VETPATH

2nd Prato Conference on the Pathongenesis of Bacterial Diseases of Animals

PROGRAM & ABSTRACT BOOK

9th - 12th October 2012 Prato, Italy

MSD Animal Health

Ceva

MONASH University
Medicine, Nursing and Health Sciences

CONTENTS

The program and abstracts can also be viewed on the smart phone / mobile device 'APP' using the following address: http://vetpath-2012-prato.m.asnevents.com.au

WELCOME

On behalf of the VetPath 2012 Organising Committee I welcome you to Prato for the 2nd Prato Conference on the Pathogenesis of Bacterial Diseases of Animals. The first Prato meeting was established in 2010 by the ARC Centre of Excellence in Structural and Functional Microbial Genomics to bring together researchers working in areas relevant to the mechanisms by which bacteria cause disease in animals. There has been an explosion of information over the past few years as genomic and proteomic technologies have developed, as genetic methodology has progressed to the extent that most bacterial pathogens can be genetically manipulated, as structural biologists have focussed on the analysis of virulence factors and as we understand more about host-pathogen interactions. However, bacterial pathogenesis conferences generally focus on human pathogens. This conference will focus on veterinary pathogens and will involve an integrated examination of the latest exciting data on disease epidemiology, bacterial adhesion, intracellular pathogens, extracellular pathogens and toxins, host-pathogen interactions, innate and acquired immunity, and vaccines, all as they apply to bacterial pathogens of animals.

I am sure that you will agree that the scientific program that has been put together is just outstanding, for which we have to thank you the registrants for submitting such high quality work for presentation. The previous meeting was remarkable for its high quality science, its friendly environment and excellent spirit of scientific cooperation. Please take advantage of the breaks between the talks and posters, and the social functions, to interact with your colleagues and develop new collaborations. I ask the senior researchers to make a special effort to talk to the postgraduate students and early career researchers about their research. I ask the more junior registrants to be brave; go up to the so-called heavies in the field and tell them about your work and question them about their research. Go up to someone that you don't know, introduce yourself and ask them about their project. In this way you will get the maximum benefit from your attendance at this meeting.

Julian Rood
Chair, Organising Committee

ORGANISING COMMITTEE

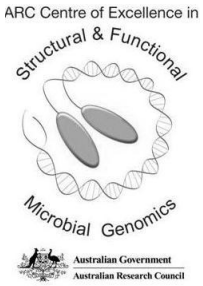
CONFERENCE CHAIR
Julian Rood (Chair) - Monash University, Australia

COMMITTEE MEMBERS
Ben Adler, Monash University, Australia
Viveca Baverud, National Veterinary Institute, Sweden
Glenn Browning, University of Melbourne, Australia
Joachim Frey, University of Bern, Switzerland
Desmond Gul, Monash University, Australia
Josée Harel, Université de Montréal, Canada
Tom Inzana, Virginia Tech, USA
Rob Moore, CSIRO Livestock Industries, Australia
John Prescott, University of Guleph, Canada

CONFERENCE SECRETARIAT
Mike Pickford
ASN Events Pty Ltd
3056 Frankston-Flinders Rd (PO Box 200), Balnarring Vic 3926, Australia
Phone: +61 (0)3 5983 2400
Fax: +61 (0)3 5983 2223
Email: mp@asnevents.net.au

SPONSORSHIP

This conference proudly acknowledges the sponsorship from the following companies;



DELEGATE INFORMATION

REGISTRATION DESK
The registration desk is located in Sala Caminetto. The room is adjacent to Salone Grollo, located on the first floor of the Monash University Prato Centre. Any enquiries can be directed to ASN staff at the registration desk.

The registration desk will be open:

Tuesday 9th October	3:00 pm – 6:15 pm
Wednesday 10th October	8:00 am – 4:30 pm
Thursday 11th October	8:00 am – 2:00 pm
Friday 12th October	8:00 am – 1:00 pm

WHAT YOUR REGISTRATION INCLUDES
Delegate registrations include:

- Access to all sessions
- Program and abstract book
- All conference catering (not including the conference dinner)
- Welcome Reception
- Wireless internet in the Grollo Room
- Use of the *Vetpath* smart phone APP (http://vetpath-2012-prato.m.asnevents.com.au)

SOCIAL PROGRAM
Welcome Reception*:*
The Welcome Reception will be held on the first evening, Tuesday 9th October on the terrace from 6:15pm to 7:30pm. Drinks and finger food will be provided. If you would like to purchase an additional welcome ticket, please see ASN staff at the registration desk. Ticket costs AUD$30.

Conference Dinner:
On the last night (Friday 12th October), the Conference Dinner will be held from 7:00 pm – 10:30 pm (buses departing at 6:30pm as described further below) at the beautiful 16th century Villa La Ferdinanda. The dinner is not included in the cost of registration; however, tickets are available for purchase (AUD$90) if you did not purchase a ticket during the registration process. The price is inclusive of a welcome cocktail, starters, main meal, selection of wines, beers and non-alcoholic beverages, and return bus transfers.

Bus Transfers to/from Villa La Ferdinanda
A bus will collect delegates at 6:30 pm from Piazza delle Carceri for the 30 minute journey to Villa La Ferdinanda. For the return journey, the bus will depart Villa La Ferdinanda at 10:30 pm, arriving in Prato around 11:00 pm. The bus will stop off at Piazza delle Carceri for all delegates who are accommodated at Hotel Flora, Hotel San Marco, and Hotel Giardino (All hotels are very close to Piazza delle Carceri). Delegates accommodated at the Art Hotel will be dropped off directly at the hotel.

SPEAKER PREPARATION INSTRUCTIONS
All speakers are using the same conference room "Salone Grollo". Speakers can upload their talk on the presentation computer in the Grollo Room, either on a break prior to your session starting or on the morning of your presentation. Please bring your presentation on a USB thumb drive or flash drive. If you wished to present using keynote you will need to bring your MAC along with the necessary adapators. Mike Pickford from ASN will be on hand each morning to load the talks if you can arrive first thing. Desmond Gul from the ARC Centre of Excellence will be available for assistance during the day.

DISPLAYING YOUR POSTER
Posters will be displayed throughout the Meeting on panels in the 'Sala Veneziana' room. The poster session will be held on Thursday 11th October from 5pm to 6:30pm. Drinks and nibbles will be provided. Poster authors are requested to locate their abstract number for correct positioning and your poster can be mounted for the whole duration of the conference. The maximum size provided is 1.0 m wide by 1.2 m high. The approved method for attaching posters is with velcro. Please visit the registration desk for additional supplies of velcro.

EMAIL AND INTERNET ACCESS
Complimentary Wi-Fi will be available throughout the conference in the "Salone Grollo" room. For username and password, please see ASN staff at the registration desk. There are also three computer rooms available to visitors to the Monash University Prato Centre. All computers are networked, with high speed internet access, and are connected to networked printers.

SETTLING YOUR ROOM ACCOUNT WITH YOUR HOTEL
At the time of registration, delegates were asked to provide credit card details for on-forwarding to hotels to secure their reservation. If you booked accommodation during the registration process, you will be asked to settle your account directly with the hotel.

USEFUL PHONE NUMBERS

Monash University Prato Centre:	
Mike Pickford, ASN on-site office	+61 411 699 422
Monash University Prato Centre, Palazzo Vaj, Via Pugliesi, 26, Prato	+39 0574 43691
Cathy Crupi, Centre Manager, Monash Prato Centre	+39 0574 436921
Hotels:	
Hotel Flora, ia. B. Cairoli, 31 – 59100, Prato	+39 0574 33521
Hotel San Marco, Piazza San Marco, 48, Prato	+39 0574 21321
Art Hotel Milano, Via Tiziano, 15, Prato	+39 0574 23371
Giardino Hotel, Via Magnolfi, 2/4/6 – 59100, Prato	+39 0574 26189
Emergency Services:	
Emergency number – general emergency	113
Emergency number – Carabinieri (police)	112
Emergency number – Fire	115
Emergency number – Ambulance	118
Hospital – Piazza dell'Ospedale	+39 0574 4341
Other:	
Prato Tourist Board, Piazza Santa Maria delle Carceri, 15, Prato	+39 0574 24112
Taxi – Radiotaxi Prato (ranks located at central train station and Piazza Duomo)	+39 0574 5656
Maggiore Car Hire, Via di Ponzano, 24d, Prato Web: http://www.webcarhire.com	+39 0574 536000
Australian Embassy in Rome, via Antonio Bosio 5, 00161, Rome Opening hours: Monday-Friday, 9:00 am - 5:00 pm (except for public holidays) Web: http://www.italy.embassy.gov.au	+39 06 852721

ABOUT THE MONASH UNIVERSITY PRATO CENTRE
The Monash University Prato Centre occupies the ground and first floors of an 18th century palazzo, called Palazzo Vaj, after the Vaj family who were the original owners. Parts of the building are believed to be much older with 15th century frescoes having been discovered on one of the outer walls of the palazzo. The present owners, L'Arte della Lana or 'Wool Guild', purchased the building from the Vaj family in the 1920's. Between 1875 and 1999, the area which has become the Monash Centre was home to a prestigious club of local businessmen. The club was primarily a gaming venue and much of the centre's interior architecture and fittings still reflect this purpose. The main fit-out was executed in the 1950's by the famous Italian architect, Italo Gamberini, and because of this, the first floor and its fittings are preserved under the Italian equivalent of the Heritage Commission.

BRIEF BACKGROUND TO PRATO

Prato is a small but thriving Tuscan city, with a beautiful historic centre and a vibrant cultural and economic life. Famous for its textile industry, the city has a population of some 180,000 inhabitants, a growing percentage of whom are immigrants from mainland China, the Indian subcontinent, north and west Africa and elsewhere. The communal, provincial and regional governments, progressive in outlook, are active in European community affairs, and have welcomed the presence of an Australian academic institution in the heart of their city. The city is home to a number of great monuments and buildings, museums, theatres and other places of interest.

WHERE TO EAT

Listed below is a selection of restaurants, cafes, bars and gelaterias which have been tried and recommended. Please note many restaurants are closed on Tuesdays and some on Mondays.

Restaurants/Pizzerie

Antica Fiaschetteria
Piazza Lippi 4
Ph: +39 0574 41225
This is easily the best medium priced restaurant in Prato. Mostly southern Italian inspired cooking. The service is friendly and there is a good range of wines available.

Ars Libandi
Via dell'Accademia, 49
Ph: +39 0574 401984
Typical Tuscan meals lightly revised. Wide selection of wines.

Caffé al Teatro
Via Verdi 28
Ph: +39 0574 30658
A favourite of Monash staff. Serves typical Italian dishes, simple and fresh ingredients. Cakes are gorgeous, wine and beverage list good. Prices are incredibly reasonable. Service is great and friendly. Large indoor and outdoor area. Monash discount 15%

Donchisciotte
Piazza Mercatale 38/39
Ph: +39 0574 39023
Delicious wood oven pizzas at a reasonable price, plus other main dishes including seafood.

Il Rifrullo
Piazza Mercatale 18/19
Ph: +39 0574 25062
Simple pizzeria, but good, cheap entrees and big sized pasta/rice portions. Pizzas also good and inexpensive. Can take larger groups. Outdoor section available.

King's Pub
Via Garibaldi 148 (at the Piazza Mercatale end of the street)
Ph: +39 0574 28641
A favourite with Monash students for lunch and dinner, the King's pub serves pizzas, hamburgers and pasta dishes for a reasonable price.

La Buchina degli Angeli
Piazza Mercatale 134
Ph: +39 0574 442922
From the outside it looks small but unfolds with room after room, including a large elevated outdoor dining space. Good choice and mid range prices.

La Veranda
Via dell'Arco 10 (just off Piazza San Marco)
Ph: +39 0574 38235
Serves typical Tuscan dishes and a wide variety is available. Efficient service with menus in English if needed. Main courses priced slightly higher than 'trattoria' prices.

Lo Scoglio
Via Verdi 42
Ph: +39 0574 22760
This restaurant serves big pizzas, a range of pasta dishes, plus an assortment of main courses and side dishes. They also have local wines plus decent house wines. Prices are quite reasonable. Can take large bookings.

Osteria Cibbè
Piazza Mercatale, 49
Ph: +39 0574 607509
Small restaurant serving typical Tuscan cuisine. Booking recommended.

Ristorante Porta al Serraglio
Via Cavallotti 36
Ph: +39 0574 30849
Good food with a variety of entrees and mains as well as a good wine list. Indoor or outdoor (in a marquee) tables. A reasonably priced eatery. Better for small groups.

Trattoria Pizzeria "Maria" 8 Lanterne Blu
Viccolo degli Arrigoni 2 (off Via Firenzuola)
Ph: +39 0574 33139
Serves wood fired pizza and calzone. Reasonably priced with good sized portions.

2 L Duel
Pizzeria Ristorante, Piazza Duomo 44 (cnr Via Magnolfi)
Ph: +39 0574 41980
Recommended by Monash students.

Something Different……

Doner Kebab Bar
Via G. Mazzini 38
Ph: +39 0574 604862

Rosticceria Cinese
Via Santa Margherita (near Piazza Mercatale)
Good, cheap take away Chinese food

Raja – Ristorante Indiano
Piazza del Collegio, 8/A
Ph: +39 0574 32032

Higher Quality (and Priced) Restaurants

Ristorante Pirana
Via G Valentini 110
Ph: +39 0574 25746
A seafood restaurant often recommended on internet restaurant guides to Tuscany.

Ristorante Baghino
Via dell'Accademia 9
Ph: +39 0574 27920
Another restaurant favourably mentioned by various internet sites. One minute from the Centre.

BANKS

CariPrato: Cassa di Risparmio di Prato, 2 Via Rinaldesca

This is the only bank in Prato that exchanges travellers' cheques. Go straight through the entry foyer to the large banking chamber beyond. At the doorway make sure you take a number. When your number comes up on the illuminated screen, go to the cashier with your number displayed. Remember to take your passport.

Bancomat (Automatic Teller Machine)

The closest one is located in Via Garibaldi. Turn right at the gate of the Monash Centre and then turn left into Via Garibaldi. It is about 50 metres on the left. There are also two machines in Piazza San Francesco D'Assisi. See map on following page for details.

TELEPHONES AND PHONE CARDS

International

'Happiness' telephone cards give you 180 minutes talk time to Australia for €5. They are available at Bar Formica in Via Mazzoni or ask at other tobacconists (with the 'T' out the front of the shop).

In Italy

Italian Telecom phone-cards (for use in public phones) have a corner that you must tear off before you can use the card. Look for the word 'strappare' on the card. Very few phones accept coins.

FLORENCE

It is impossible to summarise all of the cultural activities Florence has to offer. The best way to find out is to go to the tourist information office.

Tourist Information

Via Cavour,1r, Prato

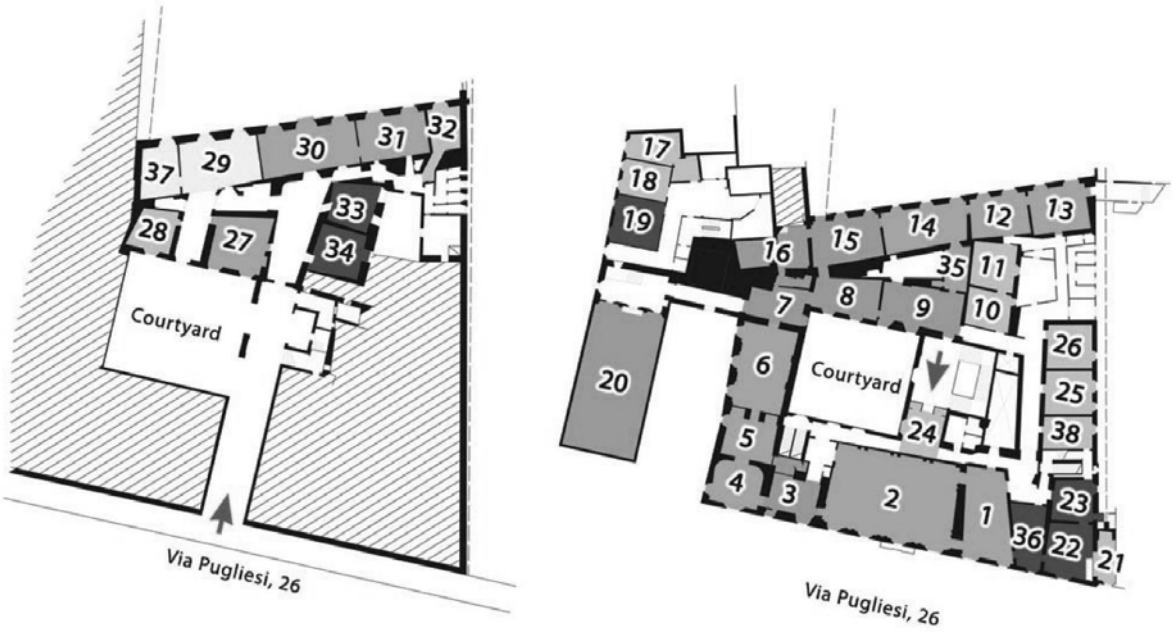
Open: Monday to Saturday from 8:30 am to 6:30 pm
Sundays and public holidays from 8:30 am to 1:30 pm

You can also browse on the internet. Try http://www.aboutflorence.com or http://www.studentsville.it.

LOCATION OF CONFERENCE HOTELS & MONASH UNIVERSITY PRATO CENTRE

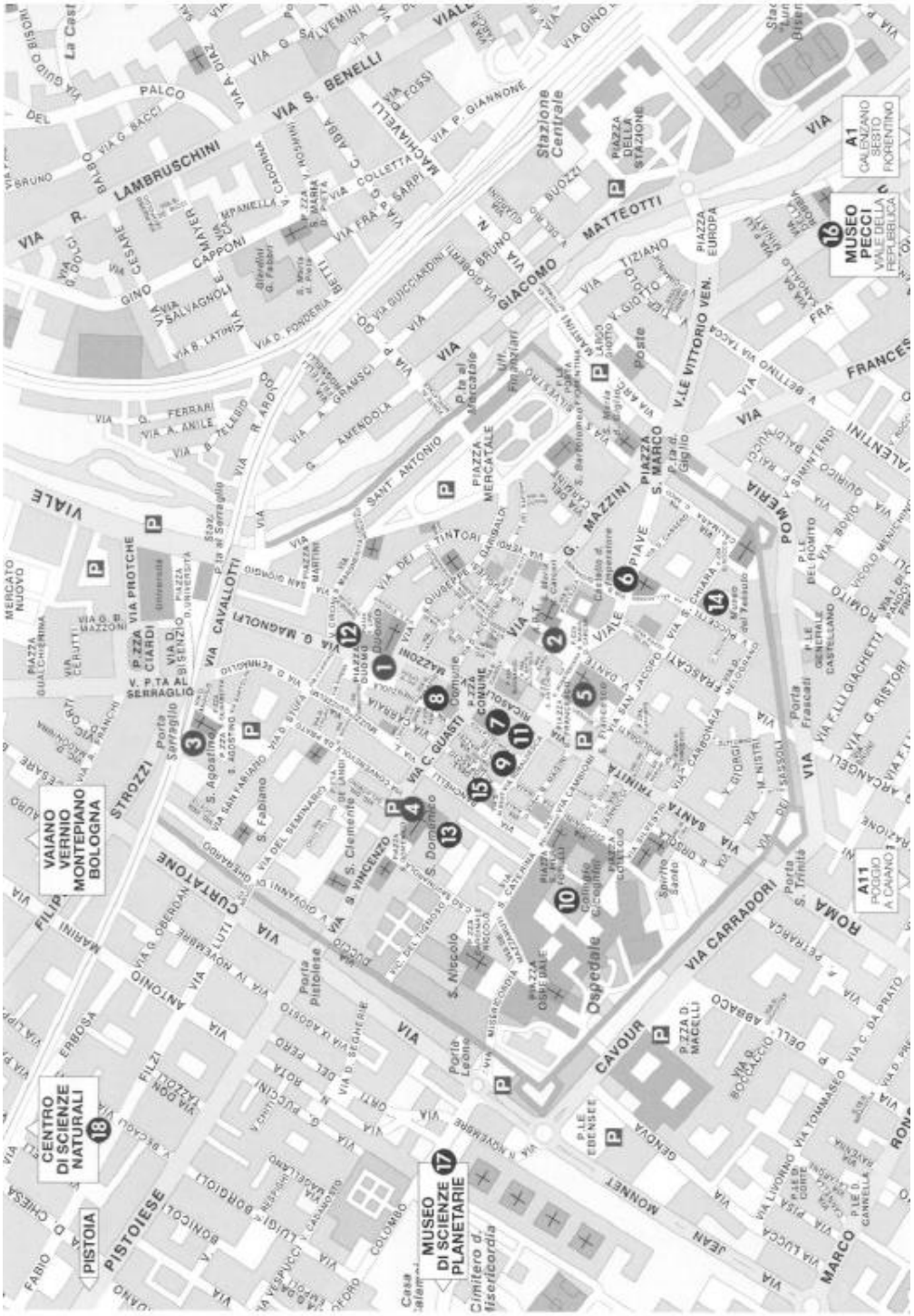
A – Monash University Prato Centre, **B –** Art Hotel, Milano, **C –** Hotel San Marco, **D –** Hotel Flora, **E –** Hotel Giardino

MAP OF MONASH UNIVERSITY PRATO CENTRE



Offices		Teaching / conference / exhibition space		Computer labs		Recreational areas		Study areas	
24	Reception	2	Salone Grollo	22	Computer lab 1	3	Piccolo Bar	37	Bill Kent Library
38	Admin office 1	1	Sala Caminetto	23	Computer lab 1	7	Main bar	29	Student Reading Room
10	Admin office 2	6	Sala Veneziana	36	Computer lab 2	8	Sala Biliardo		
11	Admin office 3	14	Sala Toscana	19	Computer lab 3	9	Sala Specchi		
25	Director	15	Sala Giochi	34	Mac lab	20	Terrace		
26	Manager	30	Seminar 2	33	WIFI lab	5	Breakout 1		
21	Visiting professor 1	31	Seminar 3			4	Breakout 2		
17	Visiting professor 2	27	Seminar 4						
28	Visiting professor 3	34	Mac lab						
32	Visiting professor 4	33	WIFI lab						
		12	Studio 2						
		13	Studio 3						
		35	Sguardi Australiani Archive						

MAP OF PRATO


EDIFICI RELIGIOSI
1 Cattedrale di S. Stefano
2 Basilica di S. Maria delle Carceri
3 Chiesa di S. Agostino
4 Chiesa di S. Domenico
5 Chiesa di S. Francesco
MONUMENTI E MUSEI
6 Castello dell'Imperatore
7 Palazzo Pretorio
8 Palazzo Comunale
9 Palazzo Datini
10 Collegio Cicognini
11 Museo Civico
12 Museo dell'Opera del Duomo
13 Museo di Pittura Murale
14 Museo del Tessuto
15 Galleria degli Alberti
16 Centro Pecci
17 Museo di Scienze Planetarie
18 Centro di Scienze Naturali
Cartografia L.A.C. - Firenze

SPEAKERS

Freddy Haesebrouck

Freddy Haesebrouck is a veterinarian and PhD graduate from Ghent University, Belgium. In 1988 he became Professor of Veterinary Bacteriology and Mycology and since 1993 he has been head of the Department of Pathology, Bacteriology and Avian Diseases at the Faculty of Veterinary Medicine, Ghent University. He is author or co-author of more than 450 articles in peer-reviewed scientific journals and associate-editor of the open access journal Veterinary Research, handling bacteriology-related manuscripts. The expertise of his research group relates primarily to the study of bacterium-host interactions and antimicrobial resistance in bacteria of veterinary and zoonotic importance, including gastric non-*H. pylori Helicobacter* species.

Michael Kogut

Michael Kogut, Ph.D. is the Lead Scientist studying the Systems Biology of the *Salmonella*-Host Interactome in Poultry and Swine within the Food and Feed Safety Research Unit at the Southern Plains Agricultural Research Center, USDA-Agricultural Research Service in College Station, Texas. Dr. Kogut obtained his Ph.D. in Poultry Science from the University of Georgia and has been with the ARS for 20 years. His research interests have focused on developing non-traditional, non-antibiotic, immunologically-based strategies to reduce *Salmonella* and *Campylobacter* colonization in poultry and swine. The lab's primary goal is to understand prospective control points to modulate innate host immune responses, thus providing the poultry and swine industries with novel, pre-harvest intervention tools to control food-borne pathogens and provide safe food products to the consumer. We have focused on elucidating the molecular and cellular mechanisms of disease resistance and defining the genetic regulation of the innate immune response. Understanding the mechanisms of the host/pathogen interactome that define the host response to bacterial intestinal persistence can then be exploited to develop new candidate targets for anti-infectives and therapeutics acting at the level of the host rather than targeting bacterial proteins.

Miia Lindström

Miia Lindström qualified as a veterinarian (1998) and obtained her PhD (2003) from the University of Helsinki and has been Professor of rocessing Hygiene since 2009. Her research interests include the diagnostics, molecular epidemiology, stress response and virulence mechanisms of psychrotrophic and spore-forming foodborne pathogenic and spoilage bacteria, with specific interest on foodborne pathogenic clostridia. The Lindström research group works in the Centre of Excellence in Microbial Food Safety Research.

Duncan Maskell

Duncan Maskell's degree (1982) and PhD (1986) are from the University of Cambridge. His post-doc at the Wellcome Research Laboratories focused on vaccine discovery, especially live attenuated *Salmonella* vaccines and *Bordetella* vaccines. After working at the Institute of Molecular Medicine, Oxford, on bacterial meningitis and at Imperial College London on various bacteria he was elected as a Professor at the Cambridge Veterinary School in 1996, and became its Head in 2004. In addition to an extensive publication record in many different areas of infectious disease research, he has also been a co-founder of two biotech companies. He continues to work on a variety of bacterial pathogens, investigating how they interact with their host animals.

Kenny Simpson

Kenny Simpson (BVM&S, PhD) is a 1984 graduate of the Royal (Dick) School of Veterinary Studies at the University of Edinburgh. He has been a faculty member at the Cornell University College of Veterinary Medicine since 1995 and has been a mentor, supervisor, and adviser to many interns, residents, and graduate students. His clinical specialty is in small animal internal medicine, with sub-specialization and research interests in gastroenterology and host-bacterial interactions, particularly *Helicobacter* spp, and *E. coli* associated with IBD He is a Past-President of the Comparative Gastroenterology Society.

Jose Vazquez-Boland

Jose Vazquez-Boland is a veterinary surgeon (1985) and PhD (1990) from Complutense University of Madrid, Spain. Before his PhD, he spent time in veterinary practice and as public health officer. After a postdoc at the Institut Pasteur in Paris, in 1993 he obtained a permanent faculty position at Madrid's Veterinary School. In 2001 he was appointed Chair of Veterinary Molecular Microbiology at the University of Bristol. Since 2007 he has been Chair and Associate Director of the Centre for Infectious Diseases at the University of Edinburgh. He is also Professor of Veterinary Microbiology at the University of Leon, Spain. His group investigates the molecular mechanisms of bacterial virulence with *Listeria monocytogenes* and *Rhodococcus equi*.

PROGRAM

Tuesday 9th October 2012

Registration
3:00 PM - 6:15 PM — Sala Caminetto

Welcome & Introduction
5:00 PM - 5:15 PM — Salone Grollo
Chair, Organising Committee: Julian Rood

Plenary session
Chair: Julian Rood
5:15 PM - 6:15 PM — Salone Grollo

This session is proudly sponsored by the ARC Centre of Excellence in Structural and Functional Microbial Genomics

5:15 PM **Freddy Haesebrouck**
Animal-associated gastric helicobacters: how do these less well-known zoonotic pathogens interact with their hosts? *abs #1*

Welcome Reception
6:15 PM - 7:30 PM — Terrace

Wednesday 10th October 2012

Session 2: Animal Diseases
Chair: Viveca Båverud
9:00 AM - 10:35 AM — Salone Grollo

The session is proudly sponsored by CEVA Corporate

9:00 AM	**Rebecca Davenport** Development of an *ex vivo* organ culture (EVOC) model of the ovine skin/hoof interface *abs #2*
9:20 AM	**Richard Zuerner** *Leptospira borgpetersenii* dissemination during experimental infection of golden Syrian hamsters *abs #3*
9:45 AM	**Thomas Inzana** Purification and preliminary characterization of the capsular polysaccharide of *Haemophilus parasuis abs #4*
10:10 AM	**Alda Natale** Viability of *Leptospira interrogans* serovar Hardjo in refrigerated raw milk *abs #5*

Tea/Coffee Break
10:35 AM – 11:15 AM — Sala Billiardo, Sala Specchi & Main Bar

This session is proudly sponsored by the University of Montreal

Faculté de médecine vétérinaire
Université de Montréal

Session 3: Genomics and molecular epidemiology
Chair: Ben Adler
11:15AM – 12:45PM — Salone Grollo

This session is proudly sponsored by FRALIN Living Science

11:15 AM	**Kenneth Simpson** Comparative genomics of adherent and invasive *Escherichia coli* associated with intestinal inflammation reveals patho-adaptive determinants of virulence *abs #6*
11:55 AM	**Mark Stevens** Comprehensive assignment of roles to *Salmonella* and *E. coli* genes in food-producing animals *abs #7*
12:20 PM	**John Prescott** Phylogeny of *Clostridium perfringens* isolates associated with avian necrotic enteritis based on microarray comparative genomic hybridization *abs #8*

Lunch at Venue
12:45 AM – 2:00PM — Sala Billiardo, Sala Specchi & Main Bar

Session 3 (Continued)
Chair: Steve Djordjevic
2:00PM – 2:50PM Salone Grollo

2:00 PM **Marianne Gilhuus**
Outbreak of ovine footrot in Norway is most likely caused by recent introduction of a virulent *Dichelobacter nodosus* strain *abs #9*

2:25 PM **Ruth Kennan**
Whole genome sequence analysis of strains from Australia and Norway reveals that virulent and benign isolates of *Dichelobacter nodosus* belong to two separate lineages *abs #10*

Session 4: Host-pathogen interactions
Chair: Miia Lindström
2:50PM – 3:55PM Salone Grollo

This session is proudly sponsored by MSD Animal Health

MSD Animal Health

2:50PM **Duncan Maskell**
Tale of the unexpected: Complex dynamics underlie apparently simple *Salmonella* infections *abs #11*

3:30 PM **Nahum Shpigel**
A novel mechanism of neutrophil entry into epithelial cells is involved in damping inflammation yet exposes a backdoor for bacterial invasion *abs #12*

Tea/Coffee Break
3:55 PM – 4:25 PM Sala Billiardo, Sala Specchi & Main Bar

Session 4 (continued)
4:25PM – 5:40PM Salone Grollo
Chair: Miia Lindström

4:25PM **Andrew Rycroft**
Colonisation of pig lymphoid tissues with avirulent *Actinobacillus pleuropneumoniae*: competitive exclusion and displacement of virulent *A. pleuropneumoniae* from the respiratory tract? abs #13

4:50 PM **Jo Stevens**
Molecular insights into actin-based motility of *Burkholderia* species abs #14

5:15 PM **Smarajit Chakraborty**
Intracellular pH measurements with a DNA nanosensor indicate that the cytoplasm of *Salmonella* Typhimurium is acidified in the macrophage vacuole (SCV) abs #15

Thursday 11th October 2012

Session 5: Mechanisms of Pathogenesis
Chair: Joachim Frey
9:00AM – 10:30AM — Salone Grollo

This session is proudly sponsored by Pfizer Animal Health

9:00 AM	**Jose Vazquez-Boland** *Rhodococcus equi* virulence: not all is the plasmid's fault abs #16
9:40 AM	**Josée Harel** Single-cell measurement of $F165_1$ and Pap fimbrial phase variation in real-time abs #17
10:05 AM	**Joanne Allen** Sequence diversity and cytotoxicity of the leukotoxin of *Mannheimia* species isolated from cases of ovine mastitis abs #18

Tea/Coffee Break
10:30AM – 11:00AM — Sala Billiardo, Sala Specchi & Main Bar

Session 5 (continued)
Chair: Robert Moore
11:00AM – 12:40PM — Salone Grollo

11:00 AM	**Steven Djordjevic** The surfaceome of *Mycoplasma hyopneumoniae* displays an extensive population of multifunctional endoproteolytic cleavage fragments derived from the P97 and P102 adhesin families abs #19
11:25 AM	**Lotte Jelsbak** Polyamines are required for virulence and expression of virulence loci in *Salmonella* Typhimurium *abs #20*
11:50 AM	**Charles Dozois** The siderophore esterases Fes and IroD contribute to virulence of avian extra-intestinal pathogenic *Escherichia coli abs #21*
12:15 PM	**John Olsen** Identification of redundant metabolic pathways essential for virulence of *Salmonella enterica* and their potential as targets for antimicrobial therapy *abs #22*

Lunch at Venue
12:40PM – 2:00PM — Sala Billiardo, Sala Specchi & Main Bar

Session 5 (continued)
Chair: Richard Zeurner
2:00PM – 3:40PM — Salone Grollo

This session is proudly sponsored by Monash University

2:00 PM	**John Boyce** Regulation of *Burkholderia pseudomallei* motility and virulence by a novel two-component signal transduction system *abs #23*

2:25 PM **Ingeborg Frans**
Identification of virulence factors of fish pathogen *Vibrio anguillarum* using a gnotobiotic model system *abs #24*

2:50 PM **Xiaoyan Han**
Identification and functional analysis of a bacitracin resistance locus from *Clostridium perfringens abs #25*

3:15 PM **Anders Bojesen**
F17-like fimbriae are important for full virulence in *Gallibacterium anatis abs #26*

Tea/Coffee Break
3:40PM – 4:10PM Sala Billiardo, Sala Specchi & Main Bar

Session 5 (continued)
Chair: Duncan Maskell
4:10 PM – 5:00PM Salone Grollo

4:10 PM **Ben Adler**
Leptospiral genomics, virulence factors, and vaccine antigens *abs #27*

4:35 PM **Marina Harper**
Pasteurella multocida lipopolysaccharide polymorphism: the implications for vaccine development *abs #28*

Poster Session
5:00PM – 6:30 PM Sala Veneziana

This session is proudly sponsored by Prevtec Microbia

Friday 12th October 2012

Session 6: Extracellular pathogens and toxins
Chair: Tom Inzana
9:00AM – 10:55AM Salone Grollo

This session is proudly sponsored by the University of Guelph

Ontario Veterinary College PATHOBIOLOGY

9:00 AM	**Miia Lindström** Botulinum neurotoxin - A friend and a foe *abs #29*
9:40 AM	**Joachim Frey** Cytotoxin CctA, a major virulence factor of *Clostridium chauvoei* conferring protective immunity against myonecrosis *abs #30*
10:05 AM	**Julian Rood** Structural and functional analysis of NetB toxin *from Clostridium perfringens abs #31*
10:30 AM	**Daniel Dubreuil** The intestinal epithelial barrier function is altered by *Escherichia coli* STb enterotoxin *abs #32*

Tea/Coffee Break
10:55AM – 11:25AM Sala Billiardo, Sala Specchi & Main Bar

Session 7: Host Responses
Chair: Josée Harel
11:25AM – 12:55PM Salone Grollo

This session is proudly sponsored by Ceva Corporate

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11:25 AM	**Michael Kogut** Alternative to antibiotics: Small cationic peptides provide protection to neonatal chickens against enteric bacterial infections through enhanced innate host defenses *abs #33*
12:05 PM	**Henk Haagsman** Immunomodulatory activities of chicken CATH-2 derived peptides *abs #34*
12:30 PM	**Sabine Totemeyer** Inflammatory response to footrot in sheep *abs #35*

Lunch
12:55PM – 2:00PM Sala Billiardo, Sala Specchi & Main Bar

Session 8: Vaccines
Chair: Glenn Browning
2:00PM – 3:40PM Salone Grollo

This session is proudly sponsored by Novartis Vaccines and Diagnostics

NOVARTIS VACCINES

2:00 PM	**Benaouda Kadra** Coxevac, Q fever vaccine:Vaccine production and control *abs #36*
2:25 PM	**Miet Vermoote** Immunoproteomics of *Helicobacter suis* and protective efficacy of a subunit vaccine in a mouse model *abs #37*
2:50 PM	**Dorien Mot** Practical pitfalls in vaccination against necrotic enteritis in broilers *abs #38*
3:15 PM	**Robert Moore** NetB-based vaccines protect broiler chickens from necrotic enteritis *abs #39*

Concluding discussions
Chair: Julian Rood
3:40PM – 5:00PM Salone Grollo

Dinner – Offsite Venue
7:00PM – 10:30PM Villa Medice La Ferdinanda

POSTER LISTINGS

Thursday 11th October 2012 Sala Veneziana
6:00pm - 7:30pm

Øystein Angen
An investigation regarding eradication and diagnosis of *Dichelobacter nodosus* and the presence of *Fusobacterium necrophorum* in footrot affected herds *abs# 50*

Agata Bancerz-Kisiel
Influence of *ymoA* gene mutations on enterotoxin Yst production by *Yersinia enterocolitica abs# 51*

Abdelali Benkirane
Primary investigations on the sero-reactivity of various animal species to major serogroups of *Leptospira interrogans* in Morocco *abs# 52*

Christopher Browne
Survivability of *Mycoplasma hyopneumoniae* on dry surfaces and dust *abs# 53*

Glenn Browning
Nucleases and nucleotide transport operons of pathogenic mycoplasmas *abs# 54*

Sara Frosth
Correlation between clinical manifestations of footrot in Swedish sheep flocks and *Dichelobacter nodosus* strains *abs# 56*

Josee Harel
Effect of human intestinal microbiota and *Bacteroides thetaiotaomicron* on *Escherichia coli* O157:H7 transcriptomic response: multiple aspects of EHEC adaptation. *abs# 57*

Andreas Koestelbauer
Screening of phytogenics against *Lawsonia intracellularis* with a flow cytometric viability assay *abs# 58*

Anna Lima
Seroprevalence of *Leptospira* spp. infections in cattle seropositive to *Brucella abortus* in oficial diagnostics tests *abs# 59*

Anna Lima-Ribeiro
Brucella spp. DNA in the semen of seronegative bulls by polymerase chain reaction *abs# 60*

Susanne Lindahl
A clonal outbreak of upper respiratory disease in horses caused by *Streptococcus equi* subsp. *zooepidemicus abs# 61*

Gareth Maglennon
Successful transformation of *Mycoplasma hyopneumoniae* strains with self-replicating plasmids and optimisation of transformation conditions *abs# 62*

Tibor Magyar
PCR-RFLP analysis of *Bordetella bronchiseptica* isolates from different animal species to detect the possible signs of host-adaptation *abs# 63*

Mohd Muzafar
Environmental reservoirs of the footrot pathogen *Dichelobacter nodosus* and transmission dynamics of the disease *abs# 64*

Alexander Panin
Genetic diversity and epizootiology of chlamydiae prevalent among the domestic and feral avian species in Russia *abs# 65*

Valeria Parreira
Complete sequence of two virulence-associated plasmids from necrotic enteritis isolates *Clostridium perfringens* type A and comparison with *C. perfringens* plasmids *abs# 66*

Sinikka Pelkonen
Clonal relationships between human and equine *Streptococcus equi* ssp. *zooepidemicus* isolates *abs# 67*

Katherine Tanaka
Aeromonas salmonicida subsp *salmonicida* genomic diversity analysis: identification of two novel genetic elements potentially related to virulence *abs# 68*

Jean Whittingham
Studies on virulence-associated proteins of the horse pathogen *Rhodococcus equi abs# 69*

ABSTRACTS

ORAL

1

Animal-associated gastric helicobacters: how do these less well-known zoonotic pathogens interact with their hosts?

Freddy Haesebrouck[1] , Bram Flahou[1] , Annemieke Smet[1] , Frank Pasmans[1] , Richard Ducatelle[1]

1. Ghent University, Merelbeke, Belgium

Large spiral shaped helicobacters, different from the human adapted *Helicobacter pylori*, have been associated with gastritis, peptic ulcers and gastric Mucosa Associated Lymphoid Tissue (MALT) lymphoma in human patients. *H. suis* is the most prevalent gastric non-*H. pylori Helicobacter* species (NHPH) in humans and is often present in the stomach of pigs. Other NHPH colonizing the human stomach are *H. felis, H. salomonis, H. bizzozeronii* and *H. heilmannii* sensu stricto (s.s.). These microorganisms are often detected in the stomach of dogs and cats. *H. suis* causes gastritis and decreased daily weight gain in experimentally infected pigs. It also appears to be one of the factors involved in gastric ulcer disease in pigs. The pathogenic significance of gastric NHPH for dogs and cats remains enigmatic and is probably *Helicobacter* species- and strain-dependent. NHPH are most likely transmitted from animals to humans through direct contact. *H. suis* can be present and survive in pork meat, suggesting that this might also act as a source of infection for humans. Recently, the genome of several gastric NHPH has been sequenced, revealing the presence of several genes involved in pathogenicity and adaptation to the hostile gastric environment. Gerbils experimentally infected with *H. suis* developed histopathological changes in their stomach similar to those described in infected humans and characterized by a marked lymphocytic infiltration in the antrum and development of MALT lymphoma-like lesions. In part, this pathology might be explained by the mainly T helper (Th)17/Th2 polarized immune response. Both in experimentally infected mice and gerbils, *H. suis* induces gastric epithelial cell death which has been associated with production of γ-glutamyl transpeptidase. This enzyme also inhibits proliferation of lymphocytes and is present in all gastric helicobacters. Its pathologic effect is at least partly related to cleavage of extracellular glutathione into pro-oxidants.

1. Haesebrouck F., Pasmans F., Flahou B., Chiers K., Baele M., Meyns T., Decostere A., Ducatelle R. (2009). Gastric helicobacters in domestic animals and nonhuman primates and their significance for human health. Clin. Microbiol. Rev. 22, 202-223

2

Development of an *ex vivo* organ culture (EVOC) model of the ovine skin/hoof interface

Rebecca Davenport[1] , Sabine Tötemeyer[1]

1. University of Nottingham, Loughborough , Leics, United Kingdom

For livestock owners, lameness causes monumental problems to the daily welfare and productivity of their animals. For the ovine industry, one of the most common causes of lameness is of infectious origin. Footrot is a bacterial disease that affects the ovine industry globally. It has detrimental effects on the welfare of sheep, causing severe lameness if not treated as well as huge economic impacts. The skin-hoof interface of the ovine claw, if succumbed to wet and damaging environments provides the perfect entry point for the pathogenic bacteria *Dichelobacter nodosus* and *Fusobacterium necrophorum* to invade, resulting in interdigital dermatitis leading to footrot. To date, there is limited literature detailing how the innate immune system of the ovine species responds to these pathogens.

The aim of the project was to develop an *Ex vivo* organ culture (EVOC) model of the ovine foot by sampling biopsies of the ovine skin/hoof interface and supporting them in a laboratory environment. A wide range of conditions were explored in order to mimic the environment *in vivo*. Nanodrop analysis and Agilent bioanalyser were used to determine RNA concentration and quality of ovine biopsies, respectively. The model was successfully sustained for 24 hours, with suitable RNA concentrations (>40ng/µl) and good RNA quality (RIN >6) obtained for further analysis.

This novel model is the first of its kind that can be used to investigate innate immune response to infectious diseases affecting the ruminant foot, especially footrot in sheep. Crucially it also addresses the principles of the 3 Rs of experimentation providing a replacement for whole animal use and ultimately aims to improve the welfare of sheep.

3

Leptospira borgpetersenii dissemination during experimental infection of golden Syrian hamsters

Richard Zuerner[1 2] , Jennifer Wilson-Welder[2] , Mitchell Palmer[2] , David Alt[2] , Richard Hornsby[2] , Ami Frank[2] , Judith Stasko[2]

1. Institute for Biomedical Sciences and Veterinary Public Health, Swedish University for Agricultural Sciences, Uppsala, Sweden
2. *Bacterial Diseases of Livestock Research Unit, National Animal Disease Center, Agricultural Research Service, USDA, Ames, USA*

Leptospirosis isone of the most widespread zoonotic infections known and is caused by a diverse group of *Leptospira* species. Until recently, attempts to demonstrate reproducible experimental infection of golden Syrian hamsters with *L. borgpetersenii* serovar Hardjo were unsuccessful. We showed that serovar Hardjo can establish either a chronic infection with few outward signs of disease or cause an acute, debilitating and lethal infection in hamsters, depending upon the strain used. The virulent strain induced neutrophilia and formed neutrophil aggregates. Neutrophil aggregation may be a unique feature of virulent serovar Hardjo; aggregates were not detected

in animals infected with either *L. interrogans* serovar Pomona or *L. kirschneri* serovar Grippotyphosa. Circulating aggregates may form microemboli and contribute to hemorrhage in various tissues, especially lung. Serovar Hardjo strains colonized brain in both acute and chronic infection models without apparent induction of inflammatory cell infiltration. Although there is limited evidence in the literature that *Leptospira* can colonize the brain, the biological consequences are unknown. Continued use of this infection model should help elucidate *Leptospira* invasion mechanisms and lead to a better understanding of the pathological consequences of infection.

4

Purification and preliminary characterization of the capsular polysaccharide of *Haemophilus parasuis*

Thomas J Inzana[1] , Anne Hyman[1] , Cristina De Castro[2] , Antonio Molinaro[2]

1. Virginia Tech, Blacksburg, VA, United States
2. *Department of Organic Chemistry and Biochemistry, University of Naples "Federico II", Naples, Italy*

Haemophilus parasuis is a Gram-negative coccobacillus and the etiologic agent of Glässer's disease (polyserositis) in young pigs and pneumonia in adults. There are 15 recognized serotypes of *H. parasuis*, but the serotype-specific antigen has not been purified or characterized. *H. parasuis* is also proposed to be encapsulated, but the capsular antigen has also not been purified or characterized, and the presence of capsule appears to be variable among strains. We have purified the capsular polysaccharide (CP) of *H. parasuis* serotypes 4, 5, 9, 13, and 14 by Cetavlon precipitation, enzyme digestion, phenol extraction, and gel filtration chromatography. The CP was a heterogeneous, high molecular size polysaccharide, as determined by gel filtration, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Alcian Blue/silver staining. The chemical composition of the CP is being analyzed. The CP was primarily present and isolated from autoclaved whole cells after 24 h of culture, but was present primarily in culture supernatant following 72 h of culture, indicating the CP is attached to the cells, but shed during late stationary phase. Mouse antiserum to the purified CP of serotype 5 reacted with serotype 5 cells and purified CP by ELISA, immunoblotting, and other immunological assays, but not with other *H. parasuis* serotypes. Following daily passage of *H. parasuis* on agar medium the expression of CP diminished. Comparative analysis of the serotype 5 genome sequences with capsule export/synthesis regions of *Escherichia coli* capsule groups I-IV identified a putative region that may encode for the proteins responsible for CP export and synthesis, and was most similar to capsule group I, rather than groups II/III capsules, which are common to other members of the Pasteurellaceae. Therefore, *H. parasuis* expresses a typical CP that is responsible for serotype-specificity, though genetically distinct from other family members, and likely enhances virulence of the bacterium.

5

Viability of *Leptospira interrogans* serovar Hardjo in refrigerated raw milk

Alda Natale[1] , Isabella Giurisato[1] , Silvia Marchione[1] , Sara Bosello[1] , Guido Di Martino[1] , Lebana Bonfanti[1] , Letizia Ceglie[1]

1. Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

Leptospira interrogans serovar hardjo (LH) is an important infectious agent that may cause abortions, stillbirths, infertility and reduction of milk production in cattle (Ellis et al, 1985), with a possible zoonotic role (Levett, 2001). Conflicting and outdated data is available regarding the survival of LH in raw milk: infected cows were reported to shed viable leptospires in their milk (Ellis et al, 1976), and milk was supposed to be the mean of LH transmission in one human-to-human case (Bolin & Koellner, 1988); on the other hand undiluted cow milk was proven to rapidly kill LH (Kirschner & Maguire, 1955). A further investigation on the serovar Pomona confirmed the lytic antileptospiral effect (Stalheim, 1965), probably due to crude milk phospholipids.

The aims of this study were to evaluate the DNA persistence and the LH survival in experimentally contaminated raw milk samples stored at 4±3°C during a ten-day study period. For that reason, a negative fresh tank milk sample was infected with a LH strain (108 cell/ml grown in EMJH liquid medium) and examined with an rrs16S gene targeting real time PCR to quantify the detectability of LH DNA in 1 ml aliquots extracted at days 1, 2, 3, 4, 8, 9 and 10. Seven aliquots at the same time intervals were inoculated into liquid and semisolid EMJH media to attempt the culture (OIE Manual procedures). Leptospiral viability and growth were optically evaluated by means of a dark field microscopy.

All the 7 aliquots resulted PCR positive, demonstrating that raw milk did not alter the detectability of the LH DNA during the study time. Moreover, all the attempted cultures were successful, showing the capability of LH to survive and grow in raw milk until day 10.

1. Ellis, W. A., O'brien, J. J., Bryson, D. G. & Mackie, D. P. (1985). Bovine leptospirosis: some clinical features of serovar hardjo infection. Veterinary Record 117:101-104.
2. Levett P.N. (2001) Leptospirosis. Clinical Microbiology Review. Apr;14(2):296-326.
3. Ellis, W. A.; O'brien, J. J.; Pearson, J. K. L.; Collins, D. S. (1976). Bovine leptospirosis: infection by the Hebdomadis serogroup and mastitis. Veterinary Record 99: 368-370.
4. Bolin, C.A.; Koellner, P. (1988). Human-to-human transmission of Leptospira interrogans by milk. Journal of Infectious Disease, 158(1):246-247.
5. Kirschner, L.; Maguire, T. (1955). Antileptospiral effect of milk. The New Zealand Medical Journal, 54: 560.
6. Stalheim, O.H.V. 1965. Leptospiral lysis by lipids of renal tissue and milk. Journal of Bacteriology 89(2): 545.

6

Comparative genomics of adherent and invasive *Escherichia coli* associated with intestinal inflammation reveals patho-adaptive determinants of virulence

Kenneth Simpson[1]

1. Cornell University, Ithaca, NY, United States

Comparative Genomics Of Adherent And InvasiveEscherichia coli Associated With Intestinal Inflammation Reveals Patho-adaptive Determinants Of Virulence Kenenth W. Simpson, College of Cornell University, Ithaca NY Intestinal bacteria are implicated increasingly as a pivotal factor in the development of Crohn's disease (CD) but the specific components of the complex polymicrobial environment driving the inflammatory response are unresolved. Using contemporary culture-independent methodologies we have discovered that the mucosal flora of people with Crohn's ileitis, Boxer dogs with granulomatous colitis, and mice with Toxoplasma and NSAID induced ileitis are selectively enriched in E. coli. Culture based characterization of E. coli from the inflamed mucosa of people, dogs and mice indicates they belong to a putative new pathogroup, Adherent and Invasive E. coli (AIEC), originally isolated from CD ileitis in France. Our findings raise the possibility that AIEC strains share common pathoadaptive determinants of virulence that promote intestinal inflammation across species.

We used comparative genomics to identify genes of AIEC strains that could provide novel insights into how these bacteria might elicit intestinal inflammation in a Crohn's susceptible individual. We sequenced and assembled the genomes of 8 AIEC strains (4 CD, 3 ileitis and 1 colitis ; 1 murine ileitis, 1 murine fecal isolate ; 2 granulomatous colitis of Boxer dogs) and one non-AIEC strain from CD ileum. Total genome sizes were 4.7 - 5.2 Mb. The sequences were compared with publicly available genome sequences of E. coli and Shigella. E. fegusonii was used as an out group.

Phylogenies based on the non-recombinant portion of the genome, involving over 600 loci, strongly supported the independent evolutionary history of AIEC strains, including AIEC from the same host and with the majority of AIEC closely related to different strains of extraintestinal pathogenic E. coli (ExPEC) e.g. UPEC and APEC. Based on gene content, AIEC as an overall pathotype did not cluster as a single group, rather AIEC strains from different species intermingled in several distinct clusters with ExPEC. Genomic comparisons across biochemical categories revealed that AIEC were overrepresented in siderophores, heme uptake, iron uptake, and biosynthesis of natural products compared to non-pathogenic strains. Compared to other E. coli pathogens AIEC were overrepresented in intracellular survival and replication, proinflamatory effects, and types II and IV secretory proteins, and lacking type III.

7

Comprehensive assignment of roles to *Salmonella* and *E. coli* genes in food-producing animals

Mark Stevens[1] **, Duncan Maskell**[2]

1. The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom
2. *Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom*

Salmonella and *E. coli* are animal and zoonotic pathogens of worldwide importance. Complete genome sequences for prevalent *S. enterica* serovars and *E. coli* pathotypes have existed for a decade, however relatively few roles have been assigned to the encoded genes in food-producing animals. Toward this aim, we recently used transposon-directed insertion-site sequencing (TraDIS) to screen a library of 8550 random mutants of *S*. Typhimurium for their ability to colonise the intestines of chickens, pigs and calves. TraDIS relies on massively-parallel sequencing of transposon flanking regions, enabling insertion sites to be precisely mapped and the abundance of the cognate mutants to be quantified from the number of corresponding sequence reads. TraDIS simultaneously assigned the identity and phenotype of >90% of mutants screened, revealing roles for 2721 different genes in three key reservoir hosts. A core set of genes mediating intestinal colonisation of each host was identified but mutations in some loci produced host-specific phenotypes that were not observed in surrogate rodent models. Independent mutations affecting the same gene or pathway produced consistent phenotypes and the data were further validated by testing of defined null mutants in chickens. We also retrospectively applied TraDIS to a library of 1805 *E. coli* O157 mutants previously screened for their ability to colonise the intestines of calves by signature-tagged mutagenesis (STM). TraDIS assigned the identity and phenotype of 1645 of the *E. coli* O157 mutants screened, providing far richer functional annotation than previously feasible. TraDIS proved to be far more sensitive, identifying tens of novel mutations affecting Type III secretion, cytotoxins and adhesins that were missed by STM analysis of the same library. The data provide valuable data for the rational design of control strategies and establish TraDIS as the method of choice for functional annotation of microbial genomes with minimal use of target hosts.

8

Phylogeny of *Clostridium perfringens* isolates associated with avian necrotic enteritis based on microarray comparative genomic hybridization

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2. *Agriculture and Agri-Food Canada , Guelph, Ontario, Canada*
3. *Lowa State University, Ames, Iowa, United States*
4. *Public Health Agency of Canada, Guelph, Ontario, Canada*

Clostridium perfringens type A causes poultry necrotic enteritis (NE), an enteric disease of considerable economic importance. A novel toxin, NetB, is critical to NE pathogenesis and closely associated with virulent strains. We recently demonstrated that netB resides on a large, conjugative plasmid within a 42 kb pathogenicity locus (NELoc-1) that, in conjunction with two other loci (NELoc-2 and 3), is highly conserved in virulent strains. Though plasmid-encoded genes are clearly important to NE pathogenesis, it is not known whether a specific chromosomal background is associated with virulent strains.

To study further the genomic signature of NE-associated strains, we used a C. perfringens microarray based on two NE-producing strains to analyse the gene content of a set of 54 C. perfringens isolates, 25 from birds with NE and 29 from asymptomatic birds. Pulsed

field gel electrophoresis (PFGE) demonstrated that the isolates are genetically distinct. A total of 128 genes significantly associated with netB were identified, including genes related to iron-acquisition and carbohydrate utilization.

Phylogenetic analysis of CGH data placed 90% of the poultry isolates into three major clusters (I-III). Group I (n=17) consisted entirely of netB-negative isolates while Groups II (n=18) and III (n=15) were comprised of 61% and 67% netB-positive isolates, respectively. Several chromosomal loci were identified that differentiate Groups II and III, including genes for dTDP-L-rhamnose biosynthesis, D-glucuronate catabolism and capsular polysaccharide.

These findings suggest that a specific chromosomal background is predominantly associated with netB-positive strains, consisting of supplementary fitness-related genes that likely enhance virulence. Furthermore, this chromosomal background can be further divided into two main lineages that are predicted to differ in terms of carbon utilization and serotype.

9

Outbreak of ovine footrot in Norway is most likely caused by recent introduction of a virulent *Dichelobacter nodosus* strain

Marianne Gilhuus[1] , Synnøve Vatn[2] , Om P Dhungyel[3] , Bereket Tesfamichael[1] , Trine L´Abée-Lund[4] , Hannah J Jørgensen[1]

1. Norwegian Veterinary Institute, Oslo, Norway
2. *Animalia – Norwegian Meat and Poultry Research Centre, Oslo, Norway*
3. *Faculty of Veterinary Science, The University of Sydney, Camden, Australia*
4. *Norwegian School of Veterinary Science, Oslo, Norway*

An outbreak of ovine footrot in Norway in 2008, the first reported since 1948, prompted action to investigate Norwegian isolates of *Dichelobacter nodosus*. Isolates from sheep, cattle and goats from approximately 100 farms located in 8 of the 19 Norwegian counties were characterized. The potential virulence of the isolates was assessed by the gelatinase gel test (GG-test) to test the heat stability of bacterial proteases[1]. Isolates that produced heat stable- and heat labile proteases, were categorized as virulent and benign, respectively. All isolates were allocated to serogroup by a multiplex PCR that differentiates *fimA* variants [2]. A subset was serogrouped by slide agglutination and genotyped by Pulsed-field gel electrophoresis (PFGE). Isolates defined as virulent by the GG-test were all from sheep from one county in the south west of Norway. In cases where extensive under-running of the sole of the hoof had been observed in sheep, isolates from the flock were invariably virulent. More than 97% of the virulent isolates belonged to serogroup A. By PFGE, the virulent isolates were genetically similar while a greater genetic diversity was observed among benign isolates. On the grounds that virulent isolates were found in one county only, and that the majority belonged to the same serogroup, and were similar by PFGE it is believed that a virulent *D. nodosus* strain was introduced to the country recently and has spread locally within one county.

1. Palmer, MA. 1993. Vet. Microbiol. Jul; 36(1-2):113-22
2. Dhungyel, OP.; Whittington, RJ.; Egerton, J. 2002. Mol. Cell Probes. Aug. 16(4); 285-96.

10

Whole genome sequence analysis of strains from Australia and Norway reveals that virulent and benign isolates of *Dichelobacter nodosus* belong to two separate lineages

Ruth M Kennan[1] , Marianne Gilhuus[2] , Dieter M Bulach[1] , Torsten Seeman[1] , John D Boyce[1] , Hannah J Jorgensen[2] , Julian I Rood[1]

1. Monash University, Clayton, VIC, Australia
2. *National Veterinary Institute, Oslo, Norway*

Ovine footrot is a contagious disease of the feet of sheep. The disease has long been recognised as a problem in the sheep industries of Australia, New Zealand, India and Nepal, and has recently re-emerged in some European countries. Severity of disease ranges from interdigital dermatitis (benign footrot), to underrunning of the horn of the hoof and separation of the horn from the underlying tissue (virulent footrot), leading to lameness and loss of body condition. The principal causative agent of footrot is the bacterium *Dichelobacter nodosus*, an anaerobic, Gram negative rod, and strains are classified as benign or virulent based on their ability to degrade elastin and their protease thermostability. We now know that these phenotypic differences are the result of a single amino acid substitution in one of the three extracellular proteases secreted by *D. nodosus.* While the proteases play an important role in disease, and are used for differential diagnosis, our comparative genomic analysis reveals a more deeply entrenched difference between benign and virulent strains. We have sequenced the genomes of 49 isolates, predominantly from Australia and Norway, using Illumina high throughput sequencing. Analysis of conserved regions of these genomes based on read mapping to the reference strain, VCS1703A, identified 9707 sites that were present in all 49 strains but variable amongst the strains. These sites were used to construct a phylogenic tree using the neighbour-joining method. This tree clearly showed that benign and virulent strains could be separated into two distinct clades, and strongly suggests that benign and virulent strains of *D. nodosus* exist as two separate lineages. These results may lead to the development of new methods for the differential diagnosis of ovine footrot.

11

Tale of the unexpected: Complex dynamics underlie apparently simple *Salmonella* infections

Duncan Maskell[1] , Andrew Grant[1] , Chris Coward[1] , Piero Mastroeni[1]

1. Department of Veterinary Medicine, University of Cambridge, Cambridge, UK

Salmonella enterica comes in many different forms and causes a wide range of diseases in different animal hosts. In addition to gastrointestinal infections invasive infections are very common with serious consequences. The progression of an invasive infection is often measured by counting the viable salmonellae present in an organ. This gives an overall picture of the dynamic nature of these infections and the host-pathogen relationship, but underlying the apparently simple growth kinetics often observed there might well be hidden complexity. Often this complexity is broken down for study by using live cells in vitro, infecting them with salmonellae and measuring the fate of both the bacteria and the cells. We have been taking this information and testing whether it is valid in true in vivo infection systems. We have used Salmonella Typhimurium infections of mice, a natural host-pathogen relationship, rather than other large animal systems for reasons of availability and cost.

We have discovered that, contrary to what might be expected from in vitro studies, salmonellae are present inside macrophages in infected mice in low numbers. A substantial increase in viable bacterial counts per organ over time, associated with a lethal infection, is not reflected in increased intracellular counts, but rather by increased numbers of infected cells. Mathematical models of this process can capture our data and the dynamic processes that can be inferred. Using this framework for analysis, we have also shown that the Salmonella Pathogenicity Island 2 Type Three Secretory System is not an absolute requirement for intra-macrophage growth of salmonellae, but appears instead to be required for bacterial escape from infected cells and spread to infect new cells. Attenuation by knocking out these genes, the basis for new live vaccines, is not therefore dependent on simply preventing intracellular growth of the bacteria.

12

A novel mechanism of neutrophil entry into epithelial cells is involved in damping inflammation yet exposes a backdoor for bacterial invasion

Nahum Shpigel[1] , Michal Mintz[1] , Hagit Salamon[1] , Dvir Mintz[1]

1. The Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel

Neutrophil mobilization is a crucial response to protect the host against invading microorganisms. Neutrophil recruitment and removal have to be tightly regulated to prevent uncontrolled inflammation and excessive release of their toxic content causing tissue damage and subsequent organ dysfunctions. Neutrophil apoptosis and phagocytosis by resident and recruited macrophages contribute to the safe disposal of engulfed bacteria and toxic metabolites and elicits the production of anti-inflammatory cytokines. Nevertheless, in many organs, macrophage mobilization into the barrier epithelium is dearth and cannot be a major mechanism for homeostasis of inflammation. We suggest that in some mucosal surfaces or barrier epithelium, like urinary and gall bladder or the mammary alveoli, phagocytosis of apoptotic neutrophils by macrophage may not be the only or most important mechanism of neutrophil safe disposal and homeostasis of inflammation. Based on our results we suggest a novel and previously unrecognized mechanism of neutrophil internalization and apoptotic death program in epithelial cells. Viable neutrophils trigger a mechanism that enables them to crawl into cytoplasmic double membrane compartments in the host cells. Thenceforth, internalized neutrophils loose the membrane compartment and undergo apoptotic cell death in the cytoplasm. We were able to demonstrate this incredible phenomena both in vitro on epithelial cell lines and in murine in vivo systems. We hypothesize that this mechanism contributes to safe disposal of neutrophils and for the resolution of inflammation that might have been overlooked, yet is widespread and central mechanism of inflammation control and resolution in some organs and disease processes.

Most significantly we further suggest that some pathogenic bacteria are taking advantage of neutrophil cell invasion process, using it to invade epithelial cells, where they can proliferate. Bacteria invasion to epithelia may be of prime importance in the pathogenesis of major diseases such as bovine mastitis, urinary tract infection (UTI) and typhoid fever and might account for the chronic carriage and relapsing disease caused by mammary pathogenic E. coli (MPEC), urinary pathogenic E. coli (UPEC) and Salmonella enteric subsp. enterica serovar Typhi, respectively.

13

Colonisation of pig lymphoid tissues with avirulent *Actinobacillus pleuropneumoniae*: competitive exclusion and displacement of virulent *A. pleuropneumoniae* from the respiratory tract?

Andrew N Rycroft[1] , Janine T Bossé[2] , Paul R Langford[2]

1. Royal Veterinary College, North Mymms, UK, United Kingdom
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Actinobacillus pleuropneumoniae causes a common, acute respiratory disease of pigs seen worldwide. Vaccination and antimicrobials are used to control the disease but are inadequate and the disease remains a major problem in intensively grown pigs. Signature-tagged mutagenesis was used to identify mutants of *A. pleuropneumoniae* which had reduced virulence. A number of mutants were incapable of causing experimental disease. The hypothesis was formed that a non-pathogenic mutant could occupy the niche or site of carriage of *A. pleuropneumoniae* and hence reduce or eliminate carriage and susceptibility of the pig to disease. One mutant (4074-14D5) was selected with the aim of deliberate infection and colonisation of the respiratory tract in order to (a) competitively exclude virulent *A. pleuropneumoniae* and (b) displace existing *A. pleuropneumoniae* from its niche in the crypts of the palatine tonsil lymphoid tissue. Results showed the avirulent mutant to be capable of persisting in the palatine tonsil and the nasal secretion for at least 14 days. Animals pre-colonised with 14D5 and then challenged with wild-type *A. pleuropneumoniae* were still susceptible to colonisation by wild- type *A. pleuropneumoniae*. Animals pre-colonised with wild-type *A. pleuropneumoniae* remained colonised in the face of high-

dose inoculation with the avirulent mutant. In conclusion, use of an avirulent mutant of *A. pleuropneumoniae* did not exclude or prevent wild-type infection and it could not displace established colonisation by the wild-type pathogen.

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Molecular insights into actin-based motility of *Burkholderia* species

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In common with selected species of *Listeria*, *Shigella*, *Rickettsia* and *Mycobacterium*, some members of the genus *Burkholderia* are capable of intracellular actin-based motility. In the case of *B. pseudomallei* this requires BimA, which exhibits C-terminal homology to the *Yersinia* autosecreted adhesin YadA and shares motifs with cellular actin-binding proteins and nucleation-promoting factors. Our research has established that tandem WASP-homology (WH2) domains of *B. pseudomallei* BimA ($BimA_{Bp}$) are required to bind and polymerise actin monomers in a manner independent of the cellular Arp2/3 complex. Direct repeats of a casein kinase II target site, which vary in number in natural isolates of *B. pseudomallei*, were found to be required for actin-based motility and act additively to promote actin polymerization.

Functional BimA homologues exist in the glanders pathogen *B. mallei* ($BimA_{Bm}$) and avirulent saprophyte *B. thailandensis* ($BimA_{Bt}$). $BimA_{Bt}$ appears distinct from other BimA variants in that it possesses a conserved central acidic (CA) domain. Such domains often mediate the recruitment and activation of the Arp2/3 complex. Consistent with this prediction, $BimA_{Bt}$ was found to require Arp2/3 for actin-polymerization and in-frame deletion of the CA domain of $BimA_{Bt}$ abolished this activity and the binding of the Arp2/3 complex components. These data suggest that different mechanisms for actin-based motility have evolved even among closely-related *Burkholderia* species.

Our preliminary data indicate that BimA may be modified post-translationally by phosphorylation and glycosylation. Indeed, a predicted glycosyltransferase encoded immediately 5' of *bimA* in *B. pseudomallei* (BPSS1491) is required for actin-based motility but not expression or polar localization of BimA and ongoing work aims to define the nature and consequences of glycan modification. We have also identified cellular proteins that are recruited to the surface of BimA-expressing bacteria by mass spectrometry and are dissecting the protein-protein interactions required for actin-based motility of *B. pseudomallei*.

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Intracellular pH measurements with a DNA nanosensor indicate that the cytoplasm of *Salmonella* Typhimurium is acidified in the macrophage vacuole (SCV)

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Pathogens encounter extreme changes in pH during the course of infection. For example acidic pH in the "*Salmonella*-containing vacuole" or SCV is considered to be a major inducing signal for stimulating expression of *Salmonella* pathogenicity island 2 (SPI-2) genes. We set out to determine the consequences to *Salmonella* during growth in the SCV. A recently developed DNA nanomachine (I-switch) used fluorescence resonance energy transfer to map spatiotemporal pH changes during endosomal maturation in eukaryotes (1). This high performance pH sensor (pH range 5.5 – 7) is equally efficient *in vitro* and in the cytoplasm. We employed the I–switch first with *E. coli* in order to measure intracellular pH changes in response to osmotic signalling. The donor/acceptor ratio (D/A) of the *in vitro* FRET curve showed perfect agreement with the intracellular standard curve. The intracellular pH decreased ~ 1 unit (from 7.08 to 6.05) upon 400 mM salt stress, whereas the osmotic regulator mutant strain Δ*ompR* showed < 0.4 pH unit decrease, indicating that OmpR is a major regulator of osmolality in *E. coli*. In *Salmonella enterica* serovarTyphimurium, a similar pH decrease ~ 1 unit (from 6.78 to 5.75) was observed upon salt stress, but the Δ*ompR* strain exhibited an even lower response to osmotic stress than in *E. coli*. We then used the I-switch to measure the internal pH of *Salmonella* in the macrophage vacuole during systemic infection. *Salmonella* intracellular pH decreased ~ 1 unit (from 6.78 to 5.80) that was apparent twenty minutes after infection. Our study represents a novel application of an autonomous DNA nanomachine and indicates that the trigger of virulence genes in *Salmonella* upon entry inside macrophages likely results from changes in intracellular pH. Supported by Mechanobiology Institute, NUS and VA 5I01BX000372 to LJK.[1]

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Rhodococcus equi virulence: not all is the plasmid's fault

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The genus *Rhodococcus* belongs to one of the largest microbial groups on earth, the *Actinobacteria*, which includes "friendly" antibiotic and bioactive compound producers but also major pathogens such as the agents of tuberculosis (TB), leprosy or diphtheria. The rhodococci are widely distributed in the environment and are useful in biotechnology due to their extraordinary metabolic versatility and biodegradative properties. The genus also includes an animal pathogen, *Rhodococcus equi*, and the phytopathogenic species *Rhodococcus fascians*, thus providing an attractive paradigm for studying the evolution of niche breadth and virulence in *Actinobacteria*. Since the seminal work by Shinji Takai's and John Prescott's groups in the 1990's, research into *Rhodococcus equi* pathogenesis and

vaccinology has revolved around the virulence plasmid. This extrachromosomal replicon is required for intracellular proliferation in macrophages and within-host survival. Recent molecular epidemiological evidence suggests the virulence plasmid also plays a key role in the determination of *R. equi* host tropism, with specific plasmid types being associated with equine (pVAPA plasmid), porcine (pVAPB plasmids) and bovine isolates (pVAPN plasmids). This is a novel paradigm in microbial pathogenicity and a finding that shifts the "equinocentric" focus of *R. equi* research towards a more realistic perception of *R. equi* as multihost bacterial pathogen. Indeed, *R. equi* not only affects horses but causes pyogranulomatous pathology in a variety of animal species, including life-threatening opportunistic TB-like infections in humans. Isolates from humans in which the infection is opportunistic can carry any of the three animal host-adapted virulence plasmid types. While the fundamental importance of the virulence plasmid in *R. equi* pathogenesis is indisputable, the recent sequencing of the *R. equi* genome has uncovered additional novel virulence determinants on the bacterial chromosome. This presentation will summarise what we have learned about *R. equi* virulence from the genome sequence. Our comparative genomic analyses between *R. equi* and closely related environmental rhodococci provide key insights into the mechanisms of niche-adaptive genome plasticity and virulence evolution in this bacterial group. Our findings illustrate how actinobacterial virulence evolves by co-option of core microbial traits following gain by lateral transfer of a few critical genes that provide access to the host niche. Unpublished data will be presented on a novel horizontally acquired (HGT) genomic island of *R. equi*, encoding the biogenesis of Flp type IVb subfamily pili essential for virulence and lung colonization in vivo.

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Single-cell measurement of $F165_1$ and Pap fimbrial phase variation in real-time

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Background: Fimbriae $F165_1$ (*foo*)synthesized by septicemic *Escherichia coli* strains belong to the type P (*pap*) family of adhesive factors and are under the control of a methylation-dependent transcriptional regulation, called phase variation. This mechanism arises from competition between two global regulatory proteins, Lrp and Dam, within the regulatory region and gives an heterogenous phenotype among a clonal population. If $F165_1$ and Pap share similar regulatory mechanisms, $F165_1$ expression however has its own specificity, showing a more heterogeneous phenotype, with a predominant ON state. Here, we adapted single cell measurement approaches to distinguish changes during phase variation of *foo* and *pap* operons. **Methods and Results**: To characterize thephase variation, we measured it using transcriptional fusions between the regulatory regions of *pap* and *foo* operons and the T7 RNA polymerase (T7 RNAP). Expression of T7 RNAP activates a specific promoter regulating the expression of *gfp* located on a plasmid. This enabled single cells in the ON and OFF states to be distinguished by flow cytometry and fluorescence microscopy. Flow cytometry confirmed previous analysis of phase variation using *lacZ* reporter fusions but gave additional information. Each profile showed three distinct bacterial populations, corresponding to ON cells, OFF cells and intermediate level of fluorescence. While *pap* cells were mostly in the OFF state, *foo* cells showed a more heterogeneous phenotype. Similar patterns were observed when Pap and $F165_1$ phase variations were monitored in real time on individual *E. coli* using fluorescence microscopy. **Conclusion:** Following single cells in real time, we have shown distinctive patterns of expression of the *pap and foo* operons. Moreover investigation of the behavior of single cells, confirmed Pap as a slow switcher and $F165_1$ as a fast switcher. Therefore, the rapid fimbrial switch of $F165_1$-ExPEC strains may enhance their adaptation to fluctuating environments and contribute their fitness.

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Sequence diversity and cytotoxicity of the leukotoxin of *Mannheimia* species isolated from cases of ovine mastitis

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Of all the virulence factors associated with *Mannheimia* species, leukotoxin is the most characterised. A panel of *Mannheimia* sp. isolates obtained from cases of ovine mastitis in south eastern Australia were initially assessed for the presence of the gene encoding the toxin, *lktA*. The sequences of the *lktA* gene generated from these isolates were compared. Cross neutralising capacities of rat antisera raised against the leukotoxin of one *M. glucosida*, one haemolytic *M. ruminalis* and two *M. haemolytica* isolates were investigated.

A higher overall nucleotide distance was found between the *lktA* sequences of the *M. haemolytica* isolates than between those of the *M. glucosida* isolates. Based on the sequence of the *lktA* gene, the *M. haemolytica* isolates could be categorised in 2 groups (A and B).

The neutralising capacities of polyclonal sera were tested against homologous and heterologous leukotoxins. A neutralisation titre of 32 was observed for each antiserum against its homologous leukotoxin, whereas the titres differed when tested against heterologous toxins. The antigenic similarity coefficient calculated from the cross neutralisation data revealed that the leukotoxins from the two *M. haemolytica* isolates had the least similarity among the strains tested. The leukotoxin of *M. glucosida* had the highest similarity to the toxins produced by *M. haemolytica* (Group A) and the haemolytic *M. ruminalis*.

Our findings suggest that a vaccine based on *M. glucosida* may offer the broadest protection against ovine mastitis cause by *Mannheimia* species.

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The surfaceome of *Mycoplasma hyopneumoniae* displays an extensive population of multifunctional endoproteolytic cleavage fragments derived from the P97 and P102 adhesin families

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Mycoplasmas are the smallest self-replicating life forms and are attracting considerable interest as model organisms for systems studies. Omics technologies have revealed a variety of transcriptional control mechanisms involving various RNA species, extensive protein-protein interaction pathways and complex post translational protein modification mechanisms. Size and phase variation of dominant surface antigens have also been documented in a number of species. *Mycoplasma hyopneumoniae* is a primary pathogen in the porcine respiratory disease complex, an economically important disease of swine. We have shown that the archetype adhesin protein P97, operon partner P102 and their paralogs are highly expressed, multifunctional proteins that are displayed on the surface as endoproteolytic cleavage fragments. The process of endoproteolysis is complex with events that are executed with varying efficiency and generate fragments displaying different motif patterns. To date, three cleavage motifs have been characterised but the proteases responsible have not been identified. We have begun to intensively map the surface proteome of *M. hyopneumoniae* using trypsin shaving and protein biotinylation methodologies. LC-MS/MS analysis of biotinylated surface proteins captured using avidin chromatography led to the identification of an unprecedented number of endoproteolytic cleavage fragments belonging to these adhesin families. The archetype cilium adhesin P97 is extensively processed. Biochemical studies show that P97 is a multifunctional adhesin and that the C-terminus encodes cilia, extracellular matrix and host circulatory molecule binding domains. Binding studies with chemically synthesized overlapping peptides spanning the C-terminus of P97 allowed us to delineate the identity of residues critical for binding fibronectin and plasminogen. To our knowledge, *M. hyopneumoniae* is unique in the microbial world in that it generates surface protein diversity through controlled endoproteolysis of highly expressed, large mass adhesin families generating a complex array multifunctional fragments that are retained on the cell surface.

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Polyamines are required for virulence and expression of virulence loci in *Salmonella* Typhimurium

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Sensing and responding to environmental cues is a fundamental characteristic of bacterial physiology and virulence. Here we identify polyamines as novel environmental signals essential for virulence of Salmonella enterica serovar Typhimurium, a major food borne intracellular pathogen and a model organism for studying typhoid fever. Central to its virulence are two major virulence loci Salmonella Pathogenicity Island 1 and 2 (SPI1 and SPI2). SPI1 promotes invasion of epithelial cells, whereas SPI2 enables S. Typhimurium to survive and proliferate within specialized compartments inside host cells. In this study, we show that an S. Typhimurium polyamine mutant is defective for invasion, intracellular survival, killing of the nematode Caenorhabditis elegans and systemic infection of the mouse model of typhoid fever. Virulence of the mutant could be restored by genetic complementation, and invasion and intracellular survival could, as well, be complemented by the addition of exogenous polyamines to the bacterial cultures prior to infection. Experiments addressed at elucidating the roles of polyamines in infection revealed that expression of genes from both of the major virulence loci SPI1 and SPI2 responded to exogenous polyamines and was reduced in the polyamine mutant. Further molecular characterization of polyamine dependent expression of virulence revealed complex cross-talk between known regulators of virulence expression and polyamines. Together our data demonstrate that polyamines play a critical role in controlling virulence in S. Typhimurium most likely through stimulation of expression of essential virulence loci. Moreover, our data implicate polyamines as key signals in S. Typhimurium virulence.

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The siderophore esterases Fes and IroD contribute to virulence of avian extra-intestinal pathogenic *Escherichia coli*

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Extra-intestinal pathogenic *E.coli* is an important pathogen of humans, poultry, and other animals. Siderophores sequester iron and are important for virulence. In pathogenic *E. coli,* the catecholate siderophores enterobactin and salmochelins are degraded by esterases (Fes, IroD, and IroE) to release iron for bioavailability within the bacterial cell. The purpose of this study was to investigate the role of esterases for virulence of avian pathogenic *E.coli* (APEC) in chickens. Growth of strains was also tested in iron poor minimal medium.

APEC strain X7122 which produces the siderophores enterobactin, salmochelins, and aerobactin was used to generate mutants in the esterase encoding genes *fes*, *iroD*, and *iroE* by allelic exchange of single and multiple esterase encoding genes. These mutants were tested in three-week old chickens via intra air sac inoculation. Bacterial counts were determined in the blood, liver, spleen and lungs 48 hrs post-infection.

-Despite the presence of a functional aerobactin system which alone can contribute to APEC virulence, the esterases Fes and IroD were shown to be important for growth in iron poor medium. Loss of catecholate siderophore synthesis in the esterase mutants resulted in a regain in growth. The fes and iroD single mutants were reduced in the blood lungs, liver and spleen. By contrast loss of iroE had no significant effect on bacterial numbers in blood and tissues. Loss of iroD had the most marked attenuation. Cumulative loss of *fes*, *iroD*, and *iroE* did not result in an additive decrease in virulence in the chicken infection model.

Results demonstrate that esterases IroD and Fes contribute to virulence during systemic infection by APEC strain X7122. As these siderophore systems are conserved among APEC and other pathogenic bacteria, esterases may be important targets for inhibiting extra-intestinal virulence in poultry and other animals.

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Identification of redundant metabolic pathways essential for virulence of *Salmonella enterica* and their potential as targets for antimicrobial therapy

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There is an urgent need for new targets for development of antibiotics. Pathways that are non-essential because the bacterium has more than one alternative to perform the synthesis have been overlooked in this search. We hypothesise that redundancy targets may be useful, provided one can identify all components of the redundant pathway and block them in parallel.

To test this hypothesis we developed a genome scale model of *Salmonella* Typhimurium and used this to identify possible redundancy pairs. We then selected two predicted pairs: Spermidine-uptake and spermidine biosynthesis genes (*potCD* and *speE*) and ammonia and glutamate dependent asparagine synthetase genes (*asnA* and *asnB*). Single and double mutants, and double mutants complemented *in trans*, were constructed and characterized for growth and virulence. Single mutants were fully virulent or only marginally attenuated, while double mutants were severely attenuated, showing that spermidine and asparagine are essential for virulence of *S*. Typhimurium. The virulence could be restored by *in trans* complementation. Corresponding mutants in *S*. Gallinarum and *S.* Dublin behaved similarly when tested for virulence in chicken and mice.

Knock out of spermidine biosynthesis in a spermidine-transporter knock-out background caused reduced expression of *sseA* encoded from SPI-2, suggesting that the *Salmonella* dependence of spermidine for full virulence relates to the function of SPI-2. The *asnA/asnB* double mutant grew normally in rich medium but was asparagine auxotrophic in minimal medium, and the attenuation may relate to lack of asparagine at sites of infection.

In conclusion, we have developed an *in silico* approach to identification of redundancy genes. Using this model, we predicted that spermidine biosynthesis and spermidine transportation as well as asparagine synthesis via *asnA* and *asnB* would be essential for infection in *S*. Typhimurium and this prediction was proven correct. The results suggest that redundancy genes may be useful targets for infection control.

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Regulation of *Burkholderia pseudomallei* motility and virulence by a novel two-component signal transduction system

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Burkholderia pseudomallei is the cause of melioidosis, a serious invasive disease of humans and other animal species with a mortality rate of 10-50%. Screening of a *B. pseudomallei* transposon library identified a transposon mutant that was significantly attenuated for virulence in BALB/c mice. This mutant had a transposon insertion in the gene *bvrS* (BPSS0687), predicted to encode the sensor kinase component of a two-component signal transduction systems (TCSTS). TCSTS are important regulators of bacterial gene expression. An independent, directed *bvrS* mutant was also highly attenuated for virulence. To identify genes regulated by this TCSTS, we used RNA sequencing to compare the transcriptomes of the directed *bvrS* mutant and the wild-type parent; 104 genes were up-regulated and 22 down-regulated in the *bvrS* mutant. Amongst this set of differentially regulated genes we identified a number predicted to play a direct role in *B. pseudomallei* virulence. These included four genes involved in flagella biosynthesis, six in chemotaxis, six in phenylacetic acid (PA) catabolism, 19 involved in polyketide biosynthesis (four separate operons) and one encoding a thermolysin metalloprotease. We assayed the motility of the wild-type and mutant strains and the *bvrS* mutants demonstrated significantly reduced motility compared to the wild-type strain; complementation with intact *bvrS* restored motility. Furthermore, electron microscopy analysis indicated that the *bvrS* mutants produced highly reduced numbers of surface flagella. Therefore, BvrS is a component of a critical virulence-associated TCSTS that plays a vital role in regulating expression of flagellin synthesis and controlling motility.

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Identification of virulence factors of fish pathogen *Vibrio anguillarum* using a gnotobiotic model system

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Vibrio (Listonella) anguillarum is the causative agent of vibriosis, a highly fatal haemorrhagic septicaemic disease affecting many economically important fish, bivalves and crustaceans. In both aquaculture and larviculture, this disease is responsible for severe

economic losses worldwide. Because of its high morbidity and mortality rates, substantial research activity has been carried out in order to unravel the virulence mechanisms of this pathogen and to develop rapid detection and identification techniques. Nevertheless, the virulence mechanisms of this pathogen and the critical virulence factors for pathogenesis are still insufficiently known. In this study, the virulence of 16 wild-type V. anguillarum strains, isolated from different hosts or ecological niches was studied using a standardized gnotobiotic model system with axenic European sea bass (Dicentrarchus labrax) larvae as hosts. Larvae were challenged with 105 colony forming units of bacteria per ml of water, and larval survival was monitored by microscopical analysis at regular intervals. Significant differences in virulence were observed among the 16 tested V. anguillarum isolates. Eleven strains caused a significantly higher mortality compared to five other isolates and the axenic control.

Based on these results, three highly virulent V. anguillarum strains, VIB15, HI610 and 87-9-117 were selected to generate knock-out mutations in potential virulence factors, such as empA, encoding a zinc metalloprotease, pilA, encoding the type IV pilin, and qseC, enocoding a sensor kinase involved in quorum sensing. In addition to the selected strains, NB10, a well-studied V. anguillarum strain, was also included in the study.

Both the wild-type strains and knock-out mutants have been tested with the gnotobiotic system to screen for differences in virulence. This challenge test showed that the gnotobiotic model system can be used to study host-microbe interactions and more particularly to identify virulence genes that play an essential role in the pathogenesis of V. anguillarum.

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Identification and functional analysis of a bacitracin resistance locus from *Clostridium perfringens*

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Bacitracins are a mixture of structurally related cyclic polypeptides with clinically useful antibiotic properties. They act by indirectly interfering with the biosynthesis of the bacterial cell wall. In this study, we analysed an avian necrotic enteritis strain of *Clostridium perfringens* that was resistant to bacitracin. We identified, for the first time in this genus, a putative bacitracin resistance locus that resembles a bacitracin resistance determinant from *Enterococcus faecalis*. It contains the structural genes *bcrABD* and a putative regulatory gene, *bcrR*. Conjugation experiments provided evidence that this locus was located on a mobile genetic element that had the ability to transpose onto both conjugative and non-conjugative plasmids or onto the chromosome. Whole genome high-throughput sequencing of the wild-type strain and several transconjugants revealed that the *bcr* genes were located on a potential mobile element that was related to the Tn*916* family of conjugative transposons. Our analysis identified the open reading frames (ORFs) that are responsible for recombination (Xis/Int), regulation (ORF8), conjugation (ORFs13-23) and several hypothetical genes. The bacitracin resistance locus was located between genes that were homologous to ORF19 and ORF18 from Tn*916*. In summary, we have identified a novel mobile bacitracin resistance determinant in a toxin-producing *C. perfringens* strain and our study has provided important bioinformatic and functional information on the genetic organization of this resistance locus.

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F17-like fimbriae are important for full virulence in *Gallibacterium anatis*

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Gallibacterium anatis is a recently recognized, yet major, cause of salpingitis and peritonitis in egg-laying chickens, leading to decreased egg-production and animal welfare issues worldwide. High levels of drug resistance and antigenic diversity make it difficult to treat or prevent infections with *G. anatis* using traditional antimicrobial agents or vaccines. Thus, insight into the pathogenesis of disease and knowledge of important virulence factors is urgently required. A key event during colonization and invasion of mucosal surfaces is adherence, and recently, varying numbers of loci encoding putative F17-like fimbriae were identified in the genomes of *G. anatis* biovar *haemolytica*. The objective of this study was to characterize the F17-like fimbriae, encoded by the *ec4* gene, and determine their role in virulence. In vitro expression and surface-exposure of EC4 was demonstrated by flow cytometry and immunofluorescence microscopy using specific anti-EC4 antibodies. The predicted function of EC4 as a F17-like fimbrial subunit was confirmed by immunogold electron microscopy. Two fimbrial expression phenotypes were identified: one expressing low numbers of fimbriae per cell and another with hyper-fimbriation. Finally, a fimbrial knock-out mutant (ΔEC4) was generated and used to confirm the in vitro results. The ΔEC4 mutant was significantly attenuated when compared to the wild-type *G. anatis* 12656-12 following intraperitoneal challenge in the natural chicken host. In conclusion, we have for the first time described a fimbrial protein precursor from *G. anatis* and demonstrated that EC4 is an important virulence factor in the pathogenesis of *G. anatis* infection.

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Leptospiral genomics, virulence factors, and vaccine antigens

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Leptospirosis is the most widespread zoonosis worldwide, caused infection with serovars of pathogenic *Leptospira* spp. However, understanding of leptospiral pathogenesis lags far behind that for many other bacterial pathogens. Current research is thus directed at identification of leptospiral virulence factors. Saprophytic *Leptospira* species are environmental organisms that never cause disease.

Comparative genomics of pathogens and saprophytes has allowed the identification of more than 900 genes unique to either *L. interrogans* or *L. borgpetersenii*; these genes potentially encode virulence-associated proteins. However, genes of unknown function are over-represented in this subset of pathogen-specific genes, accounting for 80% and 60% of open reading frames, respectively. This finding, together with the absence of virulence factor homologues among the proteins of known function, suggests that *Leptospira* possesses unique virulence mechanisms. Whole genome microarray studies have identified genes whose expression is differentially regulated under a range of simulated *in vivo* conditions, such as physiological temperature and osmolarity, low iron levels, and the presence of serum. The subset of genes identified by these studies is likely to include virulence factors. However, most such genes encode proteins of unknown function, consistent with the hypothesis that leptospiral virulence genes do not have homologues in other bacterial species. The recent development of mutagenesis systems for pathogenic *Leptospira* spp. has allowed the screening of defined mutants for attenuation of virulence in animal infection models and has identified definitively for the first time a range of virulence factors, including lipopolysaccharide, flagella, heme oxygenase, and the OmpA-family protein, Loa22. Interestingly, inactivation of a number of genes hypothesised to encode virulence factors based on in vitro virulence-associated properties did not result in attenuation of virulence, suggesting a degree of functional redundancy in leptospiral pathogenic mechanisms. The availability of multiple leptospiral genome sequences has facilitated a more rational approach to the identification of vaccine antigens. Several candidates have been reported, but a stringent analysis of the protection data for many of them does not support the claimed levels of protection.

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Pasteurella multocida lipopolysaccharide polymorphism: the implications for vaccine development

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P. multocida is a Gram-negative capsulated bacterium that is the causative agent of a wide range of animal diseases, including bovine haemorrhagic septicaemia, atrophic rhinitis in pigs and fowl cholera, a serious disease of poultry which can present in both acute and chronic forms. The increasing consumer demand for free-range and organic poultry has resulted in an increased incidence of fowl cholera. *P. multocida* strains are currently classified into 16 Heddleston serovars using the gel diffusion precipitin test with antisera raised against somatic or lipopolysaccharide (LPS) antigen. *P. multocida* LPS is both a protective immunogen and an important virulence factor. We have developed a multiplex PCR typing system to replace the Heddleston serotyping scheme and have shown that the 16 Heddleston type strains can be grouped into 8 genotypes based on their LPS outer core biosynthesis genes. The most common strains isolated from Australian poultry belong to Heddleston serovars 3 and 4. Here we report that the type strains of these two serovars produce related but distinct LPS structures and share a common LPS outer core biosynthesis locus, L3. We also show using TargeTron® mutagenesis of genes within this locus, that truncation of the LPS outer core structure can be attributed to mutations within one of the six encoded glycosyltransferases. Interestingly, LPS structural analyses of over 35 field isolates belonging to genotype L3 reveal that a further four related, but distinct, LPS structures/glycoforms are produced by field isolates belonging to L3, and in some field isolates multiple LPS glycoforms are simultaneously produced. The existence of these multiple LPS glycoform-producing strains in the field raises significant questions about the efficacy of *P. multocida* vaccine strains that elaborate only a single LPS structure.

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Botulinum neurotoxin - A friend and a foe

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Botulinum neurotoxins are the most potent natural toxins known, produced by the anaerobic spore-former botulinum and certain strains of other clostridia. The neurotoxins cleave synaptic proteins and block acetylcholine release in cholinergic nerves, causing a flaccid paralysis. While large doses of toxin, ingested with food or feed or synthesized by the bacteria in the gut or deep wounds of humans and animals, cause a life-threatening paralysis, botulism, controlled administration of minute amounts of the neurotoxin is becoming the first choice in treating an increasing number of spastic and many other disorders. Considering the severity of botulism but also the wide range of therapeutic applications, knowing the regulatory mechanisms behind botulinum neurotoxin synthesis is of utmost importance. Nevertheless, only little is known about this control. The talk will focus on the pathogenesis of botulinum neurotoxins and their therapeutic use, and gene regulation in C. botulinum.

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Cytotoxin CctA, a major virulence factor of *Clostridium chauvoei* conferring protective immunity against myonecrosis

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A novel protein toxin, named *Clostridium chauvoei* toxin A (CctA) that belongs to the family of ß-barrel pore forming toxins of the leucocidin superfamily of bacterial toxins was discovered by whole genome sequence analysis. The corresponding gene *cctA* and the secreted toxin CctA was found in all strains of *C. chauvoei* analyzed, isolated from various geographical areas over the globe during the last 50 years, but not in other pathogenic *Clostridium* species. Native CctA and recombinant rCctA produced in *Escherichia coli* are highly cytotoxic towards Embryonic Calf Nasal Epithelial (ECaNEp) cells and have high haemolytic activity against sheep and bovine

erythrocytes in standard haemolysis assays. Polyclonal anti-rCctA rabbit antibodies fully neutralize the cytotoxic and haemolytic activity, not only of rCctA but also of supernatants from cultures of the various *C. chauvoei* strains, indicating that CctA is the main cytotoxic and haemolytic substance secreted by *C. chauvoei*. Using a standard vaccine release procedure, we demonstrated that vaccination of guinea pigs with recombinant CctA in form of a fusion protein with the B subunit of the *E. coli* heat labile toxin as a peptide adjuvant (rCctA::LTB) protected the animals against challenge with spores of virulent *C. chauvoei*. This indicates that CctA is the major virulence factor of *C. chauvoei* and a valuable antigen in vaccines against blackleg.

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Structural and functional analysis of NetB toxin *from Clostridium perfringens*

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Clostridium perfringens is an anaerobic bacterium that causes avian necrotic enteritis, which is characterised by damage to the intestinal mucosa. We previously showed that the secreted pore-forming toxin, NetB, is a major virulence factor in the pathogenesis of necrotic enteritis in chickens. The *netB* gene is encoded on a conjugative plasmid that very unusually co-exists in the same cell as two other very closely related conjugative plasmids, all of which have essentially the same conjugation locus. NetB has amino acid sequence similarity to a-hemolysin, a b-barrel pore-forming toxin from *Staphylococcus aureus*. These toxins are produced as water soluble monomers that upon target cell recognition oligomerise and undergo conformational changes to produce a transmembrane pore that causes cell lysis. We have determined the crystal structure of the water soluble form of NetB to 1.8 Å. The results showed that it adopts a similar fold to a-hemolysin, however, there are key differences in the conformation of the membrane binding domain, indicating that NetB may recognise different membrane receptors or use a different mechanism for membrane-protein interactions. Using the ability of NetB to lyse red blood cells as a screen, we have carried out a random mutagenesis study that identified several residues that are critical for NetB-induced cell lysis. Key residues identified by the mutagenesis studies were mapped onto the crystal structure; most of these amino acids clustered in regions predicted to be required for oligomerisation or membrane binding. These data provide an insight into the mechanism of NetB pore formation and will contribute to our understanding of the mode of action of this important toxin.

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The intestinal epithelial barrier function is altered by *Escherichia coli* STb enterotoxin

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Escherichia coli heat-stable enterotoxin (STb) causes diarrhea in Man and animals. STb binds to sulfatide, its receptor, and is then internalized. Inside the cytosol, through a cascade of events, STb triggers the opening of ion channels allowing ion secretion and water loss leading to diarrhea. Tight junctions (TJs) are well known for controlling paracellular traffic of ions and water by forming a physical intercellular barrier in epithelial cells. The present study aimed at determining the effect of STb toxin on TJs and the barrier function in intestinal epithelial cells. Human colon intestinal epithelial cells (T84) were treated with either purified STb toxin or *E. coli* strains expressing STb. After 24h, the TransEpithelial Resistance (TER), paracellular flux of fluorescent markers and confocal microscopy were used to analyze the effect of STb toxin on TJs. *E. coli* strains expressing STb as well as purified STb caused a significant reduction of TER ($p<0.0001$) coupled with an increase in paracellular permeability to BSA-FITC ($p<0.0001$) compared to the negative controls or a commensal *E.coli* strain. The increased paracellular permeability induced by STb was associated with a marked general dissolution and condensation of F-actin stress fibers. F-actin disorganisation was accompanied by redistribution and fragmentation of occludin, claudin and ZO-1 (Zonula Occludens-1) proteins. These changes were also observed following intoxication of T84 cells with an 8 amino acids peptide found in the loop region of STb corresponding to a concensus sequence of *Vibrio cholerae* Zot toxin. A scrambled octapeptide and an inactive STb mutant (single amino acid change) did not affect the TJs proteins. Our findings suggest that STb impairs intestinal epithelial barrier function by altering tight junction proteins and these changes could contribute to the observed diarrhea. These results provide new insight into the pathogenesis of STb toxin.

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Alternative to Aantibiotics: small cationic peptides provide protection to neonatal chickens against enteric bacterial infections through enhanced innate host defenses

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Neonatal poultry exhibit a transient susceptibility to infectious diseases during the first week of life that stems from inefficient host defense mechanisms. Yet, the initial host immune response to pathogens is a critical determinant of disease resistance and susceptibility. With this context in mind, novel ways to stimulate or modulate the hosts' natural immune response is emerging as an important area of interest for food animal producers including the poultry industry. Specifically, we have been investigating new modulation strategies tailored around the selective stimulation of the host's immune system, and particularly rapid acting innate immunity, as an alternative to direct targeting of microbial pathogens. One such approach that we have been investigating is the use of

a group of cationic peptides produced by a Gram-positive soil bacterium, Brevibacillus texasporus (BT peptides). When provided as a feed additive, BT peptides significantly induced a concentration-dependent protection against cecal colonization and extraintestinal colonization by Salmonella enterica serovar Enteritidis (SE). This protection is not the result of direct antibacterial activity of the BT peptides on the SE since the concentrations used were below the minimum inhibitory concentration for SE. We also found that BT are not absorbed in the intestine, but still induce a significant up-regulation in the functional efficiency of peripheral blood heterophils and monocytes. The mechanisms of this immune modulation are unknown. Here, using in vitro models for measuring: (1) leukocyte oxidative burst, (2) changes in leukocyte cytokine and chemokines gene expression profiles, and (3) phosphorylation of the mitogen activated protein kinases (MAPKs) in leukocytes, we evaluated the role of BT peptides as priming mediators for heterophil and monocyte responses at the level of cell function, gene transcription/expression, and cell phosphorylation following stimulation with inflammatory agonists. BT peptides primed both heterophils and monocytes for an increased oxidative burst and up-regulation in transcription of the pro-inflammatory cytokines IL-1β and IL-6 and inflammatory chemokines CXCLi1 and CXCLi2 induced by inflammatory agonists. In addition, BT peptides induced a rapid (10 min) phosphorylation and activation of the extracellular signal-regulated kinase (ERK1/2) and p38 kinase pathways in primary chicken heterophils. Lastly, we assessed the effects of feeding BT peptides for the first 4 days post-hatch, on transcriptional changes on pro-inflammatory cytokines, inflammatory chemokines, and Toll-like receptors (TLR) in the ceca of broiler chickens. After BT feed removal, chickens were then challenged with SE and mucosal gene expression measured at 1 or 7 days post-infection by quantitative real-time polymerase chain reaction (qRT-PCR). Feeding BT peptides had no direct effect on TLR gene expression, the BT peptides primed cecal tissue for increased ($P< 0.05$) expression of TLR4, 15, and 21 upon infection with SE on days 1 and 7 post-infection (pi). Although feeding the BT peptides primed the cecal tissue for increased expression (2-5 fold) of pro-inflammatory cytokines (IL-1β, Il-6, IL-18) and inflammatory chemokines (CxCli1, CxCli2) in response to SE infection 1 day pi, we found that the BT peptide fed birds had a 5-16-fold increase in cytokine and chemokine expression 7 days pi with SE challenge when compared to the chickens fed the basal diet. Taken together, we conclude that BT peptides, acting through MAPK pathways, enhance leukocyte functional and pro-inflammatory cytokine and chemokine gene transcription activities. These small cationic peptides may prove useful as immune modulators in neonatal poultry.

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Immunomodulatory activities of chicken CATH-2 derived peptides

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Large quantities of conventional antibiotics are used to prevent infection in farm animals, in particular in poultry and swine. Host Defense Peptide (HDP)-based anti-infectives may be an alternative to antibiotics in veterinary medicine. These peptides have direct antimicrobial activity and are immunomodulatory. Previously we identified a chicken cathelicidin (CATH-2) and reported the antimicrobial properties of this peptide. In addition, using mammalian cells, we could demonstrate that CATH-2 has immunomodulatory properties. Next, we wished to investigate the effects of CATH-2 on avian cells.

Here we report that full-length CATH-2 peptide, C(1-26), dose-dependently induces transcription of the chemokines CXCLi2/IL-8, MCP-3 and CCLi4/RANTES, but not of pro-inflammatory cytokine IL-1β, in a chicken macrophage cell line (HD11). In addition, peptide C(1-26) effectively inhibits IL-1β transcription and nitric oxide production induced by LPS from different sources. N-terminal truncated peptides as small as 15 residues still have the capacity to selectively induce chemokine transcription, but lack LPS-neutralizing capacity. Comparative modeling illustrates the role of basic and aromatic residue motifs in endotoxin neutralization by CATH-2 analogs. Substitution of Phe- by Trp-residues introduces endotoxin neutralization capacity in previously inactive truncated CATH-2 derived peptides. Phe/Tyr substitutions result in abrogation of endotoxin neutralization and support a pivotal role for Phe and Trp residues in peptide-mediated endotoxin neutralisation.

We conclude that peptides can be designed, based on CATH-2, that selectively modulate chemokine transcription and could serve as new leads for the design of HDP-based antimicrobials with tailor-made immunomodulatory activities.

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Inflammatory response to footrot in sheep

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Ovine lameness due to foot diseases has a major impact on animal welfare and economy, estimated to cost the UK sheep industry £24–80 million p.a. in treatment and lost production. The interdigital skin of the ovine foot is constantly exposed to a wide variety of microbes, most of them commensal. In addition, interdigital skin is frequently damaged, allowing opportunistic invasion of benign microbes as well as facilitating invasion of pathogens. In sheep, footrot is attributed to a mixed infection of *Fusobacterium necrophorum* and *Dichelobacter nodosus* and develops via an intermediate interdigital dermatitis (ID). The innate immune system is of crucial importance for the host response to pathogens and in the process of inflammation. Currently, there is only very limited knowledge on the innate immune response at the ovine skin hoof interface.

This study aimed to investigate the innate immune response to footrot by using quantitative PCR to determine the expression of Toll-like receptors (TLRs) and pro-inflammatory cytokines using two different approaches: (1) Post slaughter biopsy samples of the skin-hoof interface of sheep displaying signs of ID and footrot in comparison to healthy feet. Expression of TLR2, TLR4 and IL-1β was increased in biopsies from feet with ID and significantly increased in samples displaying footrot. (2) Stimulation of primary ovine dermal fibroblasts, the main cell type of the dermis that plays an important role in modulating leukocyte behaviour and function and shape the tissue microenvironment, with heat-killed F necrophorum and *D nodosus* or both, resulting in significant increases in TLR2 expression. In summary, TLR and pro-inflammatory cytokine expression is modulated at the skin-hoof interface during the inflammatory stages of footrot and dermal fibroblasts respond to footrot bacteria and contribute to the pro-inflammatory host response, providing a first insight into the innate immune response to ovine footrot.

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Coxevac, Q fever vaccine: Vaccine production and control

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Q fever is a zoonotic disease caused by *C. burnettii,* an obligatory intracellular bacterium that produces a highly resistant "spore-like" form[1,2]. Q fever affects humans and animals and it is endemic worldwide[3] except New-Zealand[4] but its prevalence is underestimated[5]. However, as diagnostic methods became more specific and easier to use, increasing number of cases are reported (e.g. acute human cases Bulgaria and The Netherlands). The shedding of *C. burnettii* by ruminants is considered as main source of the human infections[6]. The vaccination is an effective tool to control the disease.

The protective antigen of *C. burnetii* is the full-length phase I LPS[7,8,9]. According to the statement of OIE "only vaccines containing or prepared from phase I *C. burnetii* should be considered protective". Two marketed vaccines in the world are known that consist of exclusively phase I *C. burnetii*: Q-Vax (CSL, Australia) for human and Coxevac (Ceva, France) for veterinary applications.

The production of phase I antigen-based vaccine is performed in GMP biosafety-level 3 area. The process is rather complex, composed of bacteria cultivation on embryonated egg yolk sac, harvest, several downstream steps including inactivation and chemical extraction. The process is strictly controlled through specific ELISA methods to differentiate between phase I and II antigens, and to quantify antigen and egg-derived ballast protein contents for formulation.

The efficacy of the Q fever vaccine was demonstrated by vaccination and subsequent heterologous challenge in target species. The two main parameters to be assessed are the drastic decrease of abortion rate as well as the drop in the shedding of the bacterium[10]. A marketing authorisation (under exceptional circumstances) was obtained in September 2010 by Ceva in all EU member countries.

Coxevac vaccine combined with other complementary prophylactic measures has been successfully used in many EU countries to prevent Q fever in ruminants[11,12,13,14].

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Immunoproteomics of *Helicobacter suis* and protective efficacy of a subunit vaccine in a mouse model

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Helicobacter (*H.*) *suis* is a porcine gastric pathogen, which has also been associated with human gastric disorders. Previous studies in mice showed that an *H. suis* infection does not result in protective immunity, whereas immunization with an *H. suis* whole-cell lysate (lysate) protected against a subsequent experimental infection. Therefore, in a first study two-dimensional gel electrophoresis of *H. suis* proteins was performed followed by immunoblotting with pooled sera from *H. suis*- infected mice or mice immunized with lysate. Little reactivity against *H. suis* proteins was observed in post-infection sera. Sera from lysate immunized mice, however, showed immunoreactivity against a total of 19 protein spots which were identified using ESI-Q-TOF. The *H. suis* urease subunit B (UreB) showed most pronounced reactivity against sera from immunized mice and was not detected by sera from infected mice. Other identified proteins included *H. suis* chaperonin GroEL, urease subunit A, flagellin A and elongation factor G. In a second study, the protective efficacy against an *H. suis* infection of two subunit vaccines was evaluated and compared with that of *H. suis* lysate in a standardized mouse model. Subunit vaccines consisted of either the immunoreactive *H. suis* UreB or the non-immunoreactive *H. suis*

neutrophil-activating protein A (NapA), both recombinantly expressed in *Escherichia coli* (rUreB and rNapA, respectively). Intranasal immunization of mice with rUreB induced a specific serum anti-rUreB IgG response and a significant (p<0.001) reduction of *H. suis* colonization compared to non-vaccinated *H. suis*-infected positive controls. rNapA had no significant (p=0.14) protective effect, although it induced anti-rNapA IgG. Immunization with whole-cell lysate was most effective against a *H. suis* challenge (p<0.001). Increased specific serum IgG and gastric IFN-γ mRNA expression levels were correlated with decreased gastric bacterial load. In conclusion, rUreB is a potential candidate for inclusion in vaccines against *H. suis* infections.

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Practical pitfalls in vaccination against necrotic enteritis in broilers

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Necrotic enteritis (NE), caused by netB toxin producing Clostridium perfringens type A strains, is an important disease in broiler chickens worldwide. Attempts to prevent necrotic enteritis by vaccination hitherto have insufficiently taken into account the practical limitations of broiler vaccination. Indeed, vaccination of broilers needs to be performed at day of hatch or in ovo. In most published studies on vaccination against necrotic enteritis multiple doses at different ages are administered, what is practically impossible for broilers.

In the current study, the efficacy of single and double subcutaneous vaccination using crude supernatant containing active toxin and formaldehyde inactivated crude supernatant (toxoid) of a netB positive C. perfringens strain was analyzed in a subclinical necrotic enteritis model. In addition, single vaccination at day 1 and day 3, and double vaccination at day 3 and day 12 post-hatch were compared.

Double vaccination with crude supernatant and toxoid resulted in a significant decrease in the number of chickens with necrotic enteritis lesions. However, protection induced by vaccination using toxoid was lower compared to active crude supernatant. Single vaccination with crude supernatant at day 3 resulted in significant protection, while vaccination of one-day old chickens with crude supernatant or toxoid, as required under practical conditions, did not induce protection.

In conclusion, treatment of crude supernatant of a particular C. perfringens strain with formaldehyde lowered the efficacy of the crude supernatant vaccine. The observation that vaccination of one-day old broilers with crude supernatant or toxoid was not successful indicates that practical application of vaccination of broilers to control necrotic enteritis may be problematic.

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NetB-based vaccines protect broiler chickens from necrotic enteritis

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The *Clostridium perfringens* pore-forming toxin, NetB, is a major virulence factor in the pathogenesis of necrotic enteritis in chickens and hence is an interesting protein to evaluate as a vaccine antigen. *In vitro* grown cultures of *C. perfringens* produce very little NetB and hence do not provide a good source of the protein for use in vaccines. Therefore, we used recombinant versions of the mature NetB protein to investigate its efficacy in a variety of different vaccine formulations. Birds vaccinated with recombinant NetB (rNetB) were protected from a mild necrotic enteritis challenge, but this single subunit vaccine did not offer protection when a strong *C. perfringens* challenge was applied. However, statistically significant levels of protection from a strong heterologous challenge were achieved when the vaccine formulation was enhanced by combining rNetB with whole cell proteins (bacterin) or concentrated and inactivated *C. perfringens* culture supernatant (toxoid). Clostridial vaccines have been effectively used in other animal species for many years, but the traditional toxoid style of vaccine does not provide very effective protection against necrotic enteritis in chickens. We now have shown that supplementation of such vaccines with sufficient quantity of a key antigen (NetB) provides good levels of protection. We have gone on to show that these vaccines produce high antibody titres in hens and in their eggs and hatched chicks. In conclusion, the identification of a key virulence factor in the pathogenesis of necrotic enteritis (NetB) has led to the development of an effective vaccine formulation that will be of major value to the poultry industry.

POSTERS

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An investigation regarding eradication and diagnosis of *Dichelobacter nodosus* and the presence of *Fusobacterium necrophorum* in footrot affected herds

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The objectives of the present study were to investigate the effect of parenteral treatment with gamithromycin in 3 footrot infected herds over time and to investigate the presence of DNA from *D. nodosus* and *F. necrophorum* on hoofs of footrot affected animals. Further objectives were to evaluate how the use of pooled samples to detect footrot on herd level at different herd prevalences influences the test compared to individual samples and finally to compare clinical scores and real-time PCR for diagnosing footrot.

Results from this study indicate that whole herd treatment with gamithromycin efficiently removed *D. nodosus* and associated clinical signs. All PCR tests were negative on day seven and 28 after treatment and all animals being scored healthy at day 28 by the clinical evaluation.

The prevalence of *F. necrophorum* was found to range between 6 and 33%. Association between *D. nodosus* and *F. necrophorum* and clinical score and *F. necrophorum,* respectively, was found in the herd with the highest prevalence of *F. necrophorum*. *F. necroporum* has been suspected to play a role in the pathogenesis of ovine footrot.

Analysis of pools from four and four animals, gave the same information as individual samples. This indicates that pooled samples are a precise, efficient and cost-effective method when the goal is diagnostics on herd level. However, examples in this study shows the herd level prevalence of footrot should be considered. The current approach among Danish sheep veterinarians is to sample only eight animals of a herd and to analyse these as two pools. This will only give a reliable result provided that the herd prevalence is greater than 32%. This indicates that pooled samples, as they are used today, are not sufficient when the goal is to declare a herd free from footrot if the prevalence is low.

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Influence of *ymoA* gene mutations on enterotoxin Yst production by *Yersinia enterocolitica*

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Yersinia (Y.) enterocolitica is considered as a frequent agent of human and animal diarrhea. One of the important virulence markers is the *ystA* gene encodes the production of enterotoxin YstA. However, not all the strains with *ystA* gene produce enterotoxin, which seems to be caused by the *ymoA* geneencoding the production of the YmoA protein inhibiting the expression of various genes. The purpose of our study was to evaluate the influence of the *ymoA* gene mutations on enterotoxin YstA production by *Y. enterocolitica* isolated from humans and pigs. All the studied strains obtained from pigs had the *ystA* gene which indicates that they belong to the group of strains commonly regarded as pathogenic, but the ability to produce YstA detected using infant mice bioassay was different (3 groups of strains: positive, doubtful, negative). The fragments of *ystA* gene were also detected in all *Y. enterocolitica* strainsisolated from humans and the ability to produce enterotoxin YstA were confirmed by the fact that all the strains were isolated from clinical cases of yersiniosis with diarrhea. Amplification of a fragment of the *ymoA* gene was also detected in all the studied strains, both humans and pigs, so we decided to sequencing *ystA* and *ymoA* genes to find possible mutation involving on enterotoxin YstA production. Mutations in *ystA* gene were not found, both in human and pig strains of *Y. enterocolitica*, but sequencing of *ymoA* gene fragments showed two mutations: transition A3387326G and insertion A in 3387368 position in some of examined strains. Detailed analysis of the strains belonged to each of 3 groups (positive, doubtful, negative) of *Y. enterocolitica* strains from pigs and similarly from humans showed no correlation between the occurrence of the *ymoA* gene mutations and enterotoxin YstA production by *Y. enterocolitica* strains.

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Primary investigations on the sero-reactivity of various animal species to major serogroups of *Leptospira interrogans* in Morocco

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Due to the diversity of clinical signs and the difficulties of establishing a confirmatory laboratory diagnosis, leptospirosis remains poorly investigated, particularly in the developing world. In Morocco, a descriptive study of the seroprevalence of Leptospira infection in animals has never been undertaken so far. In order to fill this gap, the current study was conducted on a subset of animals in north-western Morocco as a preliminary step to the understanding of clinical and epidemiological patterns of animal leptospirosis in the country.

The study was conducted on 289 serum samples collected between January and April 2012 from dogs, cattle, sheep, goats and donkeys in Rabat-Salé, Khémisset (low land) and Oulmès (high land). All serum samples were serologically tested by the standard MAT using two representatives of *Leptospira biflexa* (Patoc and Andaman) and 14 reference strains of the most widespread *L. interrogans* serovars. The threshold was established at 1:20. When a given serum reacted to more than one serovar and, despite cross-reactivity of various serovars, positives to more than one serovar were recorded as many times. The overall seroprevalence to *Leptospira interrogans* in cattle, sheep, goats, dogs and donkeys was respectively 15%, 18%, 20%, 21 and 20%. The following serovars were unevently distributed among different animal species: Ballum, Sejroe, Bratislava, Australis, Icterohaemorrhagiae, Poi, and Canicola. Of the 7 serovars revealed by this study, Icterohaemorrhagiae was the only serovar which have been previously reported in clinical leptospirosis in humans in Morocco. The majority of positive sera were collected from low the land area. Most sera that proved negative to all pathogen serovars reacted with *L. biflexa* Patoc and Andaman, which implies a possible presence of new, unclassified Leptospira serovars in Morocco. These will be further investigated and isolation of Leptospiras will be soon attempted in the author's laboratory.

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Survivability of *Mycoplasma hyopneumoniae* on dry surfaces and dust

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Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is the causal agent of enzootic pneumonia (EP). Transmission of *M. hyopneumoniae* is via direct contact and aerosol spread (Stärk et al., 1998). Transmission via contaminated environments may also occur but little is known about the persistence and survivability of *M. hyopneumoniae* within the environment (Friis, 1973). The aim of this work is to investigate *in vitro* thesurvivability of *M. hyopneumoniae* on surfaces and dust under conditions representative of pig accommodation. *M. hyopneumoniae* (40µl) was dried out onto 6-well plates for 0,1,2,4,8 and 16 days, and then stored at three temperature ranges (4°C, 25°C and 37°C). Growth was assessed by serial dilution in 96-well plates, measuring colour changes units (CCU) per ml. The dilutions were grown in Friis medium, incubated at 37°C, and read after the growth period. The results indicate (**figure 1**) that dried *M. hyopneumoniae* was able survive up to eight days when stored at 4°C, but only two and one days at 25°C and 37°C respectively, this is in agreement with Friis, (1973) who showed that *M. hyopneumoniae* could not survive when dried for longer than eight days (20 - 25°C).

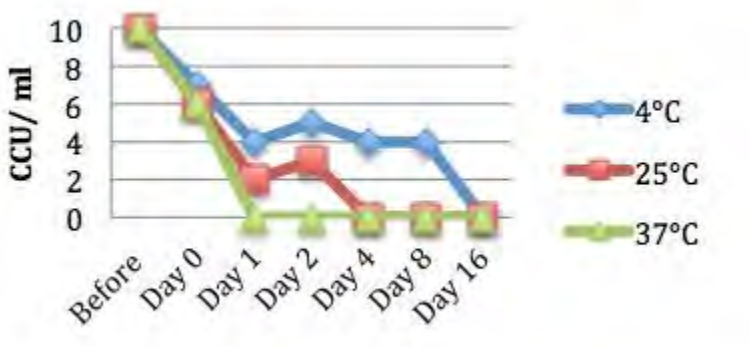

Figure 1: The survivability of *M. hyopneumoniae* when dried at 4°C, 25°C and 37°C from 0 to 16 days.

Goodwin, (1985) explored the survival of *M. hyopneumoniae* on filter paper and cloth, but not on dust, a known risk factor of EP (Stärk et al., 1998). Here, *M. hyopneumoniae* was deposited onto autoclaved pig barn dust, allowed to dry and serial dilutions were performed. The results indicated that dust inhibited *M. hyopneumoniae* growth from dilutions of 10^{-1} and 10^{-2}, but continued to grow at higher dilutions. Stärk et al., (1998) found that dust inhibited *M. hyopneumoniae* detection by nested PCR, similarly to the culture findings seen here. In conclusion, *M. hyopneumoniae* can survive for substantial periods on plastic (polystyrene) surfaces and on dust, even though dust may have an inhibitory effect. Investigations into novel control strategies, which address such environmental contamination, are warranted in the future.

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Nucleases and nucleotide transport operons of pathogenic mycoplasmas

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Mycoplasmas are obligate parasites with minimal genomes. As a consequence of their extended history of reductive evolution they have lost much biosynthetic capacity and are dependent on exogenous sources of most complex nutrients. However, the mechanisms that they use to acquire these biosynthetic substrates are poorly understood. Among these complex molecules that they must scavenge from their environment are nucleotides, and it is likely that efficient derivation and transport of these nucleic acid precursors into their cytoplasm is essential for virulence. We have used a variety of approaches to identify and characterise genes that are likely to be involved in this process and to establish the activity of their products in several pathogenic mycoplasma species of animals, including *Mycoplasma gallisepticum, Mycoplasma hyopneumoniae* and *Mycoplasma bovis*. In earlier studies cloning and site directed

mutagenesis in *E. coli* allowed us to identify a lipoprotein nuclease in *M. hyopneumoniae* (Mhp379)[1], while in recent transposon mutagenesis we have identified a distinct, major lipoprotein nuclease of *M. bovis* (MBOVPG45_0215). Gene synthesis and expression in *E. coli* have enabled us to demonstrate that the function of the product of a gene (*mslA*) shown to be required for virulence in *M. gallisepticum* binds oligonucleotides. In many mycoplasmas this gene is located in an operon that contains both the orthologue of the lipoprotein nuclease of *M. hyopneumoniae* and also genes predicted to encode an ABC transporter. Our findings suggest that the oligonucleotide binding protein and the nuclease act in concert to facilitate degradation of host nucleic acids to nucleotides for transport into these pathogenic mycoplasmas, thus enhancing their pathogenicity.

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Correlation between clinical manifestations of footrot in Swedish sheep flocks and *Dichelobacter nodosus* strains

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Ovine footrot is a contagious disease that affects the feet of sheep in which *Dichelobacter nodosus*, a Gram-negative anaerobic bacterium, is the main causative agent. In Sweden, diagnosis is based on clinical examination of sheep feet where the feet are scored according to the Australian system for scoring footrot[1], and with the definition of footrot as score ≥2 lesions. Diagnosis is often complemented by detection of *D. nodosus* by real-time PCR.

Very few of the *D. nodosus* strains isolated from more severe cases of footrot in Swedish flocks meet the criteria required for virulence in this case, the gelatin gel test[2], which leaves us questioning why the diagnostics is not consistent with the clinics for our conditions. Hence the aim of this study was to try and find a correlation between the clinical manifestations of footrot and *D. nodosus* strain differences by the comparison of strains isolated from flocks with different clinical status.

Eighteen sheep flocks were classified into four categories (A-D) according to their clinical status where categories A and B were clinically healthy and C and D affected by footrot. About 900 swab samples, 50 from each flock, were analyzed for *D. nodosus* by a specific real-time PCR assay[3] and by culturing as seen in Table 1. To summarize, *D. nodosus* was frequently found in all of the footrot affected flocks (*n*=8) but only found in three out of 10 clinically healthy flocks. The obtained *D. nodosus* strains are under characterization and the swab samples are being analyzed for the presence of other relevant bacterial species than *D. nodosus*.

Table 1 – *Dichelobacter nodosus* PCR positive samples found in 18 sheep flocks belonging to different categories (A-D) depending on their clinical manifestation of footrot

Flock	Category*	Positive samples	Positive samples in (%)	Samples in total
1	A	0	0	50
2	A	0	0	50
3	A	0	0	50
4	A	0	0	50
5	A	5	10	50
6	B	0	0	50
7	B	0	0	49
8	B	0	0	50
9	B	4	8	50
10	B	36	72	50
11	C	27	54	50
12	C	38	76	50
13	C	44	88	50
14	C	47	94	50
15	D	34	68	50
16	D	41	85	48
17	D	47	94	50
18	D	49	98	50

*The categories were as follows; A= mainly sheep with score 0 lesions but a few sheep with score 1 lesions is allowed, B=many sheep with score 1 lesions but none with more severe lesions, C=a few sheep with score 2 lesions or more severe lesions (3-5), and D=many sheep with score 2 or more severe (3-5) lesions.

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Effect of human intestinal microbiota and *Bacteroides thetaiotaomicron* on *Escherichia coli* O157:H7 transcriptomic response: multiple aspects of EHEC adaptation.

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Enterohemorrhagic *E. coli* (EHEC) is a human food-borne pathogen responsible for large outbreaks in developed countries. EHEC infections can lead to hemorrhagic colitis and to the life-threatening haemolytic and uremic syndrome mainly through the production of Shiga toxins in the gut.. Contributing to its protective effect against pathogen colonization, the human gut microbiota produces soluble factors that inhibit the expression of Stx2 encoding genes by EHEC. In particular, *Bacteroides thetaiotaomicron*, a predominant symbiont of the human gut microbiota, was shown to produce such an inhibitory factor. To increase our understanding of the influence of the human gut microbiota on EHEC transcriptome, we investigated the gene expression pattern of EHEC O157:H7 cultivated in the caecal content of germ-free rats and rats associated with the human gut microbiota of a healthy subject or *B. thetaiotaomicron* alone, using Affymetrix GeneChip *E. coli* 2.0 genome arrays and qRT-PCR. We showed that the human gut microbiota has a wide effect on EHEC O157:H7 metabolic gene expression by down-regulating several carbohydrate utilization pathways and increasing the expression of genes required for the catabolism of metabolites encountered in the digestive tract. In addition, the human gut microbiota down-regulates a large number of EHEC virulence genes involved in attaching and effacing lesion formation, including genes from the locus of enterocytes effacement (LEE) pathogenicity island and genes encoding non-LEE Type III secreted effectors. Interestingly, we also demonstrated that *B. thetaiotaomicron* strongly participates to this inhibitory effect. These results show that human gut microbiota and *B. thetaiotaomicron* negatively influence the expression of many virulence genes in EHEC. This could help developing preventive and therapeutic approaches to limit EHEC gut colonization.

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Screening of phytogenics against *Lawsonia intracellularis* with a flow cytometric viability assay

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Lawsonia intracellularis (LI) are Gram-negative intracellular pathogens that cause diseases in a wide range of animals. In pigs, LI are of economic importance as the cause of proliferative enteropathy (ileitis). While the acute form of ileitis can cause sudden mortality in finishing pigs, the chronic and subclinical forms can drastically impair the growth performance of grower pigs. For the assay described here, LI were obtained either from a live vaccine or purified from infected cell cultures, diluted in PBS and seeded in U-bottom 96 well plates. Either dilution series of samples or PBS (negative control) were added to the plates, which were then incubated for 30 min at 37 °C, 8% O_2 and 8.8% CO_2. Afterwards, the bacteria were stained with the BacLight viability kit (Invitrogen), and analyzed with an ACCURI C6 flow cytometer. Bacteria were distinguished from debris in a FSC/SSC-plot. Live and dead bacteria were distinguished by their fluorescence and gated in a green/red fluorescence plot to determine viability rates. The viability rates of sample-treated LI were compared with the mean viability of the control LI to calculate viability rates. The results of samples that showed a dose-dependent anti-bacterial effect were used to calculate EC_{50} values with a probit regression. Several phytogenic samples showed antibacterial activity. Among them silymarin and a chestnut extract performed best. *In vitro* screening assays like the one presented in this study shall be the basis for the development of a phytogenic feed additive for LI control.

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Seroprevalence of *Leptospira* spp. infections in cattle seropositive to *Brucella abortus* in oficial diagnostics tests

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Brucellosis and Leptospirosis are diseases that affect humans beings and domestic animals worldwide. In relation to the diagnosis of these two diseases, must take care, because it can bring a serious economic problem to ranchers, since animals with brucellosis, should be sacrificed, while animals infected with Leptospira can be treated. The objective of this study was to verify if exist seropositive cattle for leptospirosis, among seropositives for brucellosis in official tests. Were collected 214 blood serum samples from adult cattle of the region of Uberlândia, Minas Gerais State of Brazil, wich were subjected to the diagnostic screening test for brucellosis (Rose Bengal test) conducted at the Laboratory of Infectious Diseases, Faculty of Veterinary Medicine, Federal University of Uberlândia. Positive samples in Rose Bengal test, were referred to the confirmatory test 2-mercaptoethanol (2-ME) according to PNCEBT (Governamental Program) and microscopic agglutination test (MAT), to detect antibodies anti-Leptospira spp. Of the 214 samples taken from cattle of the region of Uberlândia, 52 (24.29%) were reactive in the screening Rose Bengal test, and of these, 33 (63.46%) were confirmed seropositives and 19 (36.53%) were negative in confirmatory diagnosis test of brucellosis, 2- Mercaphtoethanol (2-ME). Considering the samples confirmed by 2ME, 13 (39.39%) of these also reacted to the test of SAM. Thus, of the 52 positive cattle in the Rose Bengal test, 13 (25%) presented both, brucellosis and leptospirosis. Of the total samples, 162 (75.70%) showed negative results in Rose Bengal test, and among these, 109 (67.28%) were positive for one or more Leptospira interrogans serovars and 53 (32.71%) negative for leptospirosis. Those results, may be the from of a double infection, or vaccine reactions and even non specific reactions. This project

was financially supported by CNPq and Fapemig, to which we are grateful. We would also like to thank the veterinary doctors who donated the samples to this project.

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Brucella spp. DNA in the semen of seronegative bulls by polymerase chain reaction

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Brucellosis is a common zoonosis in many parts of the world, which mainly infect cattle, swine, goats, sheep, dogs and also wildlife populations. Most Brucella species are also found in semen. Males can shed these organisms for long periods or lifelong. The importance of venereal transmission varies with the species. Cryopreservation offers the advantage of allowing for the storage of semen for long periods and facilitating its distribution, but it may also can act as a vehicle of distribution of numerous pathogens. An agent that is transmissible through semen is Brucella abortus, which causes orchitis and may be associated with vesiculitis and epididymitis. However many animals can be asymptomatic carriers, which is why cattle breeders do not see brucellosis as a cause of infertility and decline in reproduction rates. The purpose of this work was to detect the presence of Brucella spp. in semen samples from bulls used both for natural mating and as semen donors, using the polymerase chain reaction (PCR) technique. Semen samples from 88 reproductively mature bulls were screened to detect the presence of Brucella spp. by polymerase chain reaction. Twenty-seven samples were found to be positive, underscoring the importance of researching brucellosis in males and the need for greater care in the selection of spermdonating bulls for semen centres.

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A clonal outbreak of upper respiratory disease in horses caused by *Streptococcus equi* subsp. *zooepidemicus*

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Streptococcus equi subsp. *zooepidemicus* (*S. zooepidemicus*) is considered a commensal of the upper airways in horses and an opportunistic pathogen causing respiratory disease. *S. zooepidemicus*, unlike *S. equi* subsp. *equi* (*S. equi*) that causes strangles, displays wide genetic variation. The importance of this genetic diversity to the pathogenicity of different strains is currently being investigated, as it appears that strains of *S. zooepidemicus* can differ in virulence. Whether certain strains of *S. zooepidemicus* can cause upper respiratory disease as a host-specific pathogen in horses, and if there are certain genogroups of *S. zooepidemicus* that are more virulent than others is of major clinical importance. Here, we describe an outbreak of upper respiratory disease that could be related to one strain of *S. zooepidemicus*.

An outbreak of upper respiratory disease occurred in a herd of 17 Icelandic horses. Twelve of the 17 horses were selected for bacterial sampling during the outbreak, of which ten displayed clinical signs of respiratory disease, while two horses were clinically healthy. Paired serum samples were collected from all 17 horses. Bacterial sampling was repeated twice after the outbreak had subsided. Samples were cultured, analysed by real-time PCR for *S. zooepidemicus* and *S. equi,* and genetically differentiated by sequencing of the SzP protein gene and MLST. Mitogenic activity and the presence of *S. zooepidemicus* superantigens SzeF, SzeN and SzeP were also investigated. Serum samples were analysed for antibodies against *S. equi* and common viral respiratory pathogens.

All horses were positive for *S. zooepidemicus* during the outbreak. All horses with clinical signs of disease carried the same strain, while the healthy horses carried other strains of *S. zooepidemicus*. None of the horses were positive for *S. equi.* Bacteriological, molecular and serological analyses support that the isolated *S. zooepidemicus* strain was likely to be responsible for the disease outbreak.

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Successful transformation of *Mycoplasma hyopneumoniae* strains with self-replicating plasmids and optimisation of transformation conditions

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Mycoplasma hyopneumoniae is the primary aetiological agent of enzootic pneumonia, a chronic respiratory disease of considerable economic importance to pig industries around the world. Advances in our understanding of the pathogenesis of this bacterium at the molecular level have been hampered by a lack of genetic tools to allow the manipulation of the mycoplasma genome. We have recently reported the first successful transformation of M.hyopneumoniae with self-replicating plasmids containing the predicted M.hyopneumoniae origin of replication (oriC) and the tetM determinant, conferring tetracycline resistance to transformed bacteria. We have used our oriC plasmids to optimise the conditions required for transformation of the 232 strain of M.hyopneumoniae by electroporation and the successful culture of transformants in solid and liquid medium. Using our optimised conditions, we have determined the susceptibility of further isolates of M.hyopneumoniae to transformation. Self-replicating plasmids have been

successfully used to generate targeted gene mutants in other mycoplasmas by homologous recombination. With this in mind, we have determined the stability of our oriC containing plasmids over serial passages in liquid culture. In an attempt to reduce the integration of plasmid DNA into the mycoplasma genome, we have determined the minimal size of the oriC region that allows plasmid maintenance as extrachromosomal DNA. As an alternative strategy to reduce integration of plasmid DNA by homologous recombination, we have produced plasmids containing the oriC regions of phylogenetically related mycoplasma species and determined their ability to transform M.hyopneumoniae. It is anticipated that our plasmids will be useful tools in allowing the generation of targeted gene mutants in M.hyopneumoniae. Furthermore, in demonstrating the amenability of M.hyopneumoniae to transformation and in optimising the conditions necessary to do so, our work is an important step in the development of other strategies such as transposon mutagenesis, that will greatly assist our study of this pathogen.

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PCR-RFLP analysis of *Bordetella bronchiseptica* isolates from different animal species to detect the possible signs of host-adaptation

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Bordetella bronchiseptica (Bb) is involved in the aetiology of atrophic rhinitis of swine, kennel cough of dogs, bordetellosis of cats, and occasionally in human respiratory diseases. Bb causes remarkable economic losses in the large-scale pig farming; however, the source of infection remains frequently unknown. The Bb isolates (43 from pigs, 38 from dogs, 4 from cats and 7 from humans) of various geographical origins were examined by PCR-RFLP on coding region of fimA, flaA and cyaA to determine correlation between profiles and hosts, and to detect the possible source of pig illnesses in Hungary. The analysis of fimA resulted uniform bands in all examined strains. The pig isolates from Hungary were uniform on flaA (B type), while the foreign strains were diverse (A, B and C types). The dominant profile of strains of canine origins (89%) was type A. The human isolate from Hungary showed unique restriction patterns (type F), while the foreign isolates represented three types (A, C and D). The analysis of pig and cat isolates on cyaA resulted uniform profiles (A), only one foreign pig isolate showed type D. In the Hungarian canine isolates, the cyaA were not detectable, while the foreign isolates were variable (type A, B and C). The strains of human origin represented three types (A, B and D). Our results point to a possible connection between Bb flaA PCR-RFLP profiles and hosts. In Hungary, the signs of the host adaptation were recognizable, each hosts had different profiles. These findings confirm the utility of molecular genetic methods in epidemiological studies. The OTKA (K81690, K83332) and the Bolyai János Scholarship (BO/330/10) of the Hungarian Academy of Sciences sponsored this work.

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Environmental reservoirs of the footrot pathogen *Dichelobacter nodosus* and transmission dynamics of the disease

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Over 95% of sheep flocks in the UK have sheep with footrot, and 8–10% of sheep are affected at any one time. The disease presents with a range of severities, from mild interdigital inflammation (interdigital dermatitis), to separation of the hoof horn from the foot, all severities are associated with lameness. The causative agent, *Dichelobacter nodosus* (Dn) is an obligate anaerobe that can survive outside the host, suggesting that the environment plays a role in disease transmission. It is possible to detect *D. nodosus* strains in samples directly using a selection of targeted gene sequences based on 16S rRNA, *rpoD* and *pgr* the latter is based on the region encoding the polymorphic proline-glycine repeat (Pgr) protein, in addition a new typing method using MLVA has also been developed to establish strain diversity. Therefore the aim of this study was to investigate the transmission dynamics of Dn at parturition using molecular markers.

Foot swabs were collected from ten lambs at birth, and from the same lambs and their dam 5-13 hours later; bedding samples were also collected from the lambing pen at the second time point. DNA was extracted from all samples using standard techniques, and both nested and quantitative PCR (qPCR) was used to determine the presence and load of Dnin each sample.

Analysis of DNA indicated that Dn was undetectable on lamb's feet at birth; however, 5-13 hours later, the pathogen was detected by nested 16S PCR. The *rpoD* qPCR indicated a lower load on the feet of lambs compared to their dams, with the load ranging from 10^3 to 10^4 in lambs and 10^4 to 10^6 in ewes. Dn load was significantly higher in ewes than their lambs (Mann-Whitney U, p-value < 0.001). The environment was found to be a potential source of infection as Dn cells were detected in bedding. Further studies are in progress to determine environmental conditions affecting survival, preliminary work indicated that Dn survival was greater in dry soil (-kPa 210), compared to wet (0 kPa).

These results indicate that the environment plays a key role in the transmission of the pathogen between dam and offspring from a very early age. It appears that lambs are colonized by Dn when they are very young, and this may have implications for lambing management.

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Genetic diversity and epizootiology of chlamydiae prevalent among the domestic and feral avian species in Russia

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To study genetic diversity and occurrence of Chlamydophila psittaci, a total of 763 samples from different species of feral and domestic birds were examined using ompA gene based PCR. Among 74 positive samples (Psittaciformes-67, Columbiformes – 4 and

Passeriformes- 3) two types of chlamydiae: C. psittaci and unknown Chlamydophila sp. were identified. A single isolate from crow of unknown Chlamydophila sp. was found phylogenetically intermediate between Chlamydophila species infecting avian and mammalian hosts. The ORF8–ORF1 region of the extrachromosomal plasmid was amplified for 12 parrot and 3 corvid isolates. A plasmid is often present in C. psittaci strains and the lack of an extrachromosomal plasmid is regarded as a characteristic feature of C. abortus strains The corvid isolates proved to lack an extrachromosomal plasmid. The analysis of the rRNA intergenic spacer support the position of one crow isolate in the group of strains intermediate between C. abortus and C. psittaci. Chlamydiosis was detected among 7.2% dead birds as well 16% clinically normal birds. It was observed that the genotype A is the most frequent among parrot chlamydiae.

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Complete sequence of two virulence-associated plasmids from necrotic enteritis isolates *Clostridium perfringens* type A and comparison with *C. perfringens* plasmids

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Clostridium perfringens is an important pathogen of humans and animals. Certain strains of type A isolates cause necrotic enteritis (NE) in broiler chickens, a common infection. A major breakthrough in understanding virulence in NE isolates of *C. perfringens* was the demonstration that a new toxin, NetB, is critical for development of the disease. A netB-positive (pNetB-NE10) and a cpb2-positive plasmid (pCpb2-CP1) obtained from NE isolates were shown to be conjugative, and the plasmids were completely sequenced. Both plasmids possessed the large conjugative region characteristic of *C. perfringens* conjugative plasmids (CpCPs). Comparative genomic analysis of nine CpCPs, including the two plasmids described here, showed extensive gene rearrangements including pathogenicity locus and accessory gene insertions around rather than within the backbone region. The pattern that emerges from this analysis is that the major toxin-containing regions of the variety of virulence-associated CpCPs are organized as complex pathogenicity loci.

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Clonal relationships between human and equine *Streptococcus equi* ssp. *zooepidemicus* isolates

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Streptococcus equi ssp *zooepidemicus* (Sez) is a zoonotic pathogen for people in contact with horses. Human infections associated with Sez are however rare, compared to the common presence of Sez in horses. Here, we compared Sez isolates from severe human infections to those isolated from horses to obtain insight into the epidemiology of Sez. Within half a year in 2011, three unrelated cases (Cases 1 to 3) of severe, disseminated Sez infections occurred in men working with horses in eastern Finland. Nasal swabs from horses related to Case 1 (stable A) and Case 2 (stable B) were collected, and Sez were isolated. The contact stable of Case 3 was not sampled. Altogether 14 Sez isolates were analysed: three from Cases 1 to 3, five from stable A, one from stable B, and five from four other stables in the region. Only three equine isolates were from diseased animals. The isolates were analysed using pulsed field gel electrophoresis with SmaI (SmaI-profile), multi locus sequence type analysis (MLST) and sequencing a part of the SzP protein gene (*szP* type). The isolate from Case 1 was identical with all methods to an equine isolate from stable A. The isolate from Case 2 was dissimilar to the equine isolate from contact stable B, but the MLST and *szP* types were identical to those of Case 1. The SmaI-profiles of the strains from Cases 1 and 2 differed by 4 bands. The isolate from Case 3 had the same *szP* type and an almost identical SmaI-profile as an isolate from an unrelated stable. In conclusion, all Sez isolates from humans were closely related to those from horses. The two human isolates were clonally closely related. The results emphasize that Sez transmitted from horses can lead to severe infectious disease in humans.

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Aeromonas salmonicida subsp *salmonicida* genomic diversity analysis: identification of two novel genetic elements potentially related to virulence

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Aeromonas salmonicida subsp. *salmonicida* is the causative agent of furunculosis, which causes septicaemia and necrosis in salmonids[4] . Although furunculosis is an important issue in aquaculture, the genomic diversity among strains of this bacterium remains to be analyzed in details. Previous studies, focusing on plasmid composition and PFGE patterns, showed that "typical" *A. salmonicida* strains are genetically homogenous[1 3]. However, it does not explain the variation in virulence among strains. *A. salmonicida* "typical" strain A449 have been fully sequenced in 2008[5]. Unfortunately, this strain is not virulent when assayed in the infection model of the alternative host *Dictyostelium discoideum* amoeba[2]. Thus, sequencing of highly virulent *A. salmomicida* strains should allow the identification of new genetic elements associated with the pathogenesis of these bacteria. Whole genome sequencing of two virulent strains, 01-B526 (Province of Quebec, Canada) and JF2267 (Switzerland), was performed by Roche/454 pyrosequencing method. A supplementary 51 kb element was identified in strain 01-B526. This phage-like element, hence named phiAS526, may contain potential virulence factors. At least one segment of phiAS526 is widespread among *A. salmonicida* strains while another portion seems specifically found in strains in Province of Québec. Moreover, an additional 6 kb element was present in strain JF2267. This element contains genes related to pilus assembly. Thus, next generation sequencing brings light on *A. salmonicida* genetic diversity and quickens research on this ichtyopathogen as demonstrated by the discovery of two new genetic elements potentially involved in bacterial virulence in the current study.

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Studies on virulence-associated proteins of the horse pathogen *Rhodococcus equi.*

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Rhodococcus equi is a soil-borne facultative bacterial pathogen which causes a severe pyogranulomatous pneumonia in young foals. Despite the availability of antibiotics, mortality rates are high and there is currently no vaccine licensed to prevent disease caused by *R. equi*. The *R. equi* bacterium grows rapidly on simple organic acids which are abundant in the faeces of grazing animals. The major route of *R. equi* infection is by inhalation of contaminated faeces particles into the lungs. Here the bacteria enter alveolar macrophages and proliferate, evading normal macrophage killing mechanisms by processes currently unknown. The ability of *R. equi* to survive in this environment is linked to the possession of a virluence plasmid encoding a family of virulence-associated proteins (VapA-VapH). These proteins are related by sequence and are unique to *R. equi*. VapA has been found to be highly immunogenic in foals and is a defining member of this protein family. Amino acid sequence analysis of the proteins predicts a common overall structure connected to a disordered N-terminal region of variable length. In VapA this region constitutes 30% of the protein while in VapD it is only 13%.

Studies to investigate the structural properties of the virulence-associated proteins will be presented. VapA, VapG and VapD were overexpressed and purified for crystallisation trials and NMR studies. Preliminary crystals were obtained for VapD. Solution studies using SEC-MALS revealed that VapA forms dimers while VapD and VapG are monomeric. An N-terminally truncated form of VapA loses some of its capacity to form dimers, suggesting a role for the N-terminus in dimer formation. These studies provide evidence that the variable N-terminal region confers different properties to the proteins which may relate to different functions *in vivo*.

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