future work involving genomic comparisons of re-emergent *B. hyodysenteriae* isolates from the US will help understand the pathogenesis and re-emergence of swine dysentery.

To support the recognition of *B. hampsonii* as a valid species, we sequenced the genomes of strains NSH-16 and NSH-24, representing genetic groups I and II, respectively. *B. hampsonii* type strain NSH-16^T^ (ATCC BAA-2463^T^) has an approximate size of 3.2 Mb (G+C content: 27.4%) and lacks a plasmid. Whole genome comparative indices clearly discriminate *B. hampsonii* from all other recognized *Brachyspira* species. A comprehensive evaluation of genotypic, genomic, phenotypic and ecological properties supports the classification of *Brachyspira hampsonii* sp. nov. as a unique species with genetically diverse genomovars (Mirajkar NS et al., manuscript under review). Future work involving genomic comparisons of *B. hampsonii* will help understand its emergence as a swine pathogen in North America.

**Conclusion**

Although swine dysentery was traditionally attributed to *B. hyodysenteriae*, advances in molecular diagnostics have now identified other strongly beta-hemolytic *Brachyspira* species (*B. hampsonii* and *B. suanatina*) that cause a clinically indistinguishable disease. Evolving microbial genomes, changing husbandry practices and susceptible hosts might provide opportunities for new pathogens to emerge. In such cases, comprehensive evaluation of microbiological, pathological and genomic characteristics can help identify and control emerging and re-emerging pathogens and diseases.

**References**

Swine dysentery in South America and genetic characterization of Brazilian Brachyspira hyodysenteriae isolates

Roberto M.C. Guedes; José Paulo H. Sato; Amanda G.S. Daniel

Introduction and Objectives

Swine dysentery has a worldwide distribution, occurring mainly in the regions with the highest density of pig production (Boye et al, 1998; Calderaro et al., 2001). Although the disease has been absent in North American swine herds in the last 20 years, since 2008, mainly due to management of production systems, several cases have been reported in the United States and Canada (Chander et al., 2012). Recent studies describe the occurrence in Europe (Dors et al., 2015; Lòbert et al., 2016) and Asia (Kajiwara et al., 2016). This manuscript describes the situation of SD in South America and analysis of isolates from Brazilian outbreaks.

Swine dysentery in South America

In Brazil, swine dysentery was described from 1980s to 1990s in sporadic reports in small herds, in very few epidemiological studies or associated to the optimization of diagnostic techniques (Barcellos et al., 2000). Since 2010, SD outbreaks have been reported in several Brazilian states, located in the regions that concentrate the largest pig production, causing significant economic losses. From early 2010 to July 2012, SD outbreaks were reported in different herds in the states of Minas Gerais, Mato Grosso, São Paulo, Paraná and Santa Catarina, with no direct epidemiological relation between them (Daniel et al., 2013). In August 2012, a major outbreak of SD happened with the contamination of a large pig integrator company in west of Santa Catarina state, which spread throughout the production chain to many different states (Daniel et al., 2013b). This outbreak was related to the delivery of clinically healthy replacement gilts there were likely recently infected with Brachyspira hyodysenteriae to several multiplier herds, some of them with no quarantine facility (Guedes and Daniel, 2013).

Upon detection of outbreaks in the period from October to November 2012, a contingency plan was implemented in the contaminated farms. The control measure most frequently used was the implementation of eradication programs based on partial depopulation with medication. All breed stock companies were able to clean their herds and now only isolated herds in specific regions are positive for swine dysentery.

Swine dysentery situation in different South American countries are still being gathered, but we could obtain some personal information from some countries. Dr. Andrés Boulanber has mentioned that there have been no swine dysentery reports in Venezuela in the last 15 years, but there is a big concern related to the reemergence of the disease due to the shortage of antimicrobial for food animals, as consequence of the political and economic situation of the country. Isolated cases of swine dysentery have been detected in Argentina by PCR, but no major outbreak or strain MLST typification was performed (Perez et al, 2016-IPVS). According to Dr. Dario Mogollon and Dr. Alvaro Ruiz there have been no cases of swine dysentery in Colombia or Chile, respectively, in the last decade, but no specific prevalence studies have been conducted.

Recent research on B. hyodysenteriae isolates in Brazil

During Brazilian outbreaks of swine dysentery since 2010 (Daniel et al, 2014), 55 B. hyodysenteriae isolates were obtained, and 45 were characterized by 16S rRNA sequencing and evaluated by Minimum inhibitory concentration (MIC) profile of antimicrobial commonly used in pig farming, which allowed the characterization of a Brazilian antimicrobial susceptibility profile.

A study has been developed to evaluate the genetic diversity, epidemiology and phylogeny among Brazilian isolates of B. hyodysenteriae obtained from outbreaks in 1990s and from 2010 to 2015. Forty-eight Brazilian isolates of B. hyodysenteriae, from 2010s outbreaks were selected from the bacteria collection of Molecular Pathology Laboratory of Universidade Federal de Minas Gerais. For a temporal evaluation at Brazilian level, seven B. hyodysenteriae isolates obtained in 1990s were also used. For isolates comparison, a Multilocus sequence typing (MLST) analysis were performed (La et al., 2009).

The 48 strains were grouped into six different clusters. Generally, the classification was related to the periods in which the samples were isolated, with specific variations between the groups. The most significant
information was observed in cluster 1, wherein the same clonal type was isolated in seven different farms and three States, without clear epidemiological connections. The cluster 5 was assigned with samples from Rio Grande do Sul/1990’s and an isolate from Minas Gerais/2015, showing a recurrence of the old genotypes in the country. The cluster 6 was composed of isolates obtained from different states in 2011. The Tajima’s Neutrality Test (D= -2.1352, P=0.05) showed a purifying selection for the seven genes evaluated and the Brazilian populations of *B. hyodysenteriae* presented a high genetic diversity (Simpson index = 0.8316). At the moment, ST analysis were performed in 34 of 48 strains and all Brazil *B. hyodysenteriae* were unique (10 novel STs assigned).

**Conclusion**

Brazilian isolates of *B. hyodysenteriae* had a high genetic diversity with new STs and samples from different states and different periods of isolation were grouped in the same clusters.

**Acknowledgements**

CNPq, CAPES and FAPEMIG for financial support.

**References**

7. Daniel et al., 2013a. VIII SINSUI Congress, p.131-139.
The answer to this question is not easy or at least it’s difficult to give an exact answer. Anyway, our opinion is that there is not an emergence of swine dysentery in our country.

The incidence of swine dysentery (SD), like any other disease, is conditioned by many epidemiological factors. Spain is currently the third largest pork producer in the world. The structure of the Spanish pig production is becoming more similar to the American pig production and is quite different from this production in other European countries, and this fact influences the incidence and epidemiology of swine dysentery.

Spanish pig production is becoming more concentrated and currently the biggest European pig producing companies are located in Spain. There is a company with 190,000 sows, two companies with more than 80,000 sows and around ten other companies with more than 50,000 sows.

These companies are producing in farms with a census of 2000-4000 sows that send weaning piglets or growers to be fattened in separate points. These farms are built in geographically isolated areas, are populated with high health status sows and have very strong biosecurity measures. Afterwards, farms get closed and only semen for genetic improvement is introduced.

It’s very difficult that this type of farms become infected with Brachyspira (B.) hyodysenteriae so, in this production system, only occasionally the disease affects some of its fattening units and specially those located in geographic areas with high concentration of pig farms.

The disease is more common in farrow-to-finish farms, which usually have fewer sows. Some of these smaller farms, mainly the new ones, are also geographically isolated and have a good biosecurity, but are still very common farms located in densely pig populated areas. SD has higher prevalence and incidence in these areas, which are located mainly in the Northeast, the Mediterranean coast and in some inland areas of Spain.

SD is also more prevalent in Iberian pigs extensively fattened on acorns and grass on the ground of their Mediterranean ecosystem (“dehesa”).
been for years legally compulsory in our country to clean and disinfect trucks after being used for transporting pigs and the driver must have an officially certified document to prove it.

Some years ago and in another research project, we proved that the usual cleaning and disinfection of trucks were not enough to eliminate Salmonella from them and actually we know several cases of swine dysentery that have been without doubt connected with trucks that fulfill these official conditions of cleaning and disinfection.

The strains isolated in different farms within the same company showed identical MLVA profiles and these farms were exclusively connected by “disinfected” trucks.

Therefore, these data support our opinion that there doesn’t seem to be an emergence of swine dysentery in Spain. The number of cases has remained or even has tended to decrease.

The growing difficulties for the treatment and control of SD are currently the main problem of the disease in our country. The antimicrobial resistance of Spanish strains of *B. hyodysenteriae* to the most commonly used antimicrobials is increasing over the time, as it does in other countries.

Surprisingly, one strain of *B. hyodysenteriae* susceptible to tylosin has been recently isolated, after hundreds of strains tested for years, but more and more frequently strains with resistance to several or all usual antibiotics are being isolated in Spain and, as a whole, our data confirm an alarming and steady increase in the resistance.

It is important to emphasize the great differences in the degree of sensitivity to antibiotics that can be found between the isolates from different farms. This is an important practical problem for the control, and it is essential to perform a sensitivity test for each isolate in order to select individually the most efficient treatment for each farm.

Looking for alternatives to control swine dysentery, autogenous vaccines are a useful and effective possibility and we have a large and extensive experience on their efficiency.

The use of phytobiotics with activity against *B. hyodysenteriae* is another possibility still not much researched. Some phytobiotics have shown an in vitro interesting antimicrobial activity and have MICs and MBCs similar to antibiotics.

In short, there doesn’t seems to be a clear emergence of swine dysentery in Spain. The situation in our country remains stable.

Mainly the big companies but also the small ones are trying to reduce the use of antibiotics as much as possible and some of them are already producing without any antibiotic in their best farms.

This facts and the increasing restrictions to the general use of antibiotics in the UE can cause changes in the epidemiological situation, mainly in the farms located in densely pig populated areas.

**References**


An investigation into the occurrence of agents of swine dysentery in Australian pig herds

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Introduction and Objectives

Swine dysentery (SD) is a disease primarily of grower/finisher pigs characterized by colitis and mucohaemorrhagic diarrhoea. The classical aetiological agent is the anaerobic spirochaete *Brachyspira hyodysenteriae*, but in recent years the related species “*Brachyspira hampsonii*” and *Brachyspira suanatina* have been identified as causing SD in North America and Europe. Furthermore, recently there have been cases where strains of *B. hyodysenteriae* have been recovered from pigs in healthy multiplier herds. This study aimed to determine whether cases of SD in Australia are caused by the newly described species; to investigate strains of *B. hyodysenteriae* from healthy herds and compare these with strains from herds with disease; and to compare contemporary Australian isolates with those recovered in previous decades.

Materials and Methods

A total of 611 faecal or colonic samples were collected from pigs in 89 Australian herds between June 2014 and July 2015. Herds either had a history of having SD, or had no signs of disease or had only mild signs not attributed to SD. Samples were cultured and any recovered spirochaetes were subjected to species-specific PCRs for *B. hyodysenteriae*, “*B. hampsonii*” and *B. suanatina*, and for six plasmid genes associated with virulence in *B. hyodysenteriae* (1). The *B. hyodysenteriae* isolates also were subjected to multilocus sequence typing (MLST), together with 40 Australian isolates recovered between the 1980s and 2010s (2). Isolates (n=29) also were tested by the agar gel dilution method for their susceptibility to tiamulin, lincomycin and tylosin.

Results

Of the three pathogenic species only *B. hyodysenteriae* was recovered, being present in 27 (30.3%) herds, including 11 (41%) that showed either no signs of disease or mild signs not attributed to SD. Some of these 11 herds previously either had given apparently “false positive” reactions in a serological ELISA for SD, or had epidemiological links to such herds (3). The other 16 infected herds had a history of having had SD in the recent past or had relevant clinical signs. There was good but not perfect agreement between a lack of virulence-associated plasmid genes and origin from herds with no or mild disease. Multilocus sequence typing on a subset of isolates indicated that they were diverse and distinct from earlier Australian isolates dating from the 1980s through 2010s, or from those from other countries. Related isolates were found in some herds with epidemiological links. Three isolates (10%) were resistant to tiamulin, and overall there appeared to be an increasing trend towards resistance. Most of the isolates (93%) were resistant to tylosin. Only a few of the isolates (17.2%) were fully susceptible to lincomycin, and over half were resistant. Three genetically distinct isolates from different Australian States were resistant to all three antimicrobials.

Discussion

The failure to detect either “*B. hampsonii*” or *B. suanatina* in this study suggests that these species are either absent or are at most rare in Australia. On the other hand, infection of herds with *B. hyodysenteriae* was found relatively frequently, including in herds where disease was not observed, or not attributed to SD.

Isolates from the herds without recorded disease were more likely to lack plasmid genes than were those from diseased herds, hence supporting the likelihood that these genes are involved in facilitating colonisation that ultimately may lead to disease.

Minimum spanning trees constructed from the MLST data suggested there has been a gradual evolution of *B. hyodysenteriae* strains away from those that were recorded in the 1980s.

Resistance to tiamulin and lincomycin occurred more commonly than in earlier Australian studies, and the existence of three multi-drug resistant isolates is of concern for future control of the infection in Australia.

References

A longitudinal study on the epidemiology of Brachyspira hyodysenteriae infection in fattening pigs.

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Introduction and Objectives

In the EU, Brachyspira hyodysenteriae is the main agent associated with swine dysentery (SD), a severe diarrheal disease. Although SD affects pigs of different ages, it is primarily observed during the growing and finishing periods.

In the last ten years, the emergence of multi-resistant B. hyodysenteriae strains has renewed the interest in methods for the control of SD alternative to antimicrobial therapy. To reach this goal, it is necessary to understand the on-farm epidemiology of this infection. However, few papers have yet been published on this topic [1].

The focus of this study was to investigate in depth the spread and the transmission of B. hyodysenteriae in an endemically infected finishing herd.

Materials and Methods

A cohort study was performed in a fattening herd with a positive history for B. hyodysenteriae. Fifty pigs were randomly selected and individually ear-tagged. Fecal samples were taken after housing, at 14, 15, 17 and 21 weeks of age and then at weekly intervals until 38 weeks of age. The samples were cultured for Brachyspira spp. as already described [2] and fecal consistency was evaluated using a standardized procedure [3]. The antimicrobial susceptibility of B. hyodysenteriae isolates was determined by the Minimum Inhibitory Concentration (MIC) described by Rohde [4]. The interpretation of MIC values was based on the criteria proposed by Ronne and Szancer [5].

Results

B. hyodysenteriae fecal shedding was first detected at 21 weeks of age and then at every time point, until 38 weeks of age. The incidence rate of shedding was 0.036 pig-week. The graph of cumulative incidence showed a linear progression of the infection in the group throughout the fattening cycle (Graph 1). Some pigs shed B. hyodysenteriae at different ages, with or without diarrhea, with an interval between the shedding periods of up to ten weeks. Animals belonging to the same box shed the bacteria in the same period and the same pig had been shedding B. hyodysenteriae for up to six weeks. Diarrhea was recorded for all the shedding period, with a maximum incidence risk of 12%. However, only 30% of the B. hyodysenteriae positive samples belonged to categories 3 and 4 (diarrheic feces), while 70% of them were of categories 1 and 2. All the B. hyodysenteriae isolates showed a similar multi-resistant profile after MIC testing.

Discussion

In our study, neither SD nor shedding were related with a specific age of the pigs, in agreement with what Patterson observed [1]. In fact, the results showed a long-term bacterial excretion as well as the insurgence of new cases of SD through the fattening cycle, despite the extensive use of antimicrobials. It is to be noted that the isolates presented a multi-resistant profile: in these conditions, no antimicrobial for the SD control was effective.

Moreover, the same pig shed the B. hyodysenteriae with diarrhea at distant time points, suggesting a lack of effective protection after the infection. This finding can be justified by the use of antimicrobials, which may have interfered with protection after infection.

References

Comparison of the luminal and mucosa-associated microbiota in the spiral colon of pigs with and without swine dysentery

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Introduction and Objectives

With the advent of affordable next generation sequencing technology, research studies exploring microbial community profiles associated with health and disease have flourished. For enteric disease states, many studies have focused on profiling the luminal contents or feces; however, these changes likely represent an indirect measure at best of what is happening at the mucosal surface where bacteria interact more intimately with the host. In the case of swine dysentery (SD), where there are profound changes in the mucosa and where the etiologic agent can be readily visualized in the mucus layer (1), it seems logical to explore the microbiome directly associated with this biological niche.

Accordingly, the microbial profiles of colonic contents and mucosal scrapings from pigs inoculated with Brachyspira hyodysenteriae or “Brachyspira hampsonii” were compared to determine differences between the microbiota of those pigs that developed SD following inoculation and those that did not.

Materials and Methods

Paired colonic contents and mucosal scrapings from pigs inoculated with either B. hyodysenteriae or “B. hampsonii” were collected at necropsy during a previous study (2). Samples were classified as either positive (n=29) or negative (n=7) for SD based on microscopic lesions and positive culture from the source pig and remained frozen at -80 C until use in this study. Extracted and purified DNA was submitted to Argonne National Laboratory for metagenomic analysis using the V4 region of the bacterial 16S rRNA gene. The diversity of microbial profiles within sample types was compared using QIIME and biological effect sizes were estimated using the linear discriminant analysis effect size (LEfSe) method (3,4). Procrustes analysis was performed to compare profiles generated from paired contents and scrapings in individual pigs.

Results

Procrustes analysis revealed similar clustering by disease classification yet with a relatively high M2 value (0.44). In both sample types, differences in richness and beta diversity were observed between disease statuses (P ≤ 0.014). The relative abundance of Campylobacterales, Enterobacteriales, Desulfovibrionales, and Brachyspirales was higher in pigs with SD for both mucosal scrapings and luminal samples while Erysipelotrichales, Clostridiales, and Fusobacteriales were more abundant in the luminal contents only. For inoculated pigs that did not develop SD, Burkholderiales were more abundant in both sample types, Bacteroidales and Synergistales were more abundant in scrapings, and Lactobacillales were more abundant in luminal contents only. LEfSe revealed Campylobacter, Brachyspira, and a Desulfovibrio spp. as differential features in mucosal scrapings from pigs with SD while Lactobacillus and a Bifidobacterium sp. were differential in pigs without disease.

Discussion

While Procrustes analysis of the microbiota revealed similar clustering of samples by disease status, the M2 value suggests the data sets are not a particularly good fit and that differences exist between the microbiota of the colonic contents and scrapings from the same pig. While not unexpected, this paired analysis emphasizes that exploration of both sample types may be warranted when studying specific intestinal diseases.

Both luminal contents and mucosal scrapings from inoculated pigs with SD had significant differences in the relative abundance of multiple bacteria at the order level when compared with samples from inoculated pigs that did not develop disease. LEfSe further revealed that both Brachyspira and Desulfovibrio spp. were differential features at the mucosal surface of pigs with SD, which is of note as Desulfovibrio spp. can degrade the sulphated mucins that comprise part of the mucus barrier. Similarly, Lactobacillus and a Bifidobacterium sp., two genera commonly investigated as probiotic agents, were differential features in the mucosa of inoculated pigs that did not develop SD. Further investigation into the role of these specific bacteria in SD expression and prebiotic approaches that impact their relative abundance is warranted.

References

3. Caporaso J, et al., 2010, Nat Methods, 7, 335-336
4. Segata N, et al., 2011, Genome Biol, 12(6), R60
Development of an in vitro swine colon culture protocol as a model for “B. hampsonii” infection

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Introduction and Objectives

Mucohaemorrhagic diarrhea in pigs has global distribution and a remarkable economic impact due to reduced performance of animals, increased mortality and medication costs. A critical knowledge gap exists regarding the disease pathogenesis, contributing to the limited resources available for disease control and prevention. Animal models restrain pathogenesis studies since the onset of the host cellular response and tissue damage stages are not clinically detected for sampling (1,2). In vitro organ culture (IVOC) involves the maintenance of tissue explants derived from healthy subjects ex vivo for several days, enabling accurate control of host exposure to pathogen and timed sampling after inoculation. Here we described the development of a colon culture protocol, factors affecting tissue survivability in vitro and its response to infection by “Brachyspira hampsonii”.

Materials and Methods

Model development

Crossbred commercial pigs (n=13), aged 5 to 10 weeks were used for this study. To define an optimal culture protocol, a set of experiments was performed to test the impact of different factors, such as pig age at euthanasia, tissue handling technique, preparation time before culture, basal media and supplements, antibiotic drugs, buffers and culture environment. Explants (n=170) were cultured on an agarose platform, creating a liquid-tissue-gas interface, incubated at 37°C, in a 95% O2 / 5% CO2 atmosphere.

Infection setup

IVOCs from porcine colon were exposed to “B. hampsonii” for 12 hours. Explants (n=200) from ten, 8-week old pigs were prepared and randomly assigned to inoculated and control groups. Inoculated explants were exposed to a 48-hour-old pure culture of “B. hampsonii”, while controls received sterile culture broth. To inoculate, a polypropylene ring was attached to the mucosal side of the explants, and sealed with tissue adhesive. Explants were fixed in 10% buffered formalin (n=2/pig/time) at 0, 2, 4, 8 and 12 hours post incubation. Samples were analysed by optical microscopy (H&E and Warthin-Faulkner stains). Apical side of explants were sampled for “B. hampsonii” culture after 12 hours.

Results

Model development

Results indicate that this method maintains viable tissue for 5 days in vitro, with normal histologic features of the colon. Pig age, time before processing and antibiotic drugs in the media played an important role in tissue survival over time, and a strong positive correlation (P<0.01) was observed between the proportion of the tissue covered by intact columnar epithelium and the number of Lieberkühn crypts.

Infection setup

The apparatus setup for infection successfully limited explant exposure to the pathogen to the mucosal site. No leakage of inoculum, from either group, was observed. The inoculated pathogen was cultured from the mucosal site of infected explants 12 hours after inoculation. Control explants had significant less necrotic cells (P<0.05, GEE) and thinner catarrhal exudate (P<0.05, GEE) than the infected group.

Discussion

We have shown that colon explants may be kept in vitro for 5 days, and that “B. hampsonii” interacts with it during 12 hours of exposure. Different factors were tested regarding their effect on tissue survivability in vitro. Minimizing tissue manipulation, use of refrigerated transport media and manipulation surfaces contributed. Isolation of the tissue from the liquid media revealed to be of primary importance, preventing tissue swelling. The combination of tissue from pigs younger than 7 weeks of age, processed immediately after euthanasia and cultured using media supplemented with calcium and gentamicin yielded explants with the most epithelial coverage. Infection of explants with pure cultures of “B. hampsonii” revealed histological abnormalities compatible with in vivo infections.

References

Optimization of a murine challenge model for "Brachyspira hampsonii"

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Introduction and Objectives

Mouse models of swine dysentery associated with Brachyspira hyodysenteriae have been used for many years, but there is only one report of a murine model used for "B. hampsonii" challenge (1). Across all models, data pertaining to Brachyspira shedding and clinical signs following oral challenge are sparse. Moreover, different mouse strains, diets, termination points and disease outcome measures are used. Murine models do not abolish the need for pig challenge experiments, but they feature lower animal care costs, faster implementation and the use of a less sentient animal. The objectives of these experiments were to optimize a murine model for swine dysentery, and to characterize fecal shedding, changes in fecal consistency, lesion severity and growth rate following oral inoculation with "B. hampsonii" clade II.

Materials and Methods

Experiment 1: Twenty-four, 4-week old CF1 mice (Charles River Laboratories) were randomly allocated to sham (Ctrl), B. hyodysenteriae strain G44 (Bhyo) or "B. hampsonii" strain 30446 (Bhamp) inoculated treatments. Within each treatment, half of the cages were provided pelleted chow (RMH 3000, Federated Coop) and half a lower zinc diet (TD85420, Harlan Laboratories; TD) ad libitum. After a 7-day acclimation period, mice were intra-gastrically inoculated with 0.2 mL of their respective treatment (Brachyspira titrated ~10⁸ GE/mL) on D0 and D1. Fecal consistency was scored twice daily (Figure 1). Mice were weighed every 2-3 days during which fecal pellets were collected to semi-quantify shedding by culture. Histologic lesion severity was assessed in caecum and colon collected following termination at D14.

Experiment 2: Using similar procedures, 8 CF1 and 9 C3H mice were randomly allocated to Ctrl or Bhamp treatments using a 2x2 factorial design. All mice were fed TD. Mice were intra-gastrically inoculated with 0.3 mL on D0, D1 and D2 following a ~6 hour fast.

Results

Experiment 1: Following inoculation, soft mucoid feces (score 2) was observed in all Bhyo and Bhamp infected mice fed TD, and in 1/4 Bhyo infected mouse fed RMH chow. Median fecal consistency scores were higher in TD fed mice across all three groups (Kruskal Wallis; P < 0.05). While numerically shorter in TD group, the incubation period (# days to fecal score 2) did not statistically differ by diet. The frequency and level of Bhyo and Bhamp fecal shedding was higher in TD versus chow diet (Kruskal Wallis; P=0.003), but average daily growth rate ADG and histological lesion severity did not differ by diet or Brachyspira species.

Figure 1. Fecal consistency scores. Score 0=formed feces, 1=formed with mucus, 2=soft mucoid, 3=very soft, mucoid with flecks of blood

Discussion

Although these experiments were limited in terms of animal numbers, the results confirm that CF1 and C3H mice develop clinical disease (soft mucoid feces) following inoculation with B. hyodysenteriae and "B. hampsonii" clade II. In our laboratory, disease was more severe in CF1 mice, and when the low zinc Harland TD85420 diet was fed. Daily fecal consistency scores were a biologically relevant outcome worthy of measurement following inoculation. Diet and mouse strain had no effect on histopathology at 14 dpi or ADG in these experiments.

References

Administration of an avirulent strain of *B. hyodysenteriae* partially protects pigs against challenge with a virulent *B. hyodysenteriae* strain

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**Introduction and Objectives**

**Introduction**

Swine dysentery (SD) caused by *Brachyspira hyodysenteriae*, causes substantial economic losses in swine producing countries worldwide. Major costs for SD comprise medical treatment, retarded growth and increased feed conversion. Treatment with antimicrobial compounds is hampered due to increasing resistance against tylosin, tiamulin and valnemulin (1).

Animals that have recovered from SD seem to have established an immunological response, since these animals can be protected from re-infection (2). Therefore, a number of different vaccine approaches have been explored with regard to swine dysentery. Several reports describe the use of whole cell bacterins or protein digests of whole cell bacterins. A major downside of the use of inactivated whole cell bacterins is that they only evoke protection against infection with a homologous serotype of *B. hyodysenteriae*.

Vaccination with recombinant proteins has been reported to induce variable levels of protection, depending on the selected protein. A preparation of BmpB, an outer membrane lipoprotein, resulted in a 50% reduction in clinical SD (3). Song et al. (2009) describe a reverse vaccinology approach to select proteins for use in a subunit vaccine. They also report a reduction in number of animals developing clinical SD (4). A *tlyA* mutant strain of *B. hyodysenteriae* has been examined for its use as a live attenuated vaccine. A 50% reduction in the number of animals developing clinical SD upon challenge with a virulent *B. hyodysenteriae* strain was demonstrated (5). However, with none of these vaccines there was a reduction in the number of animals that was colonised by the challenge strain (3,4,5).

**Objectives**

In this study we aimed to determine the protective effect of inoculation of pigs with an avirulent, weakly haemolytic, strain of *B. hyodysenteriae* before challenge with a known virulent strain of *B. hyodysenteriae*. Local and systemic antibody responses were quantified.

**Materials and Methods**

**In vivo trial: immunisation and challenge**

Twenty-one five week old pigs were randomly assigned into two groups; an immunisation group (12 animals) and a positive control group (9 animals, no immunisation). After a two week acclimatization period the immunisation group was inoculated intragastrically with the avirulent, weakly haemolytic *B. hyodysenteriae* strain on three consecutive days (d-2, d-1, d0). Correspondingly, positive control animals were intragastrically sham-inoculated with Brain Heart Infusion broth without *B. hyodysenteriae*. Three weeks later animals in both groups were intragastrically inoculated with challenge strain B204 (ATCC 31212) on three consecutive days (d19, d20, d21). Animals were observed twice daily for the presence of diarrhoea and other signs of clinical illness. Faecal consistency was scored daily and stool samples were collected three times a week. Faecal scores were determined as 0: normal, 1: softer but formed, 2: unformed semi-wet, 3: runny, 4: runny with mucus and blood. The scores (1-3) were augmented with 0.5 if blood or mucus were present. Animals were euthanized 24 hrs after the first time a faecal score of 3 or more was recorded. In order to determine the quantity of *B. hyodysenteriae* DNA of the immunising strain, and of the challenge strain in faecal and intestinal samples, primers were designed to specifically anneal with DNA of either strain.

**Enzyme-Linked Immuno Sorbent Assay (ELISA) for serum IgG and faecal IgA**

For detection of antibodies against the immunising *B. hyodysenteriae* strain or challenge strain B204, in-house whole cell ELISA’s were prepared. Nunc-immuno plates were coated with formalin inactivated *B. hyodysenteriae*. Serum IgG was quantified by using horseradish peroxidase conjugated anti-porcine IgG, faecal IgA was quantified by using horseradish peroxidase conjugated anti-porcine IgA. For detection of IgA in faeces, faecal extracts were prepared by mixing faeces with extraction buffer (PBS, 0.5% Tween 20%, 0.05% NaN₃) after which proteinase inhibitor was added to the supernatant of this mixture. Faecal IgA was determined three times a week in the period post-immunisation – pre-challenge. Blood samples were taken for determination of the presence
of *B. hyodysenteriae* reactive serum IgG on three occasions: before immunization (d-13), before challenge (d16), and at the end of the trial (d30-40).

**Results**

**Period 1: post-immunisation – pre-challenge**

Of the pigs inoculated with the immunising strain, one animal had to be removed before challenge due to unrelated causes. Of the remaining 11 inoculated animals, five animals shed the immunising strain in a quantifiable amount at one to four sampling occasions, spanning 10 days at most. During this period, animals had a faecal score of 0 or 1. For the remaining six animals, the immunising strain could not be detected in their faecal samples, and these animals also showed faecal scores of 0 or 1. The animals that were sham-inoculated had negative faecal samples on all sampling occasions and showed faecal scores of 0 or 1. All animals in both groups were clinically healthy and no signs of anorexia or depression were noted during the period following immunisation until challenge (d-2 - d21).

**Period 2: post-challenge**

Five of nine positive control animals developed swine dysentery (SD) after an incubation period of 7-13 days after the last day of inoculation with the challenge strain. Of the 11 immunised pigs, 2 developed SD after an incubation period of 12 or 13 days. Both animals showed typical clinical signs of SD and had lesions on necropsy. The remaining 9 animals had faecal scores of 0 or 1 and did not shed the challenge strain on any sampling occasion. Despite the trend that immunised pigs were less prone to acquire a *B. hyodysenteriae* infection, the difference in SD incidence between the positive control group and the immunised group was not statistically significant (p=0.10).

The average daily faecal score and the cumulative average faecal score for both groups during the post challenge period were determined. The cumulative average faecal score for immunised pigs is statistically significantly lower compared to the cumulative average faecal score for non-immunised positive control pigs (p=0.048).

**ELISA**

An increase in serum IgG could not be demonstrated in pigs of both groups. There was no increase in IgA levels in the faecal extracts of the animals in the positive control group. For the animals that were colonized by the immunising strain, an increase in faecal IgA was noticed, on average two days after they started shedding the immunising strain in their faeces.

**Discussion**

The reduction in number of animals developing clinical SD after immunisation with the avirulent, weakly haemolytic strain is promising. The use of the immunising strain was safe, and significantly reduced the average faecal score of the immunised group in the post-challenge period. In contrast to most vaccine approaches for SD, the reduction in clinical SD was accompanied by a reduction of colonisation by the challenge strain of *B. hyodysenteriae*.

Since acquired antimicrobial resistance is increasing for *B. hyodysenteriae*, an efficient vaccine could be an important tool to manage swine dysentery in the future. The possibility of a vaccine to not only prevent clinical symptoms, but also prevent colonization and faecal shedding would be beneficial to reduce the exposure to *B. hyodysenteriae* of the pig herd.

**References**

Effect of coated calcium butyrate on the course of swine dysentery: an experimental study

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Introduction and Objectives
The increasing emergence of multiresistant strain of B. hyodysenteriae highlights the need for alternative strategies to control Swine Dysentery (SD).
Recently butyric acid demonstrated to inhibit the growth of B. hyodysenteriae in vitro [1].
We aimed at testing if the administration in feed of calcium butyrate modifies the insurgence or the development of SD.

Materials and Methods
Experimental design, methods and statistics.

Body temperature was monitored and individual fecal samples collected on day -7 (first day of treatment), 0 (day of infection), 5, 13, 21, 28, 35 and 42. Pigs were weighed on day -7 and 42.

Statistics
Qualitative data (culture+/−). Cumulative OR of Group A Vs Group B by the χ² of Mantel-Haenszel.

RT-PCR
Quantitative data. Differences AUC of Group A Vs Group B compared by Wilcoxon test

Results
- B. hyodysenteriae was more frequently isolated from fecal samples of Group B (30/72) compared to group A (20/78) (Table1). Ca butyrate treatment influenced the number of animals positive to culture. OR=2.19 (CI 95% 1.07-4.50) p=0.033.
- Animals of group A and B shed comparable amount of B. hyodysenteriae in their feces p=0.1994 (figure 2).

Discussion
Time course and clinical symptoms of SD were not affected by the administration of Ca butyrate.
On the other hand, Ca butyrate seems to favour gut colonization by B hyodysenteriae possibly modifying gut microbiome or increasing mucins secretion [2].
Another study with larger sample size would be necessary to confirm these results.

References
Poster Abstracts
The differences of colony formation between *Brachyspira pilosicoli* and *Brachyspira aalborgi*

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Introduction and Objectives:

*B. pilosicoli* spends 3-7 days for colony formation of intestinal spirochetes in biopsy samples on blood agar under anaerobic condition, while *B. aalborgi* spend 1 month at least and formed 4 different type colonies (¹) (5th Spiro.Cong., 2009) which consisted of pessary, large smooth small smooth and mucoid. If both species were present in one biopsy sample and the sample was inoculated onto one blood agar, the colonies formed by *B. aalborgi* may be covered with those by *B. pilosicoli* because *B. pilosicoli* was swarming well and grew higher than that of *B. aalborgi*. Those species can be investigated by PCR using two type primers (2,3), i.e., Acoli1F/R583 and aalF161/R596 primers. Fortunately, the life of *B. aalborgi* on blood agar at 37°C under anaerobic condition is very long and the period was at least 8 month, while that of *B. pilosicoli* was at most 5 month. My object is to simultaneously isolate *B. aalborgi* and *B. pilosicoli*. Furthermore, we attempted to compare the colonies formed by both species because we also attempted to distinguish the colonies of *B. aalborgi* from those of *B. pilosicoli* during culturing.

Materials and Methods:

Isolation of *B. pilosicoli* or *B. aalborgi* was carried out as previously described (4) (Songer et al, 1976). After the dilution of biopsy samples with triplicate soy broth (BBL, USA), the diluents were spread on the sheep blood agar containing final concentration of 400 μg/ml spectinomycin. In case of *B. pilosicoli*, after 3 to 7 days incubation at 37°C under anaerobic condition, many colonies were produced by the organisms, picked up and inoculated on fresh blood agar containing 400 μg spectinomycin. The agar was incubated until 6 month. During incubation, the culture was sometimes observed on the growth of colonies. Genetic diagnosis of the brachyspiras was carried out as previously described (2,3) (Fellstrom et al., 1995; Mikosza et al., 1999). In PCR experiments, special primers, i.e. Acoli1F/R583 and aalF161/R596 were used. In addition, F3/R500 primers were used for determination of the base alignment of 16SrDNA from the base positions 3 to 500. *B. pilosicoli* possessed a special base alignment of -TTTTTT- on the base position 150 to 180 but *B. aalborgi* did not possess. The experiment was allocated to 3 at least. Each of them was observed at the interval of one month and incubated again at 37°C under anaerobic condition. The culture was continued until 8 month at least. Colonies were picked up and cultured again on fresh blood agar. After the culture, brachyspiras forming colonies were also identified genetically by PCR.

Results:

In case of primary culture of biopsy samples, big colonies of *B. pilosicoli* were observed after one month later but after transferring to fresh blood agar, big colonies did not formed, while big colonies (Fig1A) formed by *B. aalborgi* were observed in case of mucoid type colonies among 4 colony types. The big colonies were observed not only primary cultures of biopsy samples but also after several subcultures. The colonies were gradually growing up during culturing. The big colonies consisted of druses of *B. aalborgi*. The druses of *B. aalborgi* were also confirmed by PCR. The druses were picked up, cultured and observed under dark field and phase contrast microscopy. *B. aalborgi* was also growing up on a fresh blood agar containing spectinomycin. Though the big colonies were left under aerobic condition for 2 days, the brachyspras were grown up on a fresh blood agar containing spectinomycin. Probably, the big colonies may be able to endure the tough conditions e.g. aerobic condition. On the other hand, if *B. hyodysenteriae* was left under aerobic condition for 24 hours, the organisms can be died. As one speculation, the big colonies may be covered with biofilm like material for protection against harmful. For example, in case of *B. pilosicoli*, after several months later of culture under anaerobic condition, the colonies were covered with biofilm like material (Fig1B) but the colonies did not grow big unlike those of *B. aalborgi*. We are now investigating the mechanism of the biofilm like material by electron microscopy and genetic analyses.
Fig. 1. A, B.aalborgi; B, B.pilosicoli. After inoculating into sheep blood agar containing spectinomycin, the agars were incubated at 37°C for 5 month at least. B.aalborgi formed big colonies, while B.pilosicoli formed biofilm like material on the . Arrows show the big colonies or biofilm like material.

**Discussion:**

Probably, big colonies produced by B.aalborgi may be covered with a biofilm like material and be able to endure the tough conditions e.g. aerobic condition. We could propose a new aspect on formation of big colonies covered with biofilm like material. Because the brachyspiras in colonies covered with biofilm like material could endure to the tough conditions, i.e. harmful conditions and the organism could survive under aerobic condition. In case of B.pilosicoli, after several months later, we could observe the colonies covered with biofilm like material but they were unlike B.aalborgi. The colonies produced by B.pilosicoli except for primary culture did not become big. We are now investigating the mechanism of the biofilm like materials by electron microscopy and genetic analysis.

**References:**

Introduction and Objectives

Human intestinal spirochetosis (HIS) is a colorectal bacterial infection caused by *Brachyspira* species. Recently, increased MUC5AC expression in the mucosa of the large intestine was reported in an animal *Brachyspira* infection. However, in human intestines in the *Brachyspira*-infected state, the mucosal condition as regards mucin expression has not been elucidated. The aim of this study was to examine whether mucin expression was altered in HIS.

Materials and Methods

215 specimens from 83 histology-confirmed HIS cases were examined immunohistochemically using antibodies for the mucins MUC1, MUC2, MUC4, MUC5AC, and MUC6. Also, as controls, 106 specimens taken operatively or endoscopically from non-HIS large intestines were used. In our estimation of MUC1, MUC5AC, and MUC6, even focal positive staining was considered positive. For MUC2 and MUC4, which were usually well stained, a substantial decrease in the number of immunopositive-cells was considered negative. The results of mucin immunohistochemistry were analyzed together with types of lesions and with colorectal location. Statistical analysis was performed using Fisher's exact probability test for comparisons, and a two-tailed probability *p* < 0.05 was considered significant.

Results

In most control (non-HIS) specimens, mucin immunoprofiles were MUC1(-)/MUC2(+)/MUC4(+)/MUC5AC(-or+)/MUC6(-) in normal/inflammation, MUC1(-)/MUC2(+)/MUC4(+)/MUC5AC(+)/MUC6(-or+) in hyperplasia/serrated polyp, MUC1(-or+)/MUC2(+)/MUC4(+)/MUC5AC(+or-)/MUC6(-) in conventional adenoma, and MUC1(+)/MUC2(+)/MUC4(+)/MUC5AC(+or-)/MUC6(-) in adenocarcinoma (Table).

Among HIS cases, excluding the MUC5AC(- or +) result in hyperplasia/serrated polyp, mucin immunoprofiles were almost the same as those in the controls (Table).

However, there were some differences between control and HIS. The positive rates for MUC1 in the entire and left-side large intestine were significantly lower in HIS than in control (entire large intestine, *p* < 0.005; left-side, *p* < 0.05). The positive rate for MUC5AC in hyperplasia/serrated polyp was also significantly lower in HIS than in control (*p* < 0.05). In HIS, MUC5AC-positive cells in normal/inflammation were found more frequently left-side than right (*p* < 0.005). There were no significant differences in MUC5AC-positive rates between hyperplasia/serrated polyp and adenocarcinoma, or in MUC6-positive rate between the two sides of intestine.

Discussion

From the findings made in normal/inflammation specimens, background mucosal mucin expression may be considered almost the same between HIS and control. In each type of lesion, there were some differences in mucin expression between HIS and control, although these may be subtle. The significance of these alterations in mucins remains unclear and further accumulation of findings will be required.
Introduction and Objectives
The strongly hemolytic *Brachyspira* (shB) spp. *B. hyodysenteriae*, “*B. hampsonii*” and “*B. suanatina*” are the causative agents of swine dysentery (SD), whereas weakly hemolytic species (whB) are either less virulent (*B. pilosicoli*, *B. intermedia*, *B. murdochii*, *B. alvinipulli*) or apathogen (*B. innocens*) (1). Although several hemolysin genes have been identified their contribution to the strong hemolytic phenotype are yet unresolved (2). This study aimed to determine hemolysin gene patterns among shB and whB and to explore the hemolytic activity of recombinant hemolysins.

Materials and Methods
ShB (*B. hyodysenteriae*, n=120), and whB isolates (*B. pilosicoli*, n=11; *B. intermedia*, n=11; *B. innocens*, n=12; *B. murdochii*, n=11; and *B. hyodysenteriae*-like isolate G423) were screened by PCR for known (*hlyA*, *tlyA-C*) and putative hemolysin genes (*hly*, 1870, 962, *yplQ*). Recombinant HlyA and TlyA-C, expressed in *E. coli* BLR (DE3) from a pET-24b (+) vector containing *hlyA* and *tlyA-C*, were used in growth studies.

Results
Hemolysin genes were regularly present in all *B. hyodysenteriae* isolates. Analysis of published genome sequences also confirmed the presence of highly similar hemolysin genes with highly similar nucleotide sequences in the shB species *B. suanatina* (90-99%) and “*B. hampsonii*” (87-99%). The whB possessed quite different hemolysin gene patterns and generally lacked *yplQ*. Also isolate G423 had all but the *yplQ* gene. Hemolysins of shB isolates were secreted for a short time at the beginning of exponential growth. They were most active at 37°C, heat labile and could be inhibited by non-hemolytic, heat stable substances obtained from the supernatant during stationary growth phase. The whB expressed a soluble hemolysin which could not be extracted. Recombinant HlyA and TlyA-C did not lyse sheep red blood cells in PBS at 37°C.

Discussion
Our data indicate that *yplQ* might be involved in the hemolytic phenotype of shB. Although recombinant HlyA and TlyA-C failed to cause hemolysis, other approaches suggest a contribution of TlyA to the strong hemolytic phenotype (3, 4). Functional assays using knock-out mutants will be performed to clarify the role of *yplQ* and TlyA in the strong hemolytic phenotype of *B. hyodysenteriae*.

Table 1: Presence of hemolysin encoding genes in strongly and weakly hemolytic *Brachyspira* spp.

<table>
<thead>
<tr>
<th>Species Strain</th>
<th>Detection of hemolysin genes (PCR)</th>
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<tbody>
<tr>
<td></td>
<td><em>hlyA</em></td>
</tr>
<tr>
<td><strong>B. hyo</strong></td>
<td></td>
</tr>
<tr>
<td>B204</td>
<td>+</td>
</tr>
<tr>
<td>B. <em>hyo</em> Isolates (n=120)</td>
<td>+</td>
</tr>
<tr>
<td><strong>B. hampsonii</strong> 5364-4x</td>
<td>+</td>
</tr>
<tr>
<td><strong>B. suanatina</strong> AN4859-03</td>
<td>-</td>
</tr>
<tr>
<td><strong>B. hyo</strong> G423</td>
<td>+</td>
</tr>
<tr>
<td><em>B. pilosicoli</em> Isolates (n=11)</td>
<td>+</td>
</tr>
<tr>
<td><strong>B. intermedia</strong> Isolates (n=11)</td>
<td>+/−</td>
</tr>
<tr>
<td><strong>B. innocens</strong> Isolates (n=12)</td>
<td>+/−</td>
</tr>
<tr>
<td><strong>B. murdochii</strong> Isolates (n=11)</td>
<td>+/−</td>
</tr>
<tr>
<td><strong>B. alvinipulli</strong> C1</td>
<td>+</td>
</tr>
</tbody>
</table>
| +/- variable

References
Whole-genome sequencing to investigate pleuromutilin resistance and the epidemiology of *Brachyspira hyodysenteriae*  
Ewart Sheldon1,2, Roderick M. Card1, Emma Stubberfield1, Gemma Kay3, Susanna Williamson1, Nabil-Fareed Alikhan2, Muna F. Anjum1 and Mark J. Pallen2

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**Introduction and Objectives**

**Introduction**

*Brachyspira hyodysenteriae* is the causative agent of swine dysentery, an intestinal disease of pigs characterised by bloody mucoid diarrhoea and poor growth. If untreated, it can cause serious economic loss to the pig industry. The pleuromutilin antibiotics, tiamulin and valnemulin, remain a key part of effective treatment and control. However, an increased occurrence of resistance has been reported in some countries(1). The prevalence of infected pig herds in GB is unknown; outbreaks on commercial units may be captured through GB scanning disease surveillance but the disease is not notifiable. The prevalence of pleuromutilin resistance is reported annually in the UK-VARSS report; the population structure of *B. hyodysenteriae* has not been recently investigated.

**Objectives**

Archived isolates of *B. hyodysenteriae* from pigs in England were examined by genome-sequencing and subsequent analysis to investigate the phylogeny of clinical isolates. In addition, we aimed to compare the putative resistance genotype to phenotype.

**Materials and Methods**

A panel of seventy isolates, originally isolated between 2004 and 2015, were whole-genome sequenced. Whole-genome sequencing took place on an Illumina Miseq. Genomes were assembled using Spades and single nucleotide polymorphisms (SNPs) potentially linked with resistance were identified using MUMmer. SRST2 was used to identify the MLST group of each isolate. In addition, the sequenced isolates were compared to 20 genomes from NCBI, to do this ParSNP was used to make a SNP-based phylogenetic tree. Bootstrapping was done using RAxML.

To identify a pleuromutilin resistance phenotype a broth microdilution method (VetMic Brachy plates) was used to assess the tiamulin and valnemulin minimum inhibitory concentration (MIC) of 28 isolates. A clinical breakpoint for tiamulin of >4 μg/ml was used(6).

**Results**

**Phylogenetic relationship between strains**

MLST analysis did not identify a dominant sequence type; rather nine different sequence types were identified. Some of these have previously been found only in England, but five of these sequence types (ST8, 52, 87, 122 and 167) have been found in a number of European countries. A phylogenetic tree constructed from SNPs in the core genome showed similar results, with a large cluster of English isolates and a smaller group of isolates that had a wider European distribution.

**Pleuromutilin Resistance Phenotype and Genotype**

Most isolates were sensitive to tiamulin, with only three resistant isolates in the panel, all with an MIC of 8 μg/ml. The resistant isolates all also had MIC >2 μg/ml for valnemulin.

By comparing SNPs found in resistant isolates to SNPs in sensitive isolates, it was possible to identify a combination of SNPs that were present only in resistant isolates. In one of the isolates, SNPs were present in the 23S rRNA at positions 2535, 2116 and 2165 (*E. coli* numbering). The other isolates also contained the SNP at 2535 and another SNP at 2201 (*E. coli* numbering) as well as a SNP at 443 (*B. pilosicoli* numbering) in the L3 50S ribosomal protein.

**Discussion**

Phylogenetic reconstruction using WGS indicated considerable diversity in the English isolates, with no single dominant clone identified. Some English isolates also clustered with global isolates.

The level of pleuromutilin resistance within England is relatively low. Analysis of resistant isolates suggests that a single mutation in the 23S rRNA may not always be enough to confer complete resistance to tiamulin.

**References**

1. Hidalgo, AH; Cavajal, AC; Vester, BV; Pringle, MP; Naharro, GM and Rubio, PR, 2011, Antimicrobial Agents Chemotherap, 55, 3330-3337
Characterization of *tlyA* and *tlyC* from "*Brachyspira hampsonii""

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**Introduction and Objectives**

*Introduction and Rationale*

Hemolysins produced by "*Brachyspira hampsonii*" and *Brachyspira hyodysenteriae* are believed to be important virulence factors in the pathogenesis of swine dysentery. To date four hemolysins present in both species have been identified, termed *tlyA*, *tlyB*, *tlyC*, and *hlyA*. Homologues of *tlyA* have been identified in important pathogens such as *Mycobacterium tuberculosis* (1) and *Helicobacter pylori* (2), and these purified proteins have been shown to exhibit haemolytic activity *in vitro*. Despite this, the *tlyA/B/C* and *hlyA* proteins have not yet been cloned, overexpressed, and purified from "*Brachyspira hampsonii*" or *Brachyspira hyodysenteriae*.

**Objectives**

1. Clone relevant hemolysin genes from "*Brachyspira hampsonii*" clade II strain 30446 genomic DNA into expression vectors (completed)
2. Overexpress and purify functional *tlyA/B/C* and *hlyA* proteins (completed for *tlyA* and *tlyC*).
3. Examine haemolytic activity of purified protein on sheep erythrocytes (completed for *tlyA* and *tlyC*).
4. Examine effects of purified protein on cultured porcine colonic epithelial cells (ongoing).

**Materials and Methods**

*Cloning of tlyA/B/C and hlyA*

Relevant genes were amplified from "*Brachyspira hampsonii*" strain 30446 genomic DNA via PCR and ligated into pET-28a expression vectors containing N-terminal His-tag and Maltose binding protein affinity tags.

*Overexpression and purification of protein products*

Broth cultures of *E. coli* -containing the relevant gene were induced with IPTG, lysed, and purified by Ni²⁺ and amylose affinity chromatography.

*Quantification of haemolytic activity*

Purified protein was added to a 1% (v/v) sheep erythrocyte suspension and the mixture was incubated overnight at room temperature, after which point haemoglobin release was quantified spectrophotometrically.

**Results to date**

*Purified tlyC exhibited no haemolytic activity*

*TlyC* protein at concentrations up to 11 µg/mL exhibited no more haemolytic activity than a PBS control.

*Purified tlyA exhibits haemolytic activity in vitro*

When added to a 1% sheep erythrocyte suspension *tlyA* exhibits dose dependent haemolytic activity. One notable finding was a higher specific activity in c-terminal His-tagged *tlyA* than untagged *tlyA*, with the his-tagged variant causing ~49% hemolysis at a concentration of 215 µg/mL, while the untagged variant only caused 19% hemolysis at a concentration of 123 µg/mL, the highest dose examined.

**Discussion**

Our findings for the haemolytic activity of *tlyC* were in agreement with those for *Leptospira interrogans* (3), another pathogenic spirochaete. The discrepancy in haemolytic activity found for the His-tagged and untagged variants of *tlyA* may be due to the differences in the purification processes for the protein variants, as removal of the affinity tags by TEV protease involved reducing conditions, which is believed to cause a loss of activity in the *Mycobacterium tuberculosis* *tlyA* homologue (1). Future studies will examine the effects of reducing and oxidizing conditions on the haemolytic activity of *tlyA* to determine if the reducing conditions during TEV cleavage are responsible for this loss of activity.

**References**

Looking back over five years of *Brachyspira* PCR result monitoring in French laying hens (2010-2014)

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¹ Elanco France, Rueil-Malmaison, France, ² Labofarm, Loudeac, France

**Introduction and Objectives**

In France, *Brachyspira* infections are rare in pig farms but much more common in layer and breeder poultry farms where they cause reduced growth rate, loss of egg production and diarrhoea. *Brachyspira pilosicoli*, *Brachyspira intermedia* and *Brachyspira alvinipulli* are considered to be the main pathogenic *Brachyspira* species in poultry (¹). In 2009, Labofarm and Novartis Animal Health co-developed a PCR test associated with a sampling kit dedicated to help veterinarians to diagnose avian *Brachyspira*. This test was named BrachyTest™.

**Materials and Methods**

**Sampling**

All poultry veterinarians in France can ask for a BrachyTest™ if Avian Intestinal Spirochaetosis (AIS) is suspected due to clinical signs. Sampling of 5 up to 20 fresh caecal droppings is recommended.

**Laboratory analysis**

Swabs are sent to Labofarm and analysed within 3 to 5 days. The BrachyTest™ is an association of three PCR tests. It identifies *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli*, *Brachyspira intermedia*, *Brachyspira innocens* and also the genus *Brachyspira*.

**Data analysis**

980 PCR test results have been generated from poultry farms between 2010 and 2014. 703 results out of 980 (72%) were from layers. 169 results (17%) were from breeders and the remaining 108 (11%) were from other species or the information was missing. Only the data from layers have been further analyzed.

**Results**

**Main results**

*Brachyspira* were identified in 393 out of the 703 PCR tests performed (56 %). *Brachyspira intermedia* was the main *Brachyspira* in French layers over these 5 years (254 positive results), followed by *Brachyspira pilosicoli* (133 positive results). Looking at the results profiles, it is interesting to notice that one or more pathogenic *Brachyspira* were found in 283 out of the 393 positive results (72%). In addition, 53 % of these 283 results were due to *B. intermedia* alone, 33 % to *B. intermedia* associated with *B. pilosicoli* and 10 % only to *B. pilosicoli* alone (Figure 1).

**Figure 1: Profiles of positive results**

These results have been stable throughout the five years period, even if a non-significant increase of faeces sampling and positive results was noticed from August to October each year. *Brachyspira* species were found all over the country, but the main regions asking for BrachyTests™ were Brittany and North of France.

The median age of sampled layers was 27 weeks, which was lower than the median age of positive flocks: 34 weeks for *B. intermedia* and 32 weeks for *B. pilosicoli*.

**Discussion**

Even if this isn’t a full prevalence study, the large number of data collected helps understand the disease situation in France. Some questions remain unanswered: is there a difference between free range and caged flocks? Why does south-east of France seem less contaminated? Why is the median age of positive flocks higher than the median age of sampling?

**References**

¹.  Le Roy C.I., 2016, Microb Ecol Health Dis, 26, 28853
Differentiation of *Brachyspira* spp. isolated from laying hens by PCR-based methods and MALDI-TOF-MS

M. Harms¹, V. Schmidt², T. Heydel¹, J. Hauptmann¹, R. Bergmann¹, C. Ahlers³, J. Rohde⁴ and C. Baums¹

Introduction and Objectives

Avian Intestinal Spirochaetosis (AIS) is caused by *Brachyspira* species, species infecting the caeca and the colon of poultry. The pathogenic species in poultry are *B. pilosicoli* and *B. intermedia* and possibly also *B. alvinipulli*. Clinical signs include diarrhea, weight loss and reduced egg production. High losses for the egg industry caused by AIS have been proposed in England [1]. The objective of this study is to evaluate differentiation methods for avian *Brachyspira* species and to determine prevalences of the different species in German flocks of laying hens.

Materials and Methods

In various flocks (n = 27) of poultry, fecal samples (n = 6 per flock) were collected and cultivated on specifically designed selective media for isolating avian *Brachyspira*. The flocks did not report any problems reminiscent of the clinical signs described above. The different housing systems were represented equally. Differentiation of *Brachyspira* spp. was conducted using on the one hand PCR-based methods like the nox-PCR [2], a PCR to detect the tryptophanase gene (*tnaA*) of *B. hyodysenteriae* and *B. intermedia* [3], a PCR to detect the gene encoding hippurate hydrolyase (*abgB*) of *B. alvinipulli* and *B. pilosicoli* [3] and on the other hand MALDI-TOF-MS [4] analysis. For MALDI – TOF – MS differentiation master spectra were generated first.

Results

Partial sequencing of the nox gene and MALDI-TOF-MS analysis allowed differentiation of avian *Brachyspira* spp. The combination of both methods might increase the reliability of the diagnosis in critical cases. Furthermore *tnA*- and *abgB*- PCR results were in accordance with results of *nox* sequencing and MALDI – TOF – MS results. Fourteen percent of 162 collected fecal samples allowed detection of spirochaetes by culture.

Twenty-seven percent of the *Brachyspira* isolates belonged to the known pathogenic species. The other isolates were either *B. murdochii*, *B. innocens* or *B. pulli*. *B. alvinipulli* was not isolated in any sample.

Discussion

Laying hens from clinically unobtrusive flocks in Germany might shed *Brachyspira* spp., mostly apathogenic species but at least in every fourth case one of the pathogenic species. Optimal cultivation and differentiation of avian *Brachyspira* spp. requires modification of methods established for the diagnosis of *Brachyspira* spp. in samples from pigs. It was important to create a new database for the MALDI-TOF-MS analysis with the collected isolates and to use nox sequencing for differentiation of avian *Brachyspira* spp. in critical cases.

References

3. developed by Rene Bergmann
Lactobacilli probiotics as a potential control strategy for avian intestinal spirochaetosis

Jade Passey¹, Martin J. Woodward², Jorge Gutierrez¹, Roberto M. La Ragione¹

¹ School of Veterinary Medicine, University of Surrey, Guildford, Surrey, UK, ² University of Reading, Reading, UK

Introduction and Objectives

*Brachyspira* species are the causative agent of avian intestinal spirochaetosis (AIS). AIS is a gastrointestinal disease whereby *Brachyspira* colonise the caeca and colo-rectum of poultry, resulting in diarrhoeal disease and consequently a 6-10% reduction in egg production, poor egg quality and faecally stained eggs. AIS is estimated to cost the UK poultry industry circa £18million annually. Prevalence of *Brachyspira* has increased significantly in recent years with up to 90% of free range hens testing positive for *Brachyspira* spp. Furthermore, emerging antimicrobial resistance is an increasing concern. Therefore, a better understanding of the pathobiology of AIS and novel measures to mitigate this disease are urgently required.

The objective of this study was to investigate physical and chemical interactions between *Brachyspira* and *Lactobacillus* to elucidate mechanisms by which probiotics may protect against *Brachyspira* infection in poultry.

Materials and Methods

**Growth Inhibition Assays:**
*Lactobacillus* cell free supernatant (CFS) adjusted to pH 3.8, 4.5 and 7.2 was inoculated to *Brachyspira* culture and incubated anaerobically at 37°C. Control broths were prepared with MRS at pH 3.8, 4.5, and 7.2. Optical density readings were taken at 620nm every 8 hours for 120 hours.

**NMR Analysis:**
*Lactobacillus* CFS was prepared for NMR analysis by combining with phosphate buffer. For each sample a 1H NMR spectra was acquired using a Bruker 800 spectrometer.

**Motility Assays:**
*Lactobacillus*, either heat-inactivated or viable (10⁹ CFU/ml) were mixed with *Brachyspira* in PBS, anaerobically at 37°C for 4 and 24 hours. 5μl of each mixed suspension was spotted onto *Brachyspira* selective agar and incubated anaerobically at 37°C for 8 days, the extent of motility and haemolysis was determined on the 8th day.

Results

**Growth Inhibition Assays:**

The CFS from all *Lactobacillus* strains inhibited the growth of all test *Brachyspira* in a pH dependent manner (P ≤ 0.01). The CFS from one *Lactobacillus* strain, *L. salivarius* 47 consistently decreased the growth of all test *Brachyspira* in a pH-independent manner (P ≤ 0.01), therefore this strain was used in further assays.

**NMR Analysis:**
NMR analysis of the CFS of all *Lactobacillus* strains revealed that they contained several metabolites which may contribute to the inhibition of *Brachyspira* spp. growth. These included lactic acid, acetic acid and other metabolites such as acetoin which may have inhibitory potential. The studies demonstrated that the lactobacilli which significantly inhibited *Brachyspira* growth had greater concentrations of these metabolites when compare to strains that did not inhibit *Brachyspira*.

**Motility Assays:**
Live and heat-inactivated *L. salivarius* 47 significantly impaired the motility of all test *Brachyspira* strains, irrespective of species (P ≤ 0.05).

Discussion

Due to the increasing prevalence of AIS, it has become important to understand *Brachyspira* pathobiology and to investigate novel measures to mitigate AIS.

CFS from *L. salivarius* 47 suppressed the growth of all *Brachyspira* spp. Inhibition at pH 3.8 was significantly greater than the pH matched control, suggesting the suppressive effect is not solely attributed to acidity.

NMR identified metabolites that may contribute to this suppression, these included lactic and acetic acid, in addition to metabolites such as acetoin, which have antimicrobial properties. *Lactobacillus* CFS had been previously shown to induce a lethal stress response in *Brachyspira*, this can be attributed to lactic acid production.

Both live and heat-inactivated lactobacilli were able to decrease the motility of *Brachyspira*, with live cells inhibiting motility to a greater extent. Inhibition of growth and motility had a universal effect on pathogenic and non-pathogenic *Brachyspira*, suggesting that inhibition is not related to pathogenicity of *Brachyspira*. 

Treponema ruminis sp. nov., a spirochaete isolated from the bovine rumen.

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Introduction and Objectives

Treponema species are fastidious, anaerobic spirochaetes which have been identified within the gastrointestinal (GI) tract, oral cavity and genital areas of humans, animals and insects. Treponemes are most commonly associated with infectious diseases such as bovine digital dermatitis, human periodontal disease and syphilis. However, several treponeme taxa are thought to reside within the GI tracts of animals and insects as commensal symbionts. A small number of these isolates have been taxonomically characterised, including three novel taxa which were isolated from porcine GI contents; Treponema porcinum, Treponema succinifaciens and Treponema berlinense. Although several metagenomic studies have identified a high diversity of spirochaetes within the bovine rumen based upon 16S rRNA gene sequencing, only two isolates have currently been proposed as novel Treponema species, namely Treponema bryantii and Treponema saccharophilum. Further work is required to perform detailed taxonomic appraisals and elucidate the true diversity of treponemes within the bovine GI tract.

A recent study (1) isolated seven novel spirochaetes from the GI tracts of Holstein-Friesian cattle in the United Kingdom. The isolates clustered as four novel, distinct phylotypes within the Treponema genus and each shared less than 97% identity to other characterised treponemes based upon 16S rRNA gene sequences. Suggestive that these isolates represent novel taxa, the present study aimed to further taxonomically appraise one particular phylotype, represented by strain Ru1, to consider if it was a novel Treponema species.

Materials and Methods

The novel spirochaete (Ru1) was isolated at slaughter from the rumen contents of a Holstein-Friesian bull and was cultured anaerobically (1). Morphological characteristics were observed by transmission electron microscopy and the API® ZYM system was used to determine its enzyme activity profile (1). Genomic DNA was extracted from the cultured isolate for PCR amplification, commercial sequencing and phylogenetic analysis of the 16S rRNA and recombinase A (recA) genes (1,2). A novel PCR assay was developed to amplify the recA gene using degenerate oligonucleotide primers, designed by an alignment of relevant bovine GI tract treponeme recA gene sequences. Amplified gene sequences (1,320bp and 504bp respectively) were aligned against other relevant bovine, porcine and human treponemes using CLUSTAL W (BioEdit Sequence Alignment Editor). The best-fit evolutionary models were predicted using TOPALI and were then used to infer bootstrapped maximum-likelihood trees based upon 10,000 reiterations (MEGA).

Results

Phylogenetic reconstruction of the 16S rRNA gene sequence alignment revealed that Ru1 formed a distinct phylotype within a wider, deep-branched region of porcine and bovine GI tract treponemes. Its nearest relative, based upon 86.2% 16S rRNA gene sequence similarity, was identified as the porcine GI tract isolate, T. porcinum. Phylogenetic analysis of the Ru1 recA gene identified 76.8% sequence similarity to another porcine GI tract isolate, T. succinifaciens. Despite sharing several common morphological characteristics of other treponemes, the novel bovine rumen isolate demonstrated a unique size (5-9 μm length, 0.4-5 μm width) and had 4 periplasmic flagella and 3-5 even windings. The isolate also had a unique enzyme activity profile compared to other recognised bovine, porcine and human treponemes and exhibited C8 esterase lipase, leucine arylamidase, β-galactosidase and β-glucosidase activity. Finally, its requirement for daily reinoculation and ability to grow without serum supplementation makes it distinctly different to other previously characterised treponemes.

Discussion

The novel bovine rumen isolate (Ru1) has demonstrated substantial genotypic and phenotypic diversity to other characterised treponemes and therefore warrants the proposal of a novel Treponema species, Treponema ruminis sp. nov..

References

Evaluation of an infection model of swine dysentery

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Introduction and Objectives

The ability of Brachyspira hyodysenteriae to colonize and proliferate in the pig intestine and the severity of clinical signs of swine dysentery (SD) are greatly influenced by the diet (1).

The aim of the present study was to establish an infection model for SD. This work was sponsored and directed by Aquilón CyL, an spin-off participated by the University of León (Spain), to develop a validated tool for safety and efficacy studies of autogenous vaccines or vaccines against B. hyodysenteriae.

Materials and Methods

Animals and facilities

Thirty-six 9 week-old crossbred pigs were obtained from a commercial farm without previous history of SD. The animals were housed in the animal facilities of A.M. Animalia Bianya (Girona, Spain). All of them tested negative for B. hyodysenteriae and Lawsonia intracellularis.

Experimental groups and design

Pigs were randomly allocated into three experimental groups (day -14). Animals from groups 1 (G1) and 3 (G3) were fed with high protein diet (standard feed, free of zinc oxide and antibiotics, mixed 1:1 with soya flour) from day -7 to day 0 of the experiment while animals from group 2 (G2) were fed with standard diet. Between day 1 and day 22, pigs from the three experimental groups were fed with the standard diet.

On day 0, pigs from experimental groups G1 and G2 were orally inoculated for 3 consecutive days with 50 ml of a culture of B. hyodysenteriae reference strain B204 (ATCC 31212) (5x10⁶-5x10⁷ bacteria/ml) while pigs from G3 served as non-inoculated control.

Monitoring of experimental infection

Between post-inoculation day 0 and 22 pigs were daily monitored by a blinded technician for diarrhoea (faecal score 0: normal faeces; 1: soft faeces; 2: liquid faeces/mucus; 3: presence of mucus and blood in faeces). Faecal shedding of B. hyodysenteriae was determined by culture using selective media containing antibiotics and blood as well as by qPCR directly from faeces (2).

Results

Neither diarrhoea nor B. hyodysenteriae shedding were consistently detected in the non-inoculated group. B. hyodysenteriae shedding was similar in both experimentally infected groups (G1 & G2). However, the duration and the prevalence of diarrhoea were significantly higher in the hyperproteic diet group (G1) as compared with the standard diet group (G2).

Discussion

Although a mouse model of SD has been used (3), these animals do not develop clinical disease and this model is not suitable for efficacy studies with SD vaccines or autovaccines. As previously described (4), our results suggest the essential role of the pre-challenge high protein level in the diet on the clinical outcome after experimental challenge with B. hyodysenteriae.

References

Introduction and Objectives
Swine dysentery emerged in Swedish pig herds in the late fifties and then rapidly spread within the country (1). In 2000, a certification programme in breeding herds was initiated to reduce the prevalence of the disease. During the following ten years, the numbers of infected herds decreased tremendously: in 2004 the proportion of infected herds was estimated to approximately 4% and in 2009 to 1.2% (2,3). During the last five years, the annual frequency rate of samples submitted to SVA (National Veterinary Institute) obtaining a positive result has been on a consistent level. The objective of this study was to investigate the occurrence and geographic location of swine dysentery infected herds and the epidemiological relationship between strains of Brachyspira hyodysenteriae with the long-term goal to use the result in a future eradication programme.

Materials and Methods
All isolates of Brachyspira hyodysenteriae (n=32) isolated from samples submitted to National Veterinary Institute between years 2010 and 2014 were included in the study. Information of geographic origin of the isolates was available through postal codes. Frozen stocks were subcultured anaerobically on FAA plates and DNA was extracted. Genotyping was performed by randomly amplified polymorphic DNA (RAPD) using two sets of primers P1254 and P739, and gel electrophoresis banding patterns were compared (4).

Results
All isolates came from the south – south western part of Sweden, the majority (n=30) from the counties of Skåne, Halland and Blekinge. The result from the RAPD showed that the isolates could be divided into three main genetic groups, A, B and C. Group A isolates were found every year, group B isolates years 2010 – 2013 and group C isolates years 2011 and 2012. There was no distinct difference in geographical distribution of the different genetic groups of Brachyspira hyodysenteriae isolates.

Discussion
The results indicate that swine dysentery remains in just a few herds in Sweden, located in a limited part of the country. Three main groups of isolates have been circulating between 2010 and 2014, indicating that several of the infected herds could have been infected from a common source. One of the clones seems absent the latest years which suggests that herd eradication programmes have successfully eliminated swine dysentery from affected herds.

In conclusion a total eradication of swine dysentery from Sweden is feasible considering the low number of remaining infected herds.

References
Introduction and Objectives

Brachyspira (B.) suanatina was first described by Råsbäck et al. in 2007 in samples from four Swedish and Danish pigs as well as in two samples from mallards [1]. To our knowledge so far no other isolates of this species have been described in pigs in the literature.

Since its first description in the US and Canada in 2012 and 2013 “B. hampsonii” has become a major concern in the pig industry in North America while reports from elsewhere in the world are rare [2, 3]. Both Brachyspira species have been shown to produce dysentery-like disease in pigs in experimental infections.

This work describes the occurrence of these new strongly haemolytic Brachyspira species in samples from pigs with diarrhea as well as from swine dysentery surveillance samples in Germany.

Materials and Methods

Between January 2009 and April 2016 a total of 7186 Brachyspira isolates were analysed for B. suanatina by nox-RFLP [4].

Due to the small differences in fragment sizes in nox-RFLP “B. hampsonii” cannot be differentiated from B. intermedia with this method. Therefore, 585 isolates from 2012-2016 provisionally designated as B. intermedia by nox-RFLP were tested in a species-specific conventional PCR. Primers Bhamp-f ATTGATTTATATGGGACATGAAGTA and Bhamp-r ACCATATTCAGTGCCTTCCTGTTT were designed for amplifying a 183 bp fragment of the nox-gene at an annealing temperature of 64°C. Isolates positive in this PCR were partially nox-gene sequenced using the same primers as for nox-RFLP and submitted for BLAST analysis.

A phylogenetic tree of the nox-gene of the B. suanatina and “B.hampsonii” isolates was constructed with MEGA7 software based on ClustalW alignments with the Neighbour Joining method.

Results

B. suanatina: Among the 7186 Brachyspira isolates only three isolates were identified as B. suanatina. Two isolates were identified in 2014. They showed 98% similarity of their nox-gene sequence to the nox-gene of type strain AN4859/03. Samples were from clinically healthy sows that had been bought in Denmark.

In 2015 another isolate was identified as B. suanatina with 99% similarity to the nox-gene of the type strain. This one originated from a sample form a group of grower pigs with dysentery-like diarrhoea.

“B. hampsonii”: Among the 585 presumptively identified isolates “B.hampsonii” was detected 8 times. An additional 19 PCR-positive isolates could not be confirmed as “B. hampsonii” by nox-gene sequencing. Three “B. hampsonii” isolates (5364-4x/12, 5366-2x/12 and 5369-1x/12) were from a batch of samples from pigs imported from Belgium to Germany. Another four isolates (3824-2x/12, 3824-11x/14, 3824-14x/14 and 3824-15x/14) were from a single farm, from which samples were submitted for Brachyspira surveillance. The last isolate of “B.hampsonii” (5746-2x/14) again was isolated from surveillance samples. None of the farms with “B. hampsonii” reported clinical signs of swine dysentery or diarrhoea. Isolates 5364/12, 5366/12 and 5369/12 belonged to genetic group I while isolate 3824-15x/14 was group III [5]. The last isolate 5746-2x/14 clustered with 3824-15x/14 in a nox-gene based phylogenetic tree and showed 98% similarity of the nox-gene with isolates from Spanish waterfowl.

Discussion

According to our data, strongly haemolytic Brachyspira species other than B. hyodysenteriae seem to play a minor role in German pig herds.

While at least in one of the two affected farms B. suanatina was associated with dysentery-like disease, “B.hampsonii” was only isolated from surveillance samples, in two of the three cases even in association with B. hyodysenteriae (data not shown).

References

Swine challenge trial of a Brazilian atypical *Brachyspira hyodysenteriae* isolate

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### Introduction and Objectives

*Brachyspira hyodysenteriae* is the etiologic agent of Swine Dysentery (SD). The disease causes severe mucohaemorrhagic diarrhea. Gross lesions include variable amounts of mucosal thickening, multifocal hemorrhage, necrosis associated fibrinous exudate, and variable but often excessive luminal mucus in the large intestine (1). There are a few reports of *B. hyodysenteriae* strains of low pathogenicity, which colonizes but does not induce clinical disease (2,3). In Brazil, this is the first report of such strain, which showed strong hemolysis on blood agar, PCR and nox gene sequencing of *B. hyodysenteriae*. The aim of this study was to investigate the pathogenicity of this strain in experimentally challenged pigs.

### Materials and Methods

Forty-eight 5-week-old crossbred pigs, with no previous history of *Brachyspira*-associated disease or any other confounding enteropathogen, were randomly divided in 3 groups: control (CTRL), virulent (VIR) and atypical (ATYP). The VIR strain used in this study was isolated from a pig clinically affected with diarrhea and colitis (ST179 in PubMLST database http://pubmlst.org/bhyodysenteriae/) while the ATYP strain was isolated from a health pig from disease free Brazilian herd (ST undefined). VIR and ATYP groups were inoculated by gavage with 50ml of culture broth containing 10⁸ *B. Hyodysenteriae* per ml on three successive days. All animals were monitored daily for the presence of mucohaemorrhagic diarrhea. Eighteen days post-inoculation (DPI), all pigs were humanely euthanized and necropsied. At necropsy, the entire intestinal tract was gross evaluated with special attention to the large intestine that was longitudinal opened and evaluated for the presence and distribution of luminal mucus, mucosal hemorrhage, necrosis and fibrinous exudate. Clinical and gross lesion findings were analyzed using SPSS software and Kruskal-Wallis test for comparisons among groups. P<0.05 was considered significant.

### Results

Before inoculation, one pig from ATYP group died suddenly due to valvular endocarditis and was removed from the study. Mucohaemorrhagic diarrhea was first observed 7 DPI in VIR group and 15 DPI in ATYP group. Clinical signs and gross lesions observed in each group are summarized in Table 1. When present, gross lesions in the intestinal tract were limited to the cecum and large intestines but were most common in portions of the spiral colon.

### Discussion

Atypical *B. hyodysenteriae* isolates were previously described in different studies (2,3,4). These strains were reported to appear slightly less hemolytic on blood-containing agar plate than virulent strains. Lysons et al. (1982) used three atypical strains in a challenge study and clinical signs were not observed in pigs that received two of them. The hemolytic activity of *B. hyodysenteriae* might be a major pathogenic factor. Recently, Mahu et al. (2016) sequenced seven hemolysis associated genes of *B. hyodysenteriae* strains isolated from pigs with mild to severe mucohaemorrhagic diarrhea. One weakly hemolytic strain showed sequence changes in five genes (5). The next steps of our study will be histological evaluation and full genome sequence in order to clarify the differences clinically and grossly observed in this study.

### References

4. Hampson, D.J. et al., 2015, Porcine Health Management, 1,8.

Acknowledgment: Cnpq, Capes and Fapemig.
Introduction and Objectives

Bacteriological diagnosis of swine dysentery (SD) and porcine intestinal spirochaetosis (PIS) consists of selective culture of clinical specimens (feces, colonic tissue) and subsequent classification of spirochetal isolates by PCR targeting the brachyspiral 16S rDNA and NADH Oxidase (Nox) gene, respectively (1). In addition, the presence of spirochetes prior to the PCR analysis is confirmed by examining scrapings from the bacterial lawn microscopically. However, culture-coupled PCR to detect pathogenic \textit{Brachyspira} (\textit{B}.) often requires six days or longer to be completed and many steps of sample handling. Therefore, we here aimed to optimize the workflow of \textit{Brachyspira} diagnostics through the development of a multiplex real-time PCR simultaneously detecting \textit{B. hyodysenteriae} and \textit{B. pilosicoli} and a triplet of the apathogenic \textit{Brachyspira} spp. (\textit{B. innocens}, \textit{B. intermedia}, \textit{B. murdochii}).

Materials and Methods

Sample group

Overall, a panel of 453 swabs with a positive culture of \textit{Brachyspira} spp. was arbitrarily selected from diagnostic samples. Samples were divided into two groups for comparison of culture and PCR methods. In addition, 58 microscopically \textit{Brachyspira} negative culture and swab samples were analysed with the multiplex real-time PCR for determination of the sensitivity of the diagnostic workflow.

Multiplex Real-time PCR

A primer pair amplifying a 128-bp fragment of the 23S rDNA gene of all five different proine \textit{Brachyspira} spp. (\textit{B. hyodysenteriae}, \textit{B. pilosicoli}, \textit{B. intermedia}, \textit{B. innocens}, \textit{B. murdochii}) was designed. On this amplicon, target sequences for three DNA probes specific for (i) \textit{B. hyodysenteriae}, (ii) \textit{B. pilosicoli} and (iii) a triplet of apathogenic species (\textit{B. intermedia}, \textit{B. innocens}, \textit{B. murdochii}) were identified and designed for the sub-species identification.

Results

Using DNA from fecal cultures, the multiplex real-time PCR showed excellent agreement with the previous standard diagnostic method, duplex PCR ($\kappa$=0.943 and 0.933). Analysis of 202 fecal swabs in comparison with a culture-based PCR approach confirmed the multiplex PCR suitable for high-throughput screening of porcine fecal swabs for the causative agents of SD and PIS ($\kappa$=0.853 and 0.643). Moreover, the sensitivity of the multiplex PCR was further confirmed by the analysis of \textit{Brachyspira} negative cultures, evaluated by dark-field microscopy, where 43% (25 of 58) of the samples were positive for \textit{Brachyspira} spp. in the multiplex PCR analysis. Moreover, 28% (7 of 58; 12%) of these samples were confirmed positive also when analysed direct on swab DNA.

Discussion

Both the culture and swab sample methods show high specificity, however, the culture coupled real-time PCR has the highest sensitivity. Therefore, we recommend these two analysis approaches in different setups: high-throughput screening of fecal swabs and the culture-coupled approach to identify \textit{B. hyodysenteriae} infected individuals within real-time PCR positive herds with the purpose to establish \textit{Brachyspira} strains e.g. for antimicrobial susceptibility testing. Furthermore, this study showed the intermediate microscopic evaluation step to be not essential. By analysing all samples directly with the multiplex real-time PCR, without the microscopic evaluation, the hands on time can be drastically decreased and meanwhile the diagnostic sensitivity notably increased. Together, this novel assay is robust and has a high analytical sensitivity and specificity, which leads to an improved SD and PIS diagnostic workflow. With the new optimized workflow the SD diagnosis can be delivered in half of the time (culture incubation excluded) compared to previous standard methods, even while handling with large sample groups.

References

Introduction and Objectives

The Swedish Livestock Research Centre (Lövsta) at SLU was relocated to new premises in 2012. All animals from the old facilities were culled and a new herd was established by the purchase of gilts from an SPF nucleus herd. The herd was documented free from a range of viral infections not present in Sweden, and from *Actinobacillus pleuropneumoniae*, *Brachyspira hyodysenteriae*, *Brucella suis*, *Mycoplasma hyopneumoniae*, toxigenic strains of *Pasteurella multocida*, and *Sarcoptes scabiei*. The herd had reportedly experienced outbreaks of haemorrhagic diarrhoea and been found serologically positive for *Lawsonia intracellularis*. The occurrence of other species of *Brachyspira* in the herd was not known.

At the research centre, several projects focus on feed ingredients and feed optimization strategies. Thus, it is important to control factors that may affect outcome parameters such as average daily weight gain and feed conversion ratio.

The objective of this project was to ensure the freedom of *Brachyspira* spp in the pig herd at the Lövsta Research Centre.

Materials and Methods

Approximately two months before the transfer of the 154 animals (pregnant and non-pregnant gilts, and two boars) that were purchased from the SPF nucleus herd, rectal swabs were collected from ten of the pigs. The samples were analysed in accordance with previous protocols (1). One pig was cultured positive for *Brachyspira* (*B.*) *intermedia* and one for *Brachyspira* "group III" (*B. innocens* and/or *B. murdochii*) (1,2). Before leaving the SPF herd, all animals were treated with tiamulin premix in feed 10 mg/kg b.w. (Denagard vet, Novartis Animal Health, Basel, Switzerland) for five days. The animals were transported in a clean and disinfected vehicle. At arrival, all pigs were manually washed and located into new premises, used as a temporary quarantine, and the treatment continued for additionally seven days. Thereafter, the pigs were moved into the dry-sow unit. Based on the assumption that 30% of the growing/finishing pigs would be positive, rectal swabs were collected from ten pigs six and 12 months after the end of the medication programme, and once yearly thereafter.

Results

All samples have been negative with respect to *Brachyspira* spp. at all sampling occasions during 2012-2016.

Discussion

*Brachyspira* spp were never demonstrated in the pigs kept at the old premises. Since that herd still was infected by endemic diseases such as *A. pleuropneumoniae*, *L. intracellularis* and *M. hyopneumoniae*, it was decided to instead establish an SPF herd in the new premises. In addition, it was decided to perform a medicated elimination programme before introducing the animals to the new facilities, in an attempt to also keep the new herd free from *Brachyspira* spp. In a previous study, *Brachyspira* spp. were demonstrated in 50% of the growers (3). Thus, targeting the growing pigs, 10 samples would allow the detection of *Brachyspira* spp. at 5% confidence level. However, the prevalence in gilts is not known and this might constitute a bias. Further, *Brachyspira* spp. may be transmitted by rodents and other animals such as wild boars. However, *Brachyspira* spp. has never been demonstrated in the Swedish wild boar population (3), and in SPF herds, specific precautions are undertaken to avoid contact with other animal species, which may reduce the risk for re-introduction of the microbe.

In conclusion, it seems possible to eliminate *Brachyspira* spp. from single herds. Thus, *Brachyspira* spp. does not seem to inevitably constitute a part of the normal intestinal flora.

References

Antimicrobial susceptibility of Western Canadian Brachyspira isolates

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Introduction and Objectives

Swine dysentery, characterized by mucohaemorrhagic diarrhoea has re-emerged in Western Canada since the late 2000s (1). No standardized methods for determining the antimicrobial susceptibility of Brachyspira spp. have been developed, and laboratories rely heavily on in-house protocols which may yield variable results (2, 3). The susceptibility of Canadian isolates have not been described. Our objective is to describe the antimicrobial susceptibility of Western Canadian Brachyspira isolates using an agar dilution method.

Materials and Methods

Bacterial isolates: The study included a total of 70 Brachyspira isolates from porcine diagnostic submissions where Brachyspira was suspected, isolates colonizing healthy pigs from Western Canada between 2010 and 2016 and ATCC strains. The collection included B. hyodysenteriae (n=18), “B. hampsonii” (n=15), B. murdochii (n=25) and other Brachyspira spp. (n=12).

Antimicrobial agents: Ten antimicrobials including tiamulin-TIA; valnemulin-VAL; tylosin-TYL; tylvalosin-TVL; lincomycin-LIN; chloramphenicol-CHL; nalidixic acid-NAL; tetracycline-TET; amoxicillin + clavulanic-AUG and ampicillin-AMP were included. TSA plates with 5% ovine blood containing two-fold dilutions of each drug (0.25-128µg/ml) were prepared. All testing was done in triplicate.

Agar dilution method: In our preliminary work we developed a standard curve relating OD600 of a broth culture to CFU/ml, and determined the minimum starting organism density (1-2 × 10^8 CFU/ml) which yields consistent results between replicates. Frozen isolates of Brachyspira were grown in brain heart infusion broth + 10% fetal calf serum. The OD of those cultures were measured, the density was adjusted to 1-2 × 10^8 CFU/ml, and 2 µl (2-4 × 10^5 CFU) of the culture was inoculated onto test media. Plates were incubated anaerobically for 48 hrs at 42°C. Hemolysis was used as an indicator of growth. For each organism-drug combination, the lowest two concentrations where haemolysis was not observed were sub-cultured to confirm the absence of viable organisms.

Results

Table 1: MIC distribution of 70 isolates

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</table>

*For some isolates tiamulin was only tested up to 4 µg/ml, an MIC of ≥8 is therefore the highest concentration presented. Lines denote the MIC50 for each drug.

Tiamulin and valnemulin, inhibited 57 (81%) and 64 (91%) of isolates at the lowest concentration tested. A bimodal MIC distribution was observed for ampicillin, with 29 (41%) isolates inhibited at the lowest concentration tested, and 15 (21%) uninhibited at the highest concentration tested. Susceptibility to the macrolide/lincosamide type drugs spanned the range of concentrations tested.

We found that failure to hemolyze does not reliably predict bacterial inhibition. When the MIC or the next doubling dilution were sub-cultured, live bacteria were recovered from tylosin, lincomycin, tylvalosin, ampicillin, chloramphenicol and nalidixic acid containing media. We also found that some organisms produced surface growth at low drug concentrations.

Discussion

The highly variable susceptibility of isolates highlights the importance of this testing to aid in the evidence based selection of antimicrobials and indicated the presence of acquired antimicrobial resistance. Further work is required to define a biologically relevant test endpoint.

References

Introduction and Objectives

*Brachyspira (B.) hyodysenteriae*, the agent of swine dysentery, was first detected in Switzerland in 2008 (1) and since then it has been spreading through the country. Up to now, antimicrobial susceptibility of Swiss *B. hyodysenteriae* isolates has not been investigated, although antimicrobial resistance is progressive in Europe and antimicrobial treatment remains the main therapeutic option for the treatment of swine dysentery. Therefore, antimicrobial susceptibility testing (AST) is of particular importance. However, *B. hyodysenteriae* is fastidious to culture and there is no standardised method for AST. Therefore, the VetMIC Brachy panel was evaluated for the diagnostic use and Swiss *B. hyodysenteriae* isolates were investigated for the first time.

Materials and Methods

Thirty porcine *B. hyodysenteriae* isolates were collected between 2009 and 2015 from eight Swiss cantons. The purity of isolates was controlled by native microscopy and by aerobic and anaerobic culture on blood agar. Additionally, isolates were examined by a real-time PCR (2) to avoid mixed culture of different *Brachyspira* spp. The AST was performed by using the VetMIC Brachy panel, a microbroth dilution test. The testing was performed with modifications to the manufacturer’s instructions. Every isolate was tested twice and *B. hyodysenteriae* ATCC 27164 was tested in parallel for control purposes. Furthermore, isolates were sequenced for the occurrence of mutations in the 23S rDNA which is responsible for higher minimal inhibitory concentrations for lincosamides and macrolides (3).

Results

Contamination of refrozen isolates and mixed culture of different *Brachyspira* spp. were quite common and were the main criterion for the exclusion of the study. Native microscopy as exclusive control was insufficient for the detection of contamination. For lincomycin and the macrolides, so-called skips were detected regularly. Hereby, turbid wells occur after a growth inhibition was already observed at lower antimicrobial concentrations. The phenomenon could be only seen in wild-type isolates. Subcultivated bacteria of the skipped wells of these wild-type isolates were mutated. The same could be detected for the originally susceptible control strain *B. hyodysenteriae* ATCC 27164 and additionally minimal inhibitory concentrations of >128 µg/ml for tylosin could be determined.

Regarding the antimicrobial susceptibility, all tested Swiss isolates showed good susceptibility to the pleuromutilins and with the exception of tylosin, no resistance was found to the other antimicrobial agents tested.

Discussion

The high contamination rate and the occurrence of skips aggravated the AST and mutations influenced the accurate determination of minimal inhibitory concentrations for lincomycin and the macrolides. The occurrence of skips is already described for *B. pilosicoli* but not yet for *B. hyodysenteriae* (4). Explanatory for the skips could be that mutation takes place inside of the well with the higher concentrations of the antimicrobial agent or that two clones are present in the inoculum.

Regarding the antimicrobial susceptibility of the isolates, Switzerland is still in a favourable situation compared to other European countries, where resistance to the pleuromutilins is spreading and resistance to lincomycin and macrolides is common. Although resistance monitoring and AST in routine diagnostics is recommended the establishment in a small laboratory is difficult facing the challenges in testing procedure. Optimisation of culture, AST and effective genetic screening methods for the detection of resistance are required and would facilitate the diagnostic workflow.

References

Detection of *Brachyspira hyodysenteriae* in three different parts of the intestine from slaughtered pigs in Switzerland

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Introduction and Objectives

The diagnosis of *Brachyspira hyodysenteriae* (BH) using pig faeces from herds with subclinical swine dysentery (SD) is challenging. It was reported in a previous study that diagnostic sensitivities for specific Brachyspira spp. in faecal samples from growing pigs can vary between 16.7-68.8% compared to intestinal samples (1). It is probable that the diagnostic sensitivity for BH is similar to the findings in this study. However, no data has been available to date from slaughtered pigs which often display no clinical signs of SD.

Therefore, the aim of this study was to determine the detection rate of BH in different parts of the large intestines (colon, caecum, and rectum) of slaughtered pigs. Correlation of intestinal lesions, ingesta/ faecal consistency, and time from sample collection until processing on BH detections rates should be analysed.

Materials and Methods

Intestines from 10 slaughter pigs each were randomly selected from 20 herds with previous detection of BH. The intestines were transported to a necropsy hall and scored for macroscopic lesions (0 = no lesions, up to 4 = multifocal lesions) and ingesta/ faecal consistency (score 0 = firm, up to 3 = liquid). Three samples per intestine (ingesta-mucosal scraping from the apex coil (A) and caecum (C), and faeces from the rectum (R)) were obtained and either directly submitted for cultivation or kept refrigerated until further analysis. The samples were cultured across 3 different agar plates for selection of *Brachyspira* spp. and the resulting bacteria were identified using MALDI-TOF MS. The times between sampling in the slaughterhouse (S), the pathological examination (P), and seeding the first *Brachyspira* selective agar (B) were recorded as well as the time between sampling and previous detection of BH in the herd. The data were subjected to either a Chi-square test, Fisher’s exact test or McNemar test and differences with p<0.05 were considered as statistically significant.

Results

A total of 400 ingesta-mucosal and 200 faecal samples were examined. BH was detected in 27 samples (4.5%), 8 herds (40.0%), and 18 pigs (9.0%; median per herd: 2, range 1–4). BH detection rate was highest for sample site A (n = 12 [6.0% of all A samples/ 44.4% of all positive samples]), followed by R (9 [4.5 / 33.3%]) and C (6 [3.0 / 22.2%]). Double BH proof occurred in 6 intestines: AR: 5 and AC: 1.

Most of the intestines showed no lesions at all. Lesions suspicious for SD occurred in only 3 herds testing BH positive in our analyses and only in colons (n = 7). In the herds testing BH positive, sites without lesions were more often BH positive than sites with lesions (77.8% (21/27) versus 22.2% (6/27)).

Consistency score of all sites was in median 2, of A and R 1.8, and of C 3.0; (range 0–3). In the herds testing BH positive, positive BH results were more often associated with a score of 2 or 3 than scores 0 or 1 (87.5% (21/24) versus 12.5% (3/24), p<0.01).

It was found that more BH positive test results (88.9%, 24/27) were derived from samples with a transport time (= S-P) of ≤4 hours compared to samples with S-P of 14–18 hours (11.1%, 3/27). Furthermore, more positive results (96.3%, 26/27) were derived from storing time (= P-B) of ≤60 hours compared to P-B of 240–300 hours (0.7%, 1/27). Interestingly, more BH positive results (81.5%; 22/27) were obtained from herds sampled 4-6 years after the initial diagnosis compared to <4 years (18.5, 5/27).

Discussion

BH occurred more frequently in intestinal samples than in faeces of pigs. A rapid submission to analysis enhanced detection of BH by cultural isolation. Lesions were not predictive for BH detection in our study. Although having been significant in our sample set, consistency is very variable and influenced by many factors (2) and therefore a less reliable predicting factor. The influence of time after initial diagnosis on BH detection requires further investigation. In conclusion, intestines from slaughter pigs and in particular colons are probably necessary for efficient SD diagnosis.

References

Antimicrobial resistance trends among *Brachyspira hyodysenteriae* isolates recovered from swine dysentery outbreaks in Spain (2011-2016)

Lorena Álvarez¹, Rubén Miranda², Joan Marca¹, Pedro Rubio², Ana Carvajal²

Introduction and Objectives

Swine dysentery (SD), caused by *Brachyspira hyodysenteriae*, is a major cause of diarrhoeal disease among pigs in Spain. Due to the lack of commercial vaccines, its control and treatment involves the use of antimicrobials as well as autogenous killed vaccines in this country. However, the number of effective available antibiotics is limited. Moreover, antibiotic treatment in SD is increasingly difficult due to a lowered susceptibility of *B. hyodysenteriae* field isolates to antibiotics (1, 2). Therefore, a careful use of these drugs which should include the monitoring of resistance in field isolates of *B. hyodysenteriae* is highly recommended.

The aim of this study was the monitoring of antimicrobial susceptibility of *B. hyodysenteriae* field isolates recovered from SD affected farms in Spain during the last six years.

Materials and Methods

**Bacterial strains**

A set of 164 field isolates of *B. hyodysenteriae* recovered from 164 SD outbreaks in Spain were investigated. Isolates were distributed according to the year of isolation in two groups: 2011-13 (106 isolates) and 2014-16 (58 isolates).

**Antimicrobial susceptibility tests**

The minimum inhibitory concentration (MIC) for tiamulin, valnemulin, doxycycline, tylvalosin, tylosin, and lincomycin was determined using commercial plates (VetMIC™ Brachy v2) according to the manufacturer recommendations.

**Statistical analysis**

Antimicrobial susceptibility trends were studied using a survival analysis as previously described (1).

**Results and Discussion**

MIC values that completely inhibited the growth of 50% (MIC₅₀) and 90% (MIC₉₀) of the isolates for both periods of time (2011-13 and 2014-16) are shown in Table 1.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC₅₀ (µg/ml)</th>
<th>MIC₉₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiamulin</td>
<td>1</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Valnemulin</td>
<td>1</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Tylvalosin</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>32</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Tylosin</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

Table 1. MIC₅₀ and MIC₉₀ for six antimicrobial agents against Spanish field isolates of *B. hyodysenteriae* recovered between 2011-13 and 2014-16.

When the survival curves (Fig.1) were compared using the log-rank test at $\alpha=0.05$, statistically significant differences were detected for valnemulin ($p=0.001$) and tylvalosin ($p<0.001$) between the two studied periods (2011-13 and 2014-16).

These results confirm the trend towards a lower sensitivity of *B. hyodysenteriae* isolates to some of the most commonly used antimicrobials for the treatment of SD and highlight the urgent need for new approaches in the control of this disease in swine farms.

**References**

Susceptibility to tiamulin, valnemulin and tylvalosin of Belgian *Brachyspira hyodysenteriae* field isolates collected between 2011 and 2015

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**Introduction and Objectives**

Swine dysentery (SD), a mucohaemorrhagic diarrhea caused by the anaerobic spirochaete *Brachyspira hyodysenteriae*, is commonly treated in Belgium using the pleuromutilins tiamulin and valnemulin and the macroide tylvalosin. In 1997, the European Union banned several antimicrobial growth promoters (AGP). Carbadox, olaquindox and tylosin were banned in 1999 (1). This probably contributed to an increase in the number of clinical cases of SD. A complete ban of AGPs was established in 2006. The pleuromutilins tiamulin and valnemulin and the macroide tylvalosin became the last choice antibiotics in the treatment of SD. The aim of this study was to evaluate the susceptibility to these antibiotics of *B. hyodysenteriae* field isolates obtained between 2011 and 2015 and to assess possible changes over time.

**Materials and Methods**

Minimal inhibitory concentrations (MIC, µg/ml) of tiamulin, valnemulin and tylvalosin were determined by agar dilution for 233 *B. hyodysenteriae* isolates obtained from clinical cases of SD between 2011 and 2015. Using epidemiological cut-off values for tiamulin (> 0.25µg/ml), valnemulin (> 0.125µg/ml) and tylvalosin (> 4µg/ml), the percentage of resistant isolates was determined and a temporal trend analysis was performed by means of logistic regression. The statistical analysis was performed in SPSS 23.0 for Windows.

**Results**

The percentages of isolates not belonging to the wild type population during the five consecutive years (2011 to 2015) for tiamulin were 89; 58; 73; 73; 59, for valnemulin 89; 58; 73; 73; 59 and for tylvalosin 100; 84; 83; 80; 69, respectively (Fig 1). Cross resistance was observed for the pleuromutilins. Moreover, co-resistance against the pleuromutilins and tylvalosin was detected: 92% of the obtained *B. hyodysenteriae* isolates resistant against tiamulin and valnemulin between 2011 and 2015 were also resistant against tylvalosin.

A significant (p < 0.01) decreasing trend in acquired resistance was observed for tylvalosin. However, no statistically significant trend over this 5 year period could be shown for the pleuromutilins.

**Discussion**

The results described in this study may indicate that acquired resistance against tylvalosin in Belgian *B. hyodysenteriae* isolates is decreasing. The reason for this is not clear, but more prudent use of macrolides in pigs may play a role. Regardless of this observation, the proportions of isolates with acquired resistance still remain high. Although these proportions do not necessarily equal the proportion of clinically resistant isolates, treatment or eradication by medication of SD in some herds may be problematic, especially when multiresistant *B. hyodysenteriae* isolates are involved.

**References**

1. Maron DF, Smith TJS, Nachman KE, 2013, Globalization and Health, 9, 48
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Proceedings of the 7th International Conference on
Colonic Spirochaetal Infections in Animals and Humans

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