Prato Conference on the Pathogenesis of Bacterial Diseases of Animals

6th - 9th October 2010 Monash Prato Centre

Prato, Italy

Program and Abstracts

ARC Centre of Excellence in Structural & Functional Microbial Genomics

Australian Government Australian Research Council

Intervet Schering-Plough Animal Health

Pfizer Animal Health

MONASH University Medicine, Nursing and Health Sciences

CSIRO

INDEX

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Ben Adler - Monash University

Glenn Browning - University of Melbourne

Joachim Frey - University of Bern

Rob Moore - CSIRO Livestock Industries

John Prescott - University of Guleph

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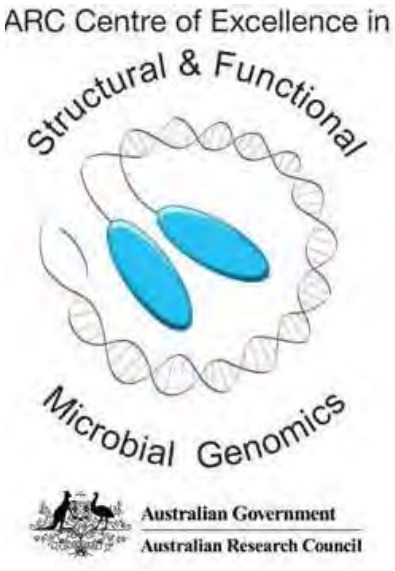

Australian Research Council Centre of Excellence in Structural and Function Microbial Genomics

The Australian Research Council (ARC) Centre of Excellence in Structural and Functional Microbial Genomics is a unique, academic, research centre elucidating key aspects of microbial pathogens and the hosts they infect. The Centre comprises a team of leading Australian researchers in the fields of bacterial genomics and genetics, bioinformatics, biochemistry, cell biology, proteomics, structural biology and vaccine immunology. Applied aspects of the Centre's research include the development of veterinary vaccines and the identification of antimicrobial drug targets. The Centre is based at Monash University, Clayton, Victoria and works in partnership with a range of institutions in Australia and internationally.

Contact: Professor Ben Adler, Director
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CSIRO Livestock Industries
The Commonwealth Scientific and Industrial Research Organisation (CSIRO) is Australia's national science agency and one of the largest and most diverse research agencies in the world. As a leading global research enterprise, CSIRO, through the Division Livestock Industries, provides innovative solutions and quality services for Australia's livestock and allied industries to enhance their sustainability, competitiveness and prosperity.

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Monash University, Faculty of Medicine, Nursing and Health Sciences
Monash University has an established international reputation for high quality innovative research. The Faculty of Medicine, Nursing and Health Sciences is the University's largest research faculty, with many world-class researchers working across a range of disciplines, from laboratory-based medical science to applied clinical, social and public health research. Ground breaking discoveries in the Faculty have contributed to advances in many areas including *in vitro* fertilisation, human stem cells, structural biology, cardiovascular physiology, drug discovery, functional genomics, infectious diseases, inflammation, neurosciences and mental health.

Contact: Professor Ian Smith, Pro Vice-Chancellor (Research and Research Infrastructure)
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Pfizer Animal Health
Pfizer Animal Health Research and Development
Now with a richer pipeline of biological and pharmaceutical products, Pfizer Animal Health is expanding its worldwide leadership role in animal health research focused on meeting unmet needs in veterinary care. Pfizer invests an estimated $300 million annually in veterinary R&D from major global centres in Australia, Belgium, India, Spain, the United Kingdom, and the United States.
Areas of R&D Focus
Livestock; Antimicrobials, Antiparasitics, Growth and production enhancement, Metabolic Disease, Respiratory and reproductive vaccines, Food Safety vaccines, Mastitis vaccines, Enteric vaccines, Parasitic vaccines
Companion Animals; Antimicrobials, Antiparasitics, Pain and Inflammation, Metabolic Disease, Cancer, Skin diseases/allergies, Infectious disease and parasitic vaccines, Internal medicine, Enteric vaccines
Pfizer Animal Health also is building a diverse and innovative portfolio and is actively seeking external opportunities for technologies, biologics and compounds in several therapeutic areas, especially those with unmet medical needs.
Areas of Interest
Allergy / Antipruritics, Antiparasitics, Appetite Regulation / CNS, Endocrine / Reproduction / Performance, Genetics, Immunomodulation, Infectious Diseases – Prevention and Therapy Infectious Diseases – Therapy, Oncology, Pain & Inflammation / Musculoskeletal Disease, Technologies, including improvements in the delivery of medicines and vaccines.

Contact: Dr Andy Allen
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BioX Diagnostics
BioX Diagnostics was created in 1990 under the name BioX. The core business of the company was then distributing diagnostic kits and kits for detection of illicit products, such as anabolics. Later, the company benefited from reseach and development from State Institutes and Universities, to develop their own products.
In 1998, the company moved from Brussels to improve the well being of it employees and changed name to BioX Diagnostics.
In 2003, the company was bought by the current owner, Dr Annita Ginter, who was then active on the commercial part of the business.
BioX Diagnostics' research and development team is highly experienced, for it has been working on the development of veterinary diagnostic tests for more than 20 years. Its development structure is flexible, enabling it to develop new reagents and products quickly and easily.
What's more, BioX Diagnostics' veterinarian surgeons enable the company to remain very close to its customers. As a result, BioX Diagnostics is able to be considered as your partner and your direct scientific and technical assistant.

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DELEGATE INFORMATION

THE ORGANISER'S OFFICE – ASN EVENTS

The organiser's office is located in the foyer on the first floor of the Monash University Prato Centre. Any enquiries can be directed to ASN staff at the organiser's office.

The Conference office hours are:

Wednesday 6 October:	3:30 pm – 7:30 pm
Thursday 7 October:	7:30 am – 10:30 am
Friday 8 October:	8:00 am – 10:30 am
Saturday 9 October:	8:30 am – 10:00 am

WHAT YOUR REGISTRATION INCLUDES

Delegate and student registrations include:

- Access to all sessions
- Delegate papers
- Morning, afternoon tea and lunches
- Welcome function on Wednesday night

SOCIAL PROGRAM

Welcome Drinks: On the first night (Wednesday 6 October), welcome drinks will be held on the terrace from 6:15 – 8:00 pm.

Conference Dinner: On the third night (Friday 8 October), the Conference Dinner will be held from 7:00 pm – 9:00 pm 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$120) 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 6:00 pm from Piazza Santa Maria delle Carceri for the one hour journey to Villa La Ferdinanda. The group will meet at 5:45pm at Monash Prato and walk together to the Piazza.

For the return journey, the bus will depart Villa La Ferdinanda at 10:30 pm, arriving in Prato around 11:30 pm. The bus will drop delegates at three drop off points: Hotel Datini, Hotel Milano and Hotel San Marco (for Accanto al Centro Apartments, Giardino Hotel).

SPEAKER PREPARATION INSTRUCTIONS

All speakers are using the same conference room "Salone Grollo". The audio-visual equipment is being supplied but no technician is on hand. Please pre-load **ALL** talks to the common laptops (a PC is linked to the projector in the room), preferably early on the day of your session. Mike Pickford from ASN will be on hand each morning to load the talks if you can arrive first thing.

DISPLAYING YOUR POSTER

Posters will be displayed on panels in the Lounge Area. There are two poster sessions - one on the afternoon of Thursday 7 October and one on the afternoon of Friday 8 October. Posters for Session 1 must be mounted by 8:30am on Thursday morning and removed immediately after the poster session. Posters for Session 2 must be mounted by 8:30am on Friday morning and also removed immediately after the poster session. Poster authors are requested to locate their abstract number for correct positioning. 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 organiser's office for additional supplies of velcro.

EMAIL AND INTERNET ACCESS

There are three computer rooms available to visitors to the Monash University Prato Centre. All computers are networked with high speed internet access. All are connected to the networked printers. Extensive wireless internet access is also available throughout the building. A password will be needed and this will be provided on site.

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 Cruoi, Acting Centre Manager, Monash University Prato Centre	+39 0574 436921
Hotels:	
Hotel Datini, Viale Marconi, 80-59100, Prato	+39 0574 562348
Hotel San Marco, Piazza San Marco, 48-59100, Prato	+39 0574 21321
Accanto Al Centro Apartments, viale Piave, 37-59100, Prato	+39 329 1581 789
Art Hotel Milano, Via Tiziano, 15-59100, Prato	+39 0574 23371
Giardino Hotel, Via Magnolfi, 2/4/6 – 59100, Prato	+39 0574 26189
Hotel Flora, Via B. Cairoli, 31 - 59100, Prato	+39 0574 33521
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 are 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

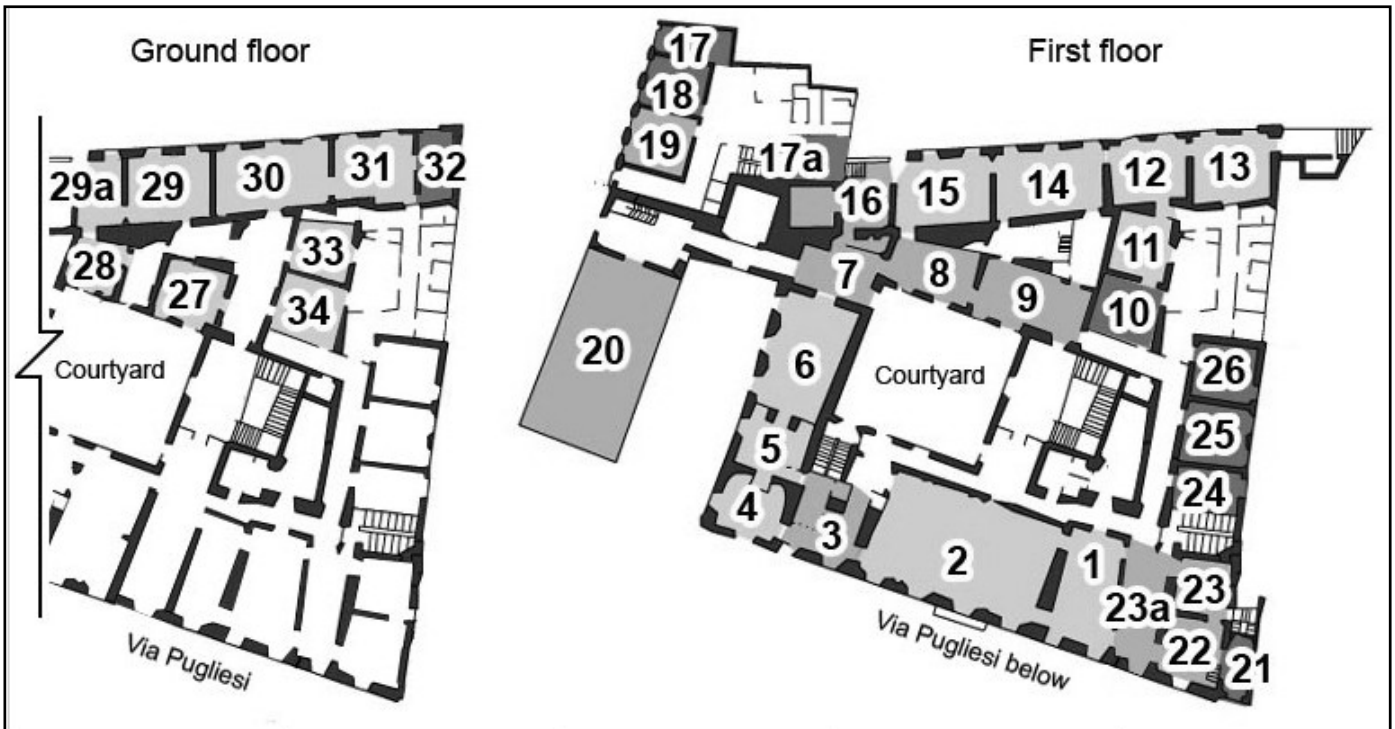
It is impossible to summarise all of the cultural activities Florence has to offer. The best way to find out is to go to the tourist information office.

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

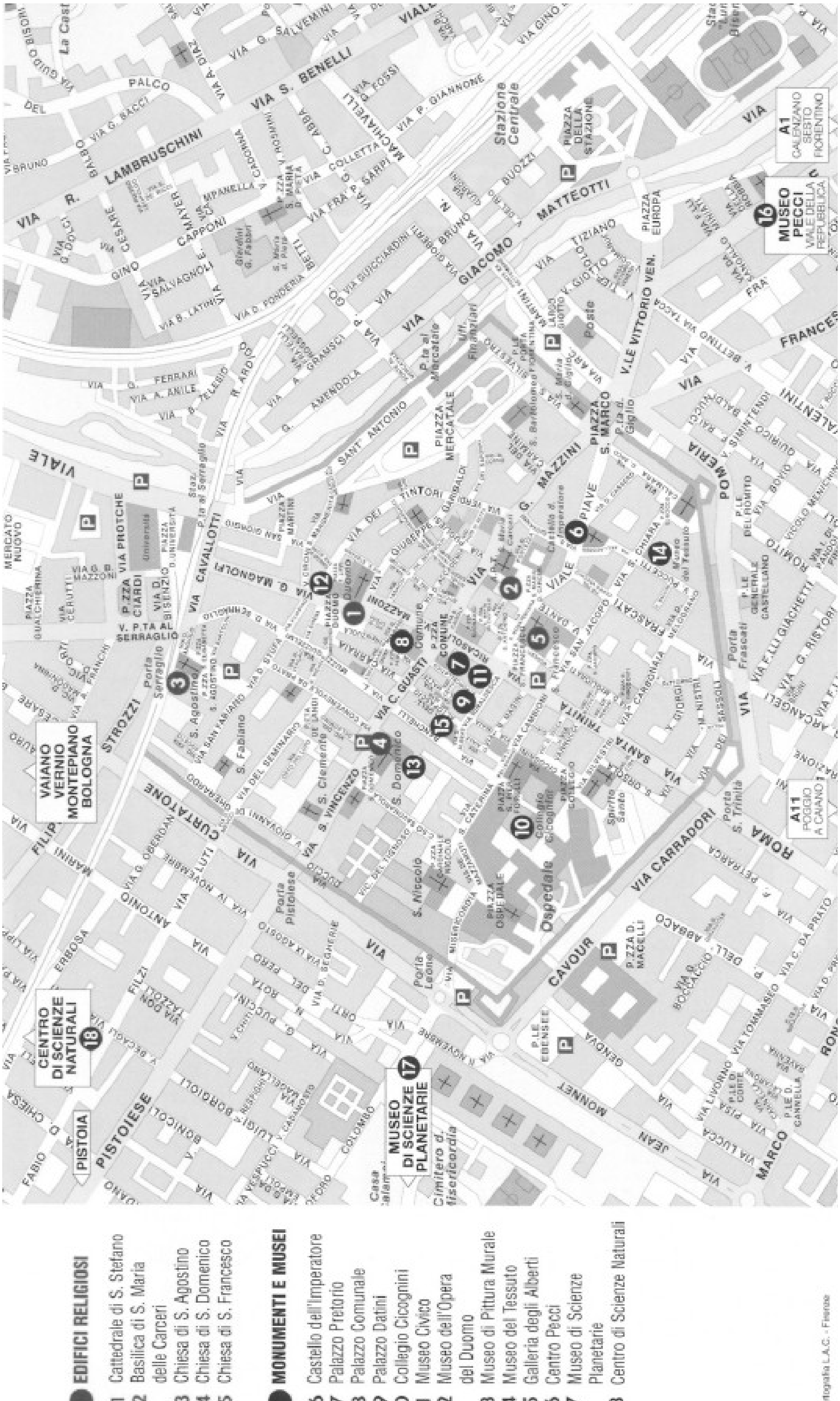
You can also browse on the internet. Try http://www.aboutflorence.com or http://www.studentsville.it.

MAP OF MONASH UNIVERSITY PRATO CENTRE



Offices	Teaching / conference	Computer labs	Recreational areas	Studio space
10 Prato staff office	1 Lobby reception	19 Computer lab 3	3 Piccolo Bar	11 Studio 1
17 Visiting Professor 2	2 Grollo room	22/23 Computer lab 1	7 Main Bar	12 Studio 2
17a Visiting Professor 3	4 Breakout lounge 2	23a Computer lab 2	8 Cafe Lounge	13 Studio 3
18 Visiting staff and postgraduates	5 Breakout lounge 1		9 Reading room	
21 Visiting Professor 1	6 Sala Veneziana		16 Kitchen	
24 Prato staff office	14 Sala Toscana		20 Terrace	
25 Prato staff office	15 Sala Giochi			
26 Prato staff office	27 Seminar 4			
32 Visiting Professor 4	28 Studio 6			
	29, 29a Seminar 1 and annexe			
	30 Seminar 2			
	31 Seminar 3			
	33 Studio 5			
	34 Studio 4			

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

INVITED SPEAKERS

Ben Adler
Monash University, Clayton, VIC, Australia
Professor Ben Adler is a Professor of the Department of Microbiology at Monash University. In additional to his managerial role as Centre Director he is recognised internationally for his work on bacterial pathogens, especially *Leptospira* and other spirochaetes, *Pasteurella* and *Shigella*. His area of scientific expertise is in the application of genomics to elucidate molecular mechanisms of bacterial pathogenesis.

Glenn Browning
University of Melbourne, Parkville, VIC, Australia
Glenn Browning is currently Professor in Veterinary Microbiology at The University of Melbourne. He graduated with a Bachelor of Veterinary Science from The University of Sydney in 1983 and spent a year as a clinical officer at the Rural Veterinary Centre in Camden, then completed his PhD on equid herpesviruses at The University of Melbourne in 1988, and spent three years as a postdoctoral scientist at the Moredun Research Institute, Scotland, working mainly on infectious causes of diarrhoea in foals. Since 1991 he has been a member of academic staff at the School of Veterinary Science at The University of Melbourne. His research interests extend across bacterial and viral pathogens of animals and humans, with a particular focus on understanding their pathogenesis and epidemiology as a route to development of improved control measures, including vaccines, diagnostic tests and changes in management. His current interests include mycoplasmoses, rhodococcal pneumonia, colibacillosis, and respiratory and immunosuppressive viral diseases.

Gadi Frankel
Imperial College London, London, United Kingdom
Gadi Frankel is currently the Professor of Molecular Pathogenesis at Imperial College, London. Some of his recent appointments include Prof. Division of Cell and Molecular Biology, Imperial College London, Reader for the Department of Biological Sciences, Imperial College London and Lecturer for the Department of Biochemistry, Imperial College, London.

Joachim Frey
University of Bern, Bern, Switzerland
Joachim Frey, studied chemistry and biochemistry at the Universities of Geneva and Uppsala (Sweden) and earned his PhD in Molecular Biology at the University of Geneva in 1980. He worked as a research fellow at the Max Planck Institute in Berlin and the University of Geneva on plasmid incompatibility and genetic engineering of soil and water bacteria. In 1986 he was an invited Professor at the University of Québec at Montréal, Canada. Since 1987 he has led the research group on molecular mechanisms of bacterial pathogenicity and vaccine developments at the Institute of Veterinary Bacteriology, University of Bern where became a Professor in 1996. Since 2000 Frey has been full Professor and Director if the Institute of Veterinary Bacteriology at the Vetsuisse Faculty of the University of Bern. He was Dean of the Faculty from 2004 - 2007.
Frey's research interests are the molecular mechanisms of bacteria that are pathogenic for animals, including *Pasteurellaceae*, where he detected RTX toxins as predominant virulence attributes of *Actinobacillus pleuropneumoniae, Mycoplasma* species where he unravelled the uptake mechanism and metabolism of glycerol as a major virulence attribute of virulence of *M. mycoides* subsp. *mycoides* SC; of, and pathogenic *Aeromonas* species where he detected Type III secretion as the central virulence attribute of A. *salmonicida* subsp. *salmonicida*, the etiological agent of furunculosis of salmon, trout and char. He is president elect of the international organisation of Mycoplasmology, IOM, member of the international committee on systematics of prokaryotes, subcommittee on the taxonomy of Mollicutes and member of the Swiss Commission of Biological Safety.

Carlton Gyles

University of Guelph, Canada

Carlton Gyles is Professor Emeritus in the Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada. He has conducted research on disease-producing *E. coli* and has edited books on *E. coli* in Animals and Humans and on Pathogenesis of Bacterial Infections in Animals. He is an Honorary Diplomate of the American College of Veterinary Microbiologists(ACVM), winner of the Canadian Society of Microbiologists Roche Award for career achievement in Microbiology in 2005; Fellow of the Canadian Academy of Health Sciences, and the ACVM Distinguished Veterinary Microbiologist in 2008. He is Editor of The Canadian Veterinary Journal and Animal Health Research Reviews.

Henk Haagsman

Utrecht University, Utrecht, Netherlands

Henk P Haagsman received his PhD degree in 1983 at Utrecht University. In 1982 he started his work on the pulmonary surfactant system. In 1985 he was the recipient of the Constantijn en Christiaan Huygens Award. From 1986-1988 he worked at the University of California at San Francisco. In 1988 he started his surfactant protein group at Utrecht University. From 1998-2006 he was Professor of Meat Science at Utrecht University. In 2006 he was appointed Professor of Molecular Host Defence at the same university. Currently his main research interests are innate host defence and muscle stem cell biology. With respect to innate host defence his research focuses on two classes of molecules: (1) collectins and (2) host defence peptides (HDPs).

Glyn Hewinson

Veterinary Laboratories Agency, United Kingdom

Glyn Hewinson is Head of the TB Research Group at the Veterinary Laboratories Agency, Weybridge and also a Visiting Professor at Imperial College, London. Glyn graduated as a microbiologist from the University of Bristol and obtained a D.Phil. at Oxford University for his work on elucidating the mechanism of cephalosporin resistance in *Pseudomonas aeruginosa*. He then spent a number of formative years studying molecular pathogenesis as an MRC Post-doctoral Fellow in Professor Moxon's Department at the Institute of Molecular Medicine, Oxford before moving into veterinary research at the VLA. Glyn leads a multi-disciplinary team involved in the development of TB vaccines for badgers and cattle and improved diagnostic tests for TB in these species. He was the leader of the *Mycobacterium bovis* genome sequencing project - a three-way collaboration between VLA Weybridge, the Sanger Institute and the Institut Pasteur. The same consortia also sequenced the genome of BCG. Glyn's Group is responsible for genotyping British isolates of *M. bovis* for epidemiological purposes and have a keen interest in the population structure and evolution of *M. bovis*. Over the past few years his Group have become involved in a number of projects investigating bovine tuberculosis in the developing world. Glyn has acted as a consultant to range of international agencies on bovine and human tuberculosis and is currently a named OIE expert on bovine tuberculosis. Glyn has published over 200 scientific papers in peer-reviewed journals and is a Section Editor of *Tuberculosis* and an Editor of *Vaccine*.

Hiroshi Kiyono

University of Tokyo, Minato-ku, Tokyo, Japan

Hiroshi Kiyono is an Associate Dean and Professor, Department of Microbiology and Immunology, the Institute of Medical Science, the University of Tokyo. At same time, he is also holds an Adjunct Professorship at the University of Alabama at Birmingham (UAB) and a Clinical Professorship at Arizona State University in USA. He has had a distinguished career in studying the molecular and cellular mechanisms controlling mucosal immune responses to infection and inflammation in the both digestive and respiratory tracts. Dr. Kiyono obtained his dental degree (DDS) from Nihon University, Japan and Ph.D from UAB. His background as a dentist combined with extensive research experience in the field of mucosal immunology at UAB, Max-Plank Institute, Osaka University and now, the University of Tokyo make him exceptionally well qualified to discuss the current and future direction of mucosal immunology for the development of mucosal vaccine and mucosal immunotherapy for the control of infectious and immunological diseases. To reflect his scientific contribution, he has been listed in ISI Highly Cited Researchers' List since 2005. He is the past President of Society for Mucosal Immunology. For the recognition of his scientific contribution in the area of mucosal immunology and mucosal vaccine development, he received of several prestigious awards including NIH New Investigator Research Award, NIH Research Career Development Award, The 2nd Japanese Society for Vaccinology, Takahashi Award, and Hideyo Noguchi Memorial Medical Science Award. He has a total of 387 publications in peer review journals and edited a total of 20 books.

Bruce McClane

University of Pittsburgh, Pittsburgh, PA, United States

Bruce McClane is a Professor in the Department of Microbiology and Molecular Genetics at the University of Pittsburgh School of Medicine. His research focuses on the toxins causing *C. perfringens* enteric infections and enterotoxemias. This includes studies of toxin action, toxin genetics, toxin regulation and virulence contributions of toxins to *C. perfringens* disease. He is a recipient of a MERIT award from the National Institute of Allergy and Infectious Diseases of NIH, is a fellow of the American Academy of Microbiology and has served on several editorial boards and grant review panels.

Rob Moore

CSIRO Livestock Industries, Clayton South, VIC, Australia

Dr Rob Moore is a molecular biologist working at the Australian Animal Health Laboratory, CSIRO Livestock Industries, Geelong, Australia. His research group studies bacterial pathogenesis (*Clostridium perfringens, Campylobacter jejuni*) and host-pathogen interactions with a view to developing vaccines for use in the poultry industry. The group is also developing *Lactobacillus* and *E. coli* strains for use as live delivery vectors for recombinant proteins. His team is also investigating the biology behind differences in chicken productivity; in particular studying gene expression in the chicken gut and the structure of the bacterial microflora present in the gut. He is a Project Leader in the Australian Poultry CRC, an Adjunct Senior Lecturer in the Department of Microbiology, Faculty of Medicine, Monash University, is a member of the ARC Centre of Excellence in Structural and Functional Microbial Genomics and serves on the Australian Government's Gene Technology Technical Advisory Committee.

Lisa Nolan

Iowa State University, Ames, Iowa, United States

Dr. Nolan received a DVM and a PhD in medical microbiology from the University of Georgia under the mentorship of Dr. Richard E. Wooley. On graduation, she moved to Fargo, N.D., as an Assistant Professor of Veterinary & Microbiological Sciences at North Dakota State University. There, she taught pathogenic microbiology, food safety and other courses to undergraduate, graduate preprofessional and pharmacy students and advised prevet students and graduate students. During her 11 winters in Fargo, she rose through the ranks from assistant to full Professor and became the founding director of the Great Plains Institute of Food Safety, which fulfilled research, service and teaching missions and provided one of the country's most comprehensive academic programs in food safety. In 2003, she left NDSU for warmer climes to become Chair of the Department of Veterinary Microbiology and Preventive Medicine at ISU, a position she was proud to hold for 5 years. During part of this time, she also served as the Interim Chair of ISU's Department of Veterinary Diagnostic and Production Animal Medicine and Executive Chair of the college. In 2008, she moved into the Associate Dean of Academic and Student Affairs position, where she oversaw the college's professional program, and in 2009, she assumed her present role as Associate Dean of Research and Graduate Studies. Throughout it all Dr. Nolan has remained an active researcher, who delights in her interactions with her post docs, students, and fellow researchers, as they study pathogenic *Escherichia coli* in all its myriad forms.

John Prescott

University of Guelph, Guelph, Canada

John F. Prescott is a veterinary bacteriologist in the Department of Pathobiology, Ontario Veterinary College, University of Guelph. He has diverse interests in bacterial infections in animals, but is best known for work in the area of *Rhodococcus equi* pneumonia in foals and in promoting stewardship of antimicrobial drug use in animals. His more recent interests are in necrotic enteritis of chickens and diseases caused by type A *Clostridium perfringens*.

Julian Rood

Professor, Department of Microbiology, Monash University, Clayton, VIC, Australia

Julian Rood holds a Personal Chair in the Department of Microbiology at Monash University in Melbourne, Australia. He is a Chief Investigator of the ARC Centre of Excellence in Structural and Functional Microbial Genomics and leads a large research group that focuses on the genetics and pathogenesis of anaerobic bacteria, including *Clostridium perfringens* and *Dichelobacter nodosus*. He is a Past-President of the Australian Society for Microbiology and Fellow of the Australian Society for Microbiology and the American Academy of Microbiology.

Glenn Songer

University of Arizona, Tucson, AZ, United States

After more than 30 years on the faculty of The University of Arizona, Glenn Songer is now Boehringer Ingelheim Professor of Food Animal Infectious Disease in the Departments of Veterinary Microbiology and Preventive Medicine and Veterinary Diagnostic and Production Animal Medicine at Iowa State University. His interests are in enteric disease in food animals and humans, with a focus on those caused by clostridia.

Judy Stabel

United States Department of Agriculture, Ames, IA, United States

Dr. Judy Stabel received her BS and MS degrees in Animal Science (ruminant nutrition) from the University of Kentucky and her PhD in Nutrition/Biochemistry from North Carolina State University in 1987. She did her postdoctoral research training at the National Animal Disease Center in nutritional immunology and subsequently accepted a permanent position. Dr. Stabel is currently a Supervisory Research Microbiologist and Lead Scientist of the Johne's Disease Research Project in the Bacterial Diseases of Livestock Research Unit at the USDA, Agricultural Research Service, National Animal Disease Center, Ames, IA. The research unit is concerned with conducting basic and applied research on the etiology, prevention and diagnosis of diseases of domestic animals caused by bacterial agents. She leads a project concerned with improving methods for identification of subclinical infection in ruminants, elucidating host-pathogen interactions, and developing safe and effective preventative strategies for *Mycobacterium avium* subsp. *paratuberculosis* infection.

James Whisstock

Monash University, Clayton, VIC, Australia

Professor James Whisstock is an ARC Federation Fellow, leader of the NHMRC Program Grant on Protease Systems Biology and Chief Investigator on the ARC Centre of Excellence in Structural and Functional Microbial Genomics. James has published over 130 papers in the fields of proteases, protease inhibitors, structural biology and Bioinformatics. James's research includes understanding how proteins control medically important processes such as blood coagulation, neurotransmission and the immune response to bacterial and viral infection. Most recently, using structural and computational biology James has demonstrated that key human immune defense molecules such as perforin and complement C9 belong to an ancient family of toxins deployed by pathogenic bacteria to destroy tissue. These data, published in Science, suggest a mechanism for perforin and complement pore formation and provide a route to control the unwanted activity of these molecules in human diseases such as transplant rejection and diabetes.

PROGRAM

Wednesday, 6 October 2010

Registration
2:30 PM - 6:00 PM — Foyer

Welcome and Introduction
5:00 PM - 5:15 PM — Salone Grollo
Julian Rood (Chair)

Plenary Session **Sponsored by Intervet Schering-Plough Animal Health**
5:15 PM - 6:15 PM — Salone Grollo
Carlton Gyles
The Problem of Relevance in Pathogenesis Research *abs#001*

Welcome Reception
6:15 PM - 8:00 PM — Terrace

Thursday, 7 October 2010

Session 2: Animal Diseases **Sponsored by BioX Diagnostics**

8:30 AM - 9:50 AM Salone Grollo

Chair: Rob Moore

8:30am **Glenn Songer**
Clostridia in swine enteric disease: always sophisticated, often brutal *abs#002*

9:00am **Francisco Uzal**
Clostridium perfringens type C and *Clostridium difficile* combined enter-typhlo-colitis in foals *abs#003*

9:20am **Joanne Platell**
Cross-over of fluoroquinolone-resistant (FQr) extra-intestinal *Escherichia co**li*** isolates from dogs and humans in Australia *abs#004*

Tea/Coffee Break

9:50 AM - 10:20 AM Lounge Area

Session 3: Genomics and Molecular Epidemiology **Sponsored by the ARC Centre of Excellence**

10:20 AM - 12:20 PM Salone Grollo

Chair: Ben Adler

10:20am **Lisa Nolan**
Exploring ExPEC Virulence, Evolution, and Host Specificity Using Pathogenomic Approaches *abs#005*

10:50am **Glyn Hewinson**
Genome downsizing and metabolic remodelling in *Mycobacterium bovis* *abs#006*

11:20am **Victor Gannon**
Analysis of the core and accessory genome of bacterial pathogens *abs#007*

11:40am **Léa Indjein**
Molecular comparisons of *Campylobacter fetus venerealis* isolates from Australian cattle to identify virulent strains *abs#008*

12:00pm **Lida Omaleki**
Molecular epidemiology and phylogenetic diversity of *Mannheimia glucosida* strains associated with ovine mastitis in Australia *abs#009*

Lunch

12:20 PM - 2:00 PM Lounge Area

Session 4: Host-pathogen Interaction I **Sponsored by Pfizer Animal Health**

2:00 PM - 4:00 PM Salone Grollo

Chair: Judy Stabel

2:00pm **Gad Frankel**
Interaction of pathogenic E. coli with plant and mammalian cells *abs#010*

2:30pm **Thomas Hannan**
Early severe lymphocyte-dependent inflammatory responses to uropathogenic *E. coli* predispose to chronic and recurrent urinary tract infection *abs#011*

2:50pm **Bernhard Kaltenboeck**
The rate of apoptosis of $CD4^+$ cells regulates immunoprotection from chlamydial infection and disease *abs#012*

3:20pm **Thomas Inzana**
The contribution of *Histophilus somni* (*Haemophilus somnus*) lipooligosaccharide to bacterial virulence and host interactions *abs#013*

3:40pm **Albert Haas**
Rhodococcus equi - how to reprogram phagosome trafficking in macrophages *abs#014*

Poster Session 1
4:00 PM - 6:00 PM Lounge Area

Friday, 8 October 2010

Session 5: Mechanisms of Pathogenesis **Sponsored by Monash University**

8:30 AM - 10:30 AM Salone Grollo

Chair: Lisa Nolan

8:30am **John Prescott**
Backing both horses: *Rhodococcus equi* as a pathogen of macrophages and a soil organism *abs#015*

9:00am **Glenn Browning**
Pathogenesis of mycoplasmosis *abs#016*

9:30am **John Boyce**
Role of the *Burkholderia pseudomallei* type III secretion system cluster 3 (TTSS3) in intracellular survival, escape from host autophagy and virulence *abs#017*

9:50am **Charles Dozois**
The secretion, but not the synthesis, of catecholate siderophores contributes to the virulence of avian extra-intestinal pathogenic Escherichia coli *abs#018*

10:10am **Josee Harel**
Increased Pho regulon activation correlates with decreased virulence of avian pathogenic *Escherichia coli* *abs#019*

Tea/Coffee Break

10:30 AM - 11:00 AM Lounge Area

Session 6: Extracellular Pathogens and Toxins I **Sponsored by CSIRO Livestock Industries**

11:00 AM - 1:00 PM Salone Grollo

Chair: Glenn Songer

11:00am **Bruce McClane**
New insights into the pathogenesis of *Clostridium perfringens* ype B, C and D diseases *abs#020*

11:30am **Horst Posthaus**
New insights into the pathogenesis of of man and pigs *abs#021*

11:50am **Joachim Frey**
The determinative role of RTX toxins in host specificity of pathogenic *Pasteurellaceae* *abs#022*

12:20pm **Anders Bojesen**
Distribution, expression and secretion of the RTX-toxin GtxA among members of the genus *Gallibacterium* *abs#023*

12:40pm **Marina Harper**
Natural selection in the chicken host identifies Kdo kinase residues essential for phosphorylation of *Pasteurella multocida* LPS *abs#024*

Lunch

1:00 PM - 2:00 PM Lounge Area

Session 7: Extracellular Pathogens and Toxins II **Sponsored by Don Whitely Scientific**

2:00 PM - 3:30 PM Salone Grollo

Chair: John Prescott

2:00pm **Julian Rood**
Structural and functional analysis of the extracellular proteases of *Dichelobacter nodosus*, the causative agent of ovine footrot *abs#025*

2:30pm **Ruth Kennan**
A two-component system modulates twitching motility in Dichelobacter nodosus *abs#026*

2:50pm **David Hampson**
Plasmid-encoded genes involved with the rhamnose biosynthetic pathway (*rfb* genes) and/or glycosylation contribute to virulence in *Brachyspira hyodysenteriae* *abs#027*

3:10pm **John Timoney**
Now you see it, now you don't - regulation of capsule and protein expression in *Streptococcus zooepidemicus* *abs#028*

Poster Session 2
3:30 PM - 5:30 PM Lounge Area

Conference Dinner
7:00 PM - 9:00 PM Villa Ferdinando

Saturday, 9 October 2010

Session 8: Host Pathogen Interactions II **Sponsored by Pfizer Animal Health**

8:30 AM - 10:30 AM Salone Grollo

Chair: Joachim Frey

8:30am **Judy Stabel**
Cellular interactions in *Mycobacterium avium* subsp. *paratuberculosis* infection *abs#029*

9:00am **Ben Adler**
Pathogenesis of leptospirosis: the influence of genomics *abs#030*

9:30am **Ruth Raspoet**
Multidrug efflux pumps confer resistance of *Salmonella* Enteritidis to egg white by the export of antimicrobial components through the tolC outer membrane channel *abs#031*

9:50am **Reda Tarabees**
The role of nitric oxide in LPS-induced muscle wasting in C2C12 murine myotubes *abs#032*

10:10am **Ragnhild Bager**
Gallibacterium anatis does not require expression of a capsule to cause lesions and mortality in intraperitoneally infected chickens *abs#033*

Tea/Coffee Break

10:30 AM - 11:00 AM Lounge Area

Session 9: Host Responses **Sponsored by ARC Centre of Excellence**

11:00 AM - 12:20 PM Salone Grollo

Chair: Gadi Frankel

11:00am **Henk Haagsman**
Host defence peptides of farm animals: functions and applications *abs#034*

11:30am **James Whisstock**
The molecular function and evolution of membrane attack complex / perforin-like (MACPF) proteins *abs#035*

12:00pm **Richard Isaacson**
Alteration of the pig distal gut Microbiota by tylosin as an antibiotic growth promoter *abs#036*

Lunch

12:20 PM - 1:20 PM Lounge Area

Session 10: Vaccines I **Sponsored by Intervet Schering-Plough Animal Health**

1:20 PM - 2:40 PM Salone Grollo

Chair: Laura Serino

1:20pm **Hiroshi Kiyono**
New prospects on the aerodigestive immunity for mucosal vaccine development *abs#037*

1:50pm **Robert Moore**
Clostridium perfringens vaccines to protect chickens against necrotic enteritis *abs#038*

2:20pm **Ramie Husneara Begum**
Development and evaluation of gamma irradiated toxoid vaccine of *Salmonella enterica* var Typhimurium *abs#039*

Tea/Coffee Break
2:40 PM - 3:10 PM Lounge Area

Session 11: Vaccines II **Sponsored by CSIRO Livestock Industries**
3:10 PM - 4:30 PM Salone Grollo
Chair: Carlton Gyles

3:10pm **Altayeb Elazomi**
Colonisation of the chicken intestine by *Salmonella Enteritidis*: analysis of *in vivo* proteins and potential for vaccination *abs#040*

3:30pm **Abdulgader Alfitouri**
Transcriptional analysis of *Salmonella* Enteritidis P4 colonisation in the chicken *abs#041*

3:50pm **Sezer Okay**
Development of new vaccine strategies against *Pasteurella multocida* *abs#042*

4:10pm **Laura Serino**
Progress towards a vaccine against extraintestinal pathogenic *Escherichia coli* *abs#043*

Concluding Discussions
4:30 PM - 5:00 PM Salone Grollo

Farewell Reception
5:00 PM - 6:00 PM Lounge Area

POSTER LISTING

Poster Session 1

4:00 PM - 6:00 PM Lounge Area

abs#101 **Salem Abureema, *RMIT University***
Factors involved in biofilm formation by *Streptococcus uberis*

abs#102 **Michael Agbaje, *University of Agriculture, Abeokuta***
Isolation of *Tatumella ptyseos* from beef in Ibadan, Nigeria

abs#103 **Sergey Artiushin, *University of Kentucky***
Mucosal and systemic immunogenicity of fusion of Se18.9 to SeM of *Streptococcus equi*

abs#104 **Annette Backhans, *Swedish University of Agriculture***
Cross-species infection among rodents and farm animals by *Brachyspira* spp

abs#105 **Agata Bancerz-Kisiel, *University of Warmia and Mazury in Olsztyn***
Evaluation of the correlation between the occurrence of the *ymoA* gene and production of the enterotoxins Yst by *Yersinia enterocolitica* using multiplex PCR and infant mice assay

abs#106 **Luca Bano, *Istituto Zooprofilattico Sperimentale delle Venezie***
Clostridium difficile survey in Italian piggeries and molecular characterization of isolates

abs#107 **John Boyce, *Monash University***
Fis is a critical regulator of virulence gene expression in *Pasteurella multocida*

abs#108 **Leo Calvo-Bado, *University of Warwick***
Detection and diversity of a putative novel heterogeneous polymorphic proline-glycine repeat (Pgr) protein in the footrot pathogen *Dichelobacter nodosus*

abs#109 **Helen Collett, *University of Nottingham***
Molecular insights into the innate immune response of bovine endometrial cells to the zoonotic abortifacient pathogen *Leptospira*

abs#111 **J. Dubreuil, *Universite de Montreal***
Escherichia coli STb enterotoxin internalization is cell type dependent

abs#112 **Sérgio Fernandes da Costa, *University of Exeter***
The multimeric pore forming structure of the epsilon toxin from *Clostridium perfringens*

abs#113 **Roya Firouzi, *Department of Pathobiology, Shiraz University***
Antibacterial effects of *Thymus vulgaris, Echinacea pallida* and *Eucalypti folium* on *E. coli* and *Salmonella* spp. isolated from chickens

abs#114 **Roya Firouzi, *Department of Pathobiology, Shiraz University***
Abortion due to *Mannheimia haemolytica* in a cow

abs#115 **Masoud Haghkhah, *Shiraz University***
Bacterial mastitis in sheep and goats and its probable relationship with bacterial flora of the mouth of *Laudakia nupta*, a small reptile, in south of Iran

abs#116 **Xiaoyan Han, *Monash University***
Functional analysis of the C-terminal domain of the extracellular protease AprV5 from *Dichelobacter nodosus*

abs#118 **Mario Jacques, *Universite de Montreal***
Use of transposon mutagenesis and microarray analysis to identify genes associated with biofilm formation in *Actinobacillus pleuropneumoniae*

abs#119 **Bernhard Kaltenboeck, *Auburn University***
Clinically asymptomatic endemic *Chlamydia pecorum* infections reduce milk production and quality in dairy cows via inflammatory liver injury

abs#120 **Bernadett Khayer, *Veterinary Medical Research Institute, Hungarian Academy of Sciences***
PCR-RFLP analysis of *Bordetella bronchiseptica* strains originated from different hosts on flaA gene

abs#121 **Jong Wan Kim, National Veterinary Research & Quarantine Service**
Immunoproteomic analysis of cell envelope proteins of *Brucella canis* for vaccine development

abs#122 **Kirstine Klitgaard, *National Veterinary Institute, Technical University of Denmark***
Intra-species variation in *Actinobacillus pleuropneumoniae* - transcriptional response to iron limitation in serotypes with different virulence potential

abs#123 **Magdalena Kostrzynska, *Agriculture and Agri-Food Canada***
Modulatory effects of probiotic lactic acid bacteria on virulence and pathogenicity of VTEC and *Salmonella typhimurium*

abs#124 **Aidin Shojaee Tabrizi, School of Veterinary Medicine, Shiraz University**
Detection of *Helicobacter heilmannii* in the gastrointestinal tract of stray cats

abs#125 **Filip Van Immerseel, Ghent University**
Salmonella Enteritidis universal stress protein (usp) gene expression is stimulated by egg white and supports oviduct colonization and egg contamination in laying hens

abs#126 **Bernard Wasinski, National Veterinary Research Institute**
Reactivity of heat-stable *Leptospira* antigenic preparation used in enzyme-linked immunosorbent assay for detection of leptospiral antibodies in swine serum

Poster Session 2

abs#151 **Roya Firouzi, *Department of Pathobiology, Shiraz University***
Study of antibacterial effects of sour and sweet pomegranate peel extracts

abs#152 **M Haghkhah, *Shiraz University***
Detection of *Fusobacterium necrophorum* in lame cattle

abs#153 **Bok Kyung Ku,** *National Veterinary Quarantine and Service*
Development of a multiplex PCR for differentiation of *Mycobacterium tuberculosis* complex and non-tuberculous mycobacteria

abs#154 **Young Ju Lee, *National Veterinary Research & Quarantine Service***
Evaluation of a fluorescence polarization assay for the diagnosis of *Salmonella* group D infection

abs#155 **Susanne Lindahl,** *National Veterinary Institute*
Mapping Swedish outbreaks of *S. equi* infection (strangles) in horses using the SeM gene

abs#156 **Catherine Logue,** *North Dakota State University*
Comparative Analysis of *Salmonella enterica* Senftenberg from humans and animals

abs#157 **Evelyn Madoroba,** *Agricultural Research Council-Onderstepoort Veterinary Institute*
Detection of *Campylobacter fetus* and *Tritrichomonas foetus* in Southern African cattle using species-specific PCR

abs#158 **Mélanie Martignon,** *Agence Française de Sécurité Sanitaire des Aliments*
Digestive response of young rabbits to an experimental reproduction of colibacillosis according to two feeding strategies

abs#159 **Gabriel Milinovich, *The University of Vienna***
A simple in vitro model for monitoring caecal microbiota alterations during the development of carbohydrate-induced equine laminitis

abs#160 **Kanitha Patarakul, *Faculty of Medicine, Chulalongkorn University***
Identification of host proteins that interact with LipL32, the major outer membrane protein of pathogenic *Leptospira*, by phage display technolog

abs#161 **Sinikka Pelkonen, *Finnish Food Safety Authority Evira***
Epidemiology of *Pasteurella multocida* in calf rearing units

abs#162 **John Pringle, *Swedish University of Agricultural Sciences (SLU)***
Is natural exposure to *Anaplasma phagocytophilum* in the horse associated with chronic clinical disease?

abs#163 **Ruth Raspoet,** Ghent University, ***Faculty of Veterinary Medicine***
Microarray-based detection of *Salmonella* Enteritidis genes involved in reproductive tract colonization

abs#164 **Mirja Raunio-Saarnisto, *Finnish Food Safety Authority***
Clostridium difficile in piglets in Finland

abs#165 **Nadra Rechidi-Sidhoum, *University of Mostaganem***

Prevalence of animals brucellosis in the Wilaya of Mostaganem, Algeria

abs#166 **Julian Rood, *Monash University***

Conjugative transfer of the NetB toxin plasmid in *Clostridium perfringens*

abs#167 **Anna Rosander, *Swedish University of Agricultural Sciences (SLU)***

Antibody response to a *Treponema phagedenis*-like recombinant protein in cattle with digital dermatitis

abs#168 **Claire Russell, *University of Bristol***

Development of a multiple loci VNTR analysis (MLVA) method for typing *Dichelobacter nodosus*, the causal agent of footrot in sheep

abs#169 **Aidin Shojaee Tabrizi, School of Veterinary Medicine, Shiraz University**

PCR, cytology, RUT: which is the method of choice for detection of *Helicobacter* spp. in feline gastric mucosa?

abs#170 **Cornelia Sperl, *Biomin Holding GmbH***

Antibacterial activity of phytogenic substances against swine pathogens

abs#171 **Olov Svartström, *Swedish University of Agricultural Sciences (SLU)***

Characterization of *Treponema pedis* isolates from necrotic skin ulcers in pigs

abs#172 **Filip Van Immerseel, *Ghent University***

Efficient oviduct colonization and egg white survival strategies of *Salmonella* Enteritidis as a possible explanation for the egg-associated pandemic

abs#173 **Ben Wade, *CSIRO***

Clostridium perfringens genes with implications for both virulence and colonisation during necrotic enteritis

abs#174 **Bernard Wasinski, *National Veterinary Research Institute***

Seroprevalence of *Leptospira* species in pigs in Poland. Increasing importance of Sejroe serogroup in etiology and pathogenesis of leptospirosis in swine?

abs#175 **Elizabeth Wellington, *University of Warwick***

Ovine pedomics- the first study of the ovine foot 16S rRNA based microbiome

abs#176 **Luci Witcomb, *University of Warwick***

Ovine footrot: detection and quantification of *Dichelobacter nodosus* and *Fusobacterium necrophorum* using real-time PCR and fluorescence *in situ* hybridization techniques

ABSTRACTS

ORAL PRESENTATIONS

001

THE PROBLEM OF RELEVANCE IN PATHOGENESIS RESEARCH

C. Gyles

Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada

Pathogenesis of bacterial diseases, involving the intricate and complex inter-relationships between pathogen, host, and environment, is a fascinating and alluring field of research. Those host-parasite-environment interactions that were relatively simple were the first to be understood. They include intoxications in which ingestion of a powerful bacterial toxin was sufficient to cause disease. In other cases bacteria occupy a variety of niches in the host and launch an attack at an opportune time. Still others have reservoirs in the environment or in an alternate host species. Some bacterial pathogens have a brief encounter with the host; others are long-term guests. This variety of spatial relationships has led to a wide range of strategies for survival and transmission of bacterial pathogens. Rapid developments in molecular genetics and genomics have facilitated understanding of the pathogens and the hosts. The examination of one gene at a time has given way to addressing the genome, the transcriptome, and the proteome. Massive information often results from such studies. The question then becomes one of determining the relevance of the vast number of genes, mRNA molecules, and proteins that are involved in the interactions. Frequently, our *in vitro* studies attempt to simulate one or two critical aspects of the environment, such as temperature, pH, iron concentration, that may provide clues as to what goes on in the host. From time to time these studies identify critical bacterial factors or other aspects of pathogenesis, but regulation of bacterial virulence and host response is complex and often not well understood. Pathogenesis is a process of continuous change in which timing and degree of gene expression are critical and are highly regulated by the environment. It is impossible to get the full picture without the use of natural or experimental infections. Experimental infections involve ethical and economic considerations which often act as a deterrent. However, there doesn't seem to be any alternative to a more complete understanding of bacterial pathogenesis.

002

CLOSTRIDIA IN SWINE ENTERIC DISEASE: ALWAYS SOPHISTICATED, OFTEN BRUTAL

G. Songer

Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Ames, Iowa, United States

Clostridial enteric disease contributes to economic loss in swine production, in spite of widespread recognition and universally advocated immunoprophylaxis. Clostridium perfringens (CP) type C causes hemorrhagic, fatal, necrotic enteritis in piglets. Affected piglets have hemorrhagic diarrhea, and hallmark lesions are profound small intestinal mucosal necrosis and emphysema. Large gram-positive bacteria may be present in deeper layers of intestinal wall. Lethal and necrotizing beta toxin is the key factor in pathogenesis, although beta2 toxin (CPB2) may also play a role. CP type A also causes enteritis in piglets. Piglets develop creamy or pasty diarrhea, and at post mortem, small intestine is flaccid, thin-walled, and gas-filled, with watery contents and no blood. Mucosal inflammation is mild, and lesions may include superficial villous tip necrosis and accumulation of fibrin. Jejunal and ileal lesions may be heavily colonized with C. perfringens. CPB2 may play a role in virulence. Clostridium difficile (CD) has become perhaps the most important uncontrolled cause of enteritis in neonatal pigs. Affected piglets typically present with a history of early-onset scours; gross lesions usually include mesocolonic edema, and large intestines may be filled with pasty-to-watery yellowish feces. Focal suppuration in colonic lamina propria is the hallmark lesion, and pathogenesis likely involves action by toxins A (TcdA) and B (TcdB). Diagnosis is by detection of toxins in feces or colonic contents. Recent advances, in some cases through application of molecular methods, promise progress in abrogation of the effects of clostridia on swine production.

(1) Bueschel DM, et al. 2003. Prevalence of cpb2, encoding beta2 toxin, in Clostridium perfringens field isolates: correlation of genotype with phenotype. Vet Microbiol, 94: 121.b

(2) Collins JE, et al. 1989. Diarrhea associated with Clostridium perfringens type A enterotoxin in neonatal pigs. J Vet Diagn Invest, 1:351-353.

(3) Gibert M., et al. 1997. Beta2 toxin, a novel toxin produced by Clostridium perfringens. Gene, 203:65–73.

(4) Songer JG, et al. 2000. Enteric infection of neonatal swine with Clostridium difficile. Swine Health Produc, 8: 185-189.

(5) Yaeger M, et al. 2002. Agents associated with neonatal diarrhea in Iowa swine including Clostridium difficile and porcine reproductive and respiratory syndrome virus. J Vet Diagn Invest, 14:281-287.

003

CLOSTRIDIUM PERFRINGENS TYPE C AND *CLOSTRIDIUM DIFFICILE* COMBINED ENTER-TYPHLO-COLITIS IN FOALS

F. Uzal[1], S. Diab[1], L. Anthenill[1], P. Blanchard[2], J. Moore[2], G. Songer[3]

[1]CAHFS, UCDavis, San Bernardino, United States
[2]CAHFS, UCDavis, Tulare, United States
[3]University of Arizona, United States

Clostridium perfringens type C is one of the most important agents of enteritis in newborn foals. *C. difficile* is now recognized as an important cause of enterocolitis in horses of all ages. While infections by *C. perfringens* type C or *C. difficile* are frequently seen in foals, we are not aware of any report describing combined infection by these two microorganisms in foals. We present here six cases of foal enterocolitis associated with *C. difficile* and *C. perfringens* type C infection. Six foals of ages ranging between one and seven days were submitted for necropsy examination to the California Animal Health and Food Safety Laboratory. The six animals had a clinical history of acute hemorrhagic diarrhea followed by death; none of these animals had received antimicrobials. Postmortem examination revealed hemorrhagic and necrotizing enter-typhlo-colitis. Histologically, the superficial mucosa of the small intestine and colon presented diffuse necrosis and hemorrhage, and it was covered by a pseudomembrane. Thrombosis was observed in mucosal vessels. Immunohistochemistry of intestinal sections of all foals showed that most large bacilli in the sections were positive for *C. perfringens*. *C. perfringens* beta toxin and *C. difficile* toxins A/B were detected in small intestinal and/or colonic contents of all animals by ELISA. *C. perfringens* (identified as type C by PCR) was isolated from the small intestine and/or colon of all animals. *C. difficile* (typed as A-/B+ by PCR) was isolated from the small intestine in 4 out of the 6 cases. This report suggests a possible synergism of *C. perfringens* type C and *C. difficile* in foal enterocolitis. Because none of the foals had received antibiotic therapy, the predisposing factor for the *C. difficile* infection remains undetermined; it is possible that the *C. perfringens* infection acted as a predisposing factor for *C. difficile* and/or viceversa. This report also stresses the need to perform a complete diagnostic work up in all cases of foal digestive disease.

004

CROSS-OVER OF FLUOROQUINOLONE-RESISTANT (FQR) EXTRA-INTESTINAL *ESCHERICHIA COLI* ISOLATES FROM DOGS AND HUMANS IN AUSTRALIA

J. L. Platell[1], R. N. Cobbold[1], P. Heisig[2], C. R. Clabots[3], J. R. Johnson[3,4], D. J. Trott[1,5]

[1]School of Veterinary Science, The University of Queensland, Gatton, QLD, Australia
[2]School of Pharmacy; Chemistry Department, University of Hamburg, Hamburg, Germany
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Fluoroquinolone-resistant (FQ^r) extra-intestinal *E. coli* isolates (120 canine, 582 human) from diagnostic laboratories (2007-2008) in Australia were assessed for inter-species transmission potential. Following PCR-based phylotyping, group B2 isolates were selectively tested for single-nucleotide polymorphisms (SNPs) indicative of sequence type ST131 and group D clonal group A and O15:K52:H1 strain types. Extended virulence genotypes (53 traits) and pulsed-field gel electrophoresis (PFGE) profiles were analysed on a subset of human isolates and all canine isolates that belonged to these clonal groups. Partial multi-locus sequence typing (MLST; *fumC*, *gyrB*, and *recA*) also revealed another clonal complex containing both human and canine isolates (CC14; O75:K+). Groups B2 and D accounted for an equal share and a collective majority of study isolates at 37% (262/702) and 38% (267/702), respectfully. Group B2 was significantly more prevalent among human (43% [250/582]) compared to canine-source (10% [12/120]) isolates ($P < 0.001$), suggesting that in Australia, dogs are a less important reservoir for such strains. ST131 was the most prevalent clonal group, accounting for the majority of both human (205/250, 82%) and canine (8/12, 67%) group B2 isolates. No clonal group A and only two O15:K52:H1 isolates were represented in the canine isolate collection. When assessed for commonality across species, PFGE analysis showed several instances of ≥ 94% similarity. Common virulence markers, including *iha, fimH, sat, fyuA, iutA, KpsII, ompT and iutA*, were found across all FQ^r ExPEC, regardless of clonal group or host species. ST131 isolates showed similar FQ resistance mechanisms, with canine strains showing a higher prevalence of the plasmid-mediated quinolone resistance determinant *qnrB*. Across-species strain similarity (by clonal group, virulence genotype, and PFGE profile) supports human-dog exchange of FQ^r *E. coli* within Australia. This, plus Australia's unique banned use of FQs in livestock, argues against the local food supply as a source for Australian FQ^r strains.

005

EXPLORING EXPEC VIRULENCE, EVOLUTION, AND HOST SPECIFICITY USING PATHOGENOMIC APPROACHES

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Extraintestinal pathogenic *Escherichia coli* (ExPEC), which encompass uropathogenic *E. coli*, neonatal meningitis-associated *E. coli*, and septicemia associated *E. coli*, are major agents of human disease. Collectively, these pathogens are responsible for significant morbidity and mortality resulting in hundreds of thousands of deaths and millions of days of lost productivity annually. ExPEC also have major impact on animal health, causing similar diseases in companion and food animals. Of particular interest in our lab is the avian ExPEC, avian pathogenic *E. coli* (APEC), which causes colibacillosis. This disease is one of the most significant infectious diseases of poultry, putting at risk one of humankind's cheapest sources of high-quality protein. Thus, no matter whether we measure the costs of ExPEC-caused diseases in terms of human and animal misery and mortality, lost productivity, or healthcare expenditures, they are unacceptably high, making effective control of these diseases highly desirable. Here, we describe our efforts to 1) define the APEC pathotype using pathogenomic approaches, 2) exploit these findings to enhance colibacillosis control, and 3) use these findings to gain insight into bacterial evolution, pathogenesis and host specificity. Finally, we will explore the potential that human ExPEC originate from APEC since recent reports have shown that avian and human ExPEC share many traits and capabilities for causing disease. As part of this discussion, we will examine the potential that APEC-contaminated poultry is a food-borne reservoir of plasmid-linked virulence and/or resistance genes contributing to ExPEC-caused diseases of human hosts.

006

GENOME DOWNSIZING AND METABOLIC REMODELLING IN *MYCOBACTERIUM BOVIS*

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Mycobacterium bovis is the causative agent of tuberculosis in a range of animal species and man, with worldwide annual losses to agriculture of $3 billion. The human burden of tuberculosis caused by the bovine tubercle bacillus is still largely unknown. *M. bovis* was also the progenitor for the *M. bovis* bacillus Calmette-Guérin vaccine strain (BCG), the most widely used human vaccine. Strikingly, the genome sequence of *M. bovis* is >99.95% identical to that of *M. tuberculosis*, but deletion of genetic information has led to a reduced genome size. Cell wall components and secreted proteins show the greatest variation, indicating their potential role in host-bacillus interactions or immune evasion. Furthermore, there are no genes unique to *M. bovis*, implying that differential gene expression may be the key to the host tropisms of human and bovine bacilli. High throughput single nucleotide polymorphism (SNP) analysis of BCG and *M. bovis* strains has identified a minimal set of mutations that may play a role in the attenuation of the BCG vaccine and have resulted in metabolic remodelling of the vaccine strain. The genome sequence therefore offers major insight into the evolution, host preference, and pathobiology of *M. bovis* and recent insights will be discussed.

007

ANALYSIS OF THE CORE AND ACCESSORY GENOME OF BACTERIAL PATHOGENS

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The pan-genome of a bacterial species consists of a core and an accessory gene pool. The accessory genome arises through gene loss and acquisition and is the most important source of genetic variability in bacterial populations. The accessory genome is thought to allow subpopulations of bacteria to better adapt to specific niches and in turn to form new, relatively stable, core genomes which are characteristic of the subgroup. Low-cost and high-throughput sequencing platforms have resulted in an exponential increase in the availability of genome sequence data for a number of bacterial pathogens. In this study, we describe a suite of new genome sequence analysis programs (http://76.70.11.198/panseq) which allow comparisons among strains *in silico* and define accessory and core components of the pan-genomes of pathogenic species. With this software, distinct clusters of strains of specific genotypes can be identified which have related phenotypic characteristics such as host preference, virulence and an association with specific disease syndromes. The genetic variability harvested from both the core and accessory genomes of pathogens using this software can also be used to construct phylogenies and suggest ways in which these pathogens may have emerged. We show that an analysis of the relationships among pathogens, such as *Salmonella enterica* and *Listeria monocytogenes*, using either core-based SNP analysis or accessory genome components yields similar phylogenies for the respective bacterial species. In addition, cluster-specific genetic elements have been defined which may be useful markers for the

identification of pathogen subtypes and strain-specific markers have been defined which may be useful for strain differentiation in epidemiological and source attribution studies.

008

MOLECULAR COMPARISONS OF *CAMPYLOBACTER FETUS VENEREALIS* ISOLATES FROM AUSTRALIAN CATTLE TO IDENTIFY VIRULENT STRAINS

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Bovine genital campylobacteriosis is caused by *Campylobacter fetus venerealis* (*Cfv)* which induces abortion and infertility in female cattle while bulls are asymptomatic carriers. To monitor disease and to maximise access to exportation, accurate detection of the organism is crucial for the Australian beef industry. Lack of correlation between molecular and cultural methods complicates diagnosis as a recent 5' Taq real-time PCR assay detected a much higher *Cfv* prevalence than indicated by culture, including herds with no history of infertility problems. This suggests that *Cfv* isolates may vary in virulence. This study aims to develop *Cfv*-specific assays as well as assays targeting putative *Cfv* virulence genes, identified after genomic and plasmid comparisons of a collection of *Cfv* isolates. To obtain isolates, the 5' Taq real-time PCR assay was used to screen 814 abattoir samples and revealed a prevalence of 27.5% (224/814) for *Cfv* from Queensland and New South Wales bulls. Culture of real-time positive samples resulted in the successful isolation of 67 *Cfv* isolates while 7 cultures were obtained from a diagnostic laboratory. As this assay targets the supposedly plasmid-borne *parA* gene, a new real-time PCR assay named AbrilTaq and based on the *Cfv*-specific insertion element ISC*fe*1 was developed and used concurrently to screen 97 abattoir samples. AbrilTaq was also used to re-evaluate the *Cfv* isolate collection. High correlation between both assays and growth of *Cfv* isolates suggested that AbrilTaq may be a new useful *Cfv*-specific diagnostic test. AbrilTaq will be used to screen specimens obtained from the field and to monitor experimental animal infection models in order to determine its specificity, sensitivity and ability to predict disease risk. The *Cfv* isolate collection is currently undergoing additional molecular screening using published conventional PCR methods as well as MLST to confirm isolate identity before further virulence testing. The collection is also being examined for the expression of a recently discovered *Cfv*-specific pathogenicity island, harbouring many virulence factors including the *parA* gene.

009

MOLECULAR EPIDEMIOLOGY AND PHYLOGENETIC DIVERSITY OF *MANNHEIMIA GLUCOSIDA* STRAINS ASSOCIATED WITH OVINE MASTITIS IN AUSTRALIA

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The genus *Mannheimia* includes several species that have been recognised as pathogens of a range of animals. *Mannheimia haemolytica* is as an important cause of pneumonia and mastitis in sheep. In a mastitis survey on Poll Dorset sheep, we isolated *M. glucosida*, another member of this genus, as frequently as *M. haemolytica* from cases of clinical mastitis. *M. glucosida* is regarded as a heterogeneous opportunistic species and has been isolated from cases of pneumonia in ruminants, and from the nasal cavity of healthy sheep.

The purpose of this study was to determine the biochemical and genetic characteristics, as well as the diversity, of nine *M. glucosida* isolates obtained from cases of ovine mastitis in five different flocks. Phylogenetic analyses of two housekeeping genes, *rrnA* and *rpoB*, and phenotypic tests were performed to study the characteristics of the isolates.

To compare strain distribution between and within flocks, genomic restriction endonuclease digestion and pulsed-field gel electrophoresis was used. The sequence of the leukotoxin (*lktA*) gene of each distinct strain was determined and these sequences compared with those of European isolates.

Diversity in phenotypic as well as genotypic characteristics was seen in the *M. glucosida* isolates. Identical pulsed-field electrophoresis patterns in three isolates from one flock indicated the possibility of horizontal transmission and the presence of a predominant strain at a particular time in this flock. The detection of six different pulsed-field electrophoresis patterns indicated that there are a number of different strains of *M. glucosida* that can cause mastitis.

Phylogenetic analysis of the two housekeeping gene sequences detected incongruence between relationships determined using these sequences and the *lktA* gene. These differences suggest the possibility of horizontal gene transfer between the *M. glucosida* strains associated with mastitis, in agreement with results obtained from similar studies on *M. haemolytica* and *M. glucosida* isolates from ovine and bovine pneumonia.

010

INTERACTION OF PATHOGENIC E. COLI WITH PLANT AND MAMMALIAN CELLS

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We are currently witnessing a dramatic change in the epidemiology of pathogens historically considered to be zoonotic (e.g. *Salmonella enterica* and *E. coli* O157), as more and more outbreaks and illnesses are traced to the consumption of contaminated fresh produce. Ready-to-eat, minimally processed vegetables, consumed raw, are becoming an important *vector* for bacterial transmission. Studying the mechanisms by which enteric pathogens bind to the surface of fresh produce implicates biofilms, flagella and the type III secretion system (T3SS), which is mainly designed for injection of virulence factors (known as effectors) into the eukaryotic host cell to facilitate bacterial attachment or invasion. Translocated effectors target diverse signaling pathways by binding eukaryotic proteins at various cellular compartments. Common targets of the T3SS effectors are the actin cytoskeleton and the innate antimicrobial responses of the host. For example, *E. coli* O157 and enteropathogenic *E. coli* (EPEC) translocate NleH, which blocks infected cells from undergoing apoptosis thus promoting bacterial colonization; and Tir, which is involved in intimate bacterial attachment, actin polymerization and formation of the characteristic attaching and effecting legions. Although EPEC is considered an extracellular pathogen, a small subset of strains translocate EspT, which activates the GTPase Rac1 leading to cell invasion. Invasive EPEC resides in a vacuole and integration of Tir into the vacuolar membrane leads to formation of a thick actin coat, which is essential for intracellular bacterial replication. I will show data of the mechanisms used by *Salmonella* and *E. coli* to bind fresh produce and describe the function of *E. coli* effectors during infection of cultured cells, *in vitro* intestinal organ cultures and animal models.

011

EARLY SEVERE LYMPHOCYTE-DEPENDENT INFLAMMATORY RESPONSES TO UROPATHOGENIC *E. COLI* PREDISPOSE TO CHRONIC AND RECURRENT URINARY TRACT INFECTION

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Chronic and recurrent urinary tract infections are an increasing problem both in dogs and humans due to the aging population and the increase in antibiotic resistant organisms. Therefore, understanding the host-pathogen interactions that result in chronic and recurrent infection is of great importance. Here, we investigate the molecular basis of chronic bacterial cystitis in a murine model. We establish that introduction of uropathogenic *E. coli* (UPEC) into the bladders of C3H mice results in two distinct disease outcomes: resolution of acute infection or development of chronic cystitis lasting months. The incidence of chronic cystitis is both host strain and infectious dose-dependent. Further, development of chronic cystitis is preceded by biomarkers of local and systemic acute inflammation at 24 hours post-infection including: severe pyuria and bladder inflammation with mucosal injury, and a distinct serum cytokine signature consisting of elevated IL-5, IL-6, G-CSF, and the IL-8 analog KC. Mice deficient in TLR4 signaling or lymphocytes lack these innate responses and are resistant, to varying degrees, to developing chronic cystitis. Furthermore, treatment of C3H mice with the glucocorticoid anti-inflammatory drug dexamethasone prior to UPEC infection also suppresses the development of chronic cystitis. Finally, individuals with a history of chronic cystitis, lasting at least 14 days, are significantly more susceptible to redeveloping severe, chronic cystitis upon bacterial challenge. Thus, we have discovered that the development of chronic cystitis in C3H mice by UPEC is facilitated by severe acute inflammatory responses early in infection, which subsequently are predisposing to recurrent cystitis, an insidious problem in both dogs and women. Overall, these results have significant implications for our understanding of how early host-pathogen interactions at the mucosal surface determines the fate of disease.

012

THE RATE OF APOPTOSIS OF CD4$^+$ CELLS REGULATES IMMUNOPROTECTION FROM CHLAMYDIAL INFECTION AND DISEASE

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Infections with *Chlamydia* (*C.*) spp. are ubiquitous in livestock (1). While most of these infections are asymptomatic (1), they cause production losses and to trigger other diseases (1). It is therefore important to understand the mechanisms that regulate chlamydial disease. Interferon-γ produced by CD4$^+$ T cells is an absolute requirement for protection against chlamydial infection. We have previously shown that reduced T cell responses in C57BL/6 mice as compared to A/J mice associate with enhanced disease (2). A possible reason for the reduced T cell responses is increased activation-induced cell death (AICD) of CD4$^+$ T cells as suggested by modeling of T helper cell regulation (3). In this investigation we used a murine model of lung disease caused by *C. pneumoniae*, a frequent pathogen in humans and marsupials (1). Immunofluorescent examination of infected lungs demonstrated a higher rate of lung CD4$^+$ cell apoptosis in immune C57BL/6 than in A/J mice. Using temporary blockade of apoptosis in C57BL/6 mice, we investigated the effect of T cell AICD on disease and immunoprotection after secondary challenge with *C. pneumoniae*. Administration of the broad-spectrum apoptosis inhibitory peptide Q-VD-OPH on days 0, 2 and 4 after intranasal challenge significantly reduced C57BL/6 lung CD4$^+$ cell apoptosis, and peak disease and *C. pneumoniae* lung load on day 10 after challenge. Inhibition of apoptosis elevated the concentrations of mRNAs for markers of memory T cells (CD45RO), and Th1 (Tim3) and Th2 (GATA3) subpopulations. In contrast, mRNA levels of IL-17, a major presumed mediator of chlamydial disease (4,5), were approximately 8-fold higher in Q-VD-OPH-treated mice on day 3, but declined rapidly and were approximately 8-fold lower on day 10. These results strongly suggest that the rate of CD4 cell apoptosis is a key regulator of protective immunity to *C. pneumoniae*.

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013

THE CONTRIBUTION OF *HISTOPHILUS SOMNI* (*HAEMOPHILUS SOMNUS*) LIPOOLIGOSACCHARIDE TO BACTERIAL VIRULENCE AND HOST INTERACTIONS

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Histophilus somni is a Gram-negative coccobacillus that is a commensal or opportunistic bovine/ovine pathogen responsible for pneumonia, myocarditis, and other systemic diseases. Our goal is to understand how the lipooligosaccharide (LOS) contributes to *H.* somni virulence in regard to host-pathogen interactions. The LOS can avoid host defenses by phase variation through slipped strand mispairing of simple sequence DNA repeats (SSR) within the open reading frame of glycosyltransferase genes. Furthermore, phosphorylcholine (ChoP) on the LOS is phase variable due to SSR within *lic1A*, and antigenic expression of ChoP correlated with adherence of *H. somni* to the bovine upper respiratory tract, whereas loss of ChoP correlated with dissemination of *H. somni* to systemic tissues. Expression of sialic acid on the LOS is not phase variable. However, at least 2 different sialyl-transferases were identified that were specific for one of two terminal galactose linkages (β1,4 or β1,3), and this linkage was susceptible to SSR-mediated phase variation. Sialylation of the LOS was also associated with reduced signaling through toll-like receptor 4, reduced complement activation, and inhibition of phagocytosis. Transposon mutants of *H. somni* have been generated in *fhaC, uspE, tolC*, and *luxS*. The *uspE* and *luxS* mutants were highly attenuated in a mouse model for *H. somni* disease, and the *fhaC* mutant was deficient in biofilm formation. The LOS of these mutants is being examined to determine if loss of virulence or biofilm formation in these mutants is correlated with LOS modification. We conclude that LOS is essential to virulence in *H. somni*, and that the bacterium can modify its LOS in response to host interactions.

014

RHODOCOCCUS EQUI - HOW TO REPROGRAM PHAGOSOME TRAFFICKING IN MACROPHAGES

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Rhodococcus equi is a Gram-positive pathogen which is closely related to mycobacteria and, like them, has a thick, hydrophobic cell wall composed of mycolic acid compounds. R. equi can cause severe -often fatal- bronchopneumonia in its natural host, the foal, but occassionally also in immunosuppressed humans. In infected foals, R. equi carrying a suitable virulence-associated plasmid multiplies in macrophages and, eventually, lyses the host cell by necrosis. We have described that, in murine macrophages, R. equi halts phagosome maturation between the early endosome-like step and the late endosome-like stage, hence effectively inhibiting phagolysosome formation. The proton-pumping ATPase is excluded from the phagosome and its pH is near-neutral at 3 h of infection. We have identified two mutants in the Fatty Acid Synthase II (FAS II) pathway that is required for the elongation of mycolic acids. In pathogenic mycobacteria, knocking-out attempts in these genes are lethal, yet R. equi mutants grow well in broth. The mutants' phagosomes are more frequently found in phagolysosomes and are more acidic. but will eventually also reach the privileged compartment. Transfer of extractable lipids from wild type versus mutant bacteria onto Escherichia coli lead to a reduced versus a normal fusion of E. coli-containing phagosomes, respectively, with lysosomes, supporting a central and direct role of these long chain (glyco-)lipids in inhibiting phagolysosome formation. As we have recently shown that VapA, a secreted protein encoded by a virulence-associated plasmid, is central in inhibiting phagosome acidification (but not phagolysosome formation or cytotoxicity), we can now experimentally dissect the mechanisms involved in inhibition of phagosome acidification and phagolysosome formation.

015

BACKING BOTH HORSES: *RHODOCOCCUS EQUI* AS A PATHOGEN OF MACROPHAGES AND A SOIL ORGANISM

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Rhodococcus equi is an important cause of pneumonia in foals, usually between four and 12 weeks of age. Infection causes a subacute or chronic abscessating bronchopneumonia, sometimes with ulcerative typhlocolitis. Tuberculosis-like lesions caused by *R. equi* may also occur in the submandibular and other lymph nodes of cattle and pigs. All isolates from foals with clinical disease possess a large plasmid encoding a 21 kb conserved pathogenicity island that is essential for virulence. The pathogenicity island encodes 6 virulence-associated proteins (Vaps), of which VapA is a crucial virulence determinant. Their function is unknown. Pig virulent isolates also possess a virulence plasmid associated with “intermediate” virulence expressing a novel Vap protein designated VapB, and other *vap* genes that share common ancestry with foal plasmid genes.

The infectivity of *R. equi in vitro* is largely or exclusively limited to cells of the monocyte-macrophage lineage. Possession of the virulence plasmid gives isolates the property of arresting phagosome maturation at a stage between the early and late endosome

This overview will summarize the emerging and rapid advances in understanding of *Rhodococcus equi* as a pathogen of animals and humans, and as a soil organism, including insights gained from a genome sequence, focused particularly on its relevance to increasing understanding of how it evades killing by macrophages and subvert the immune response. It will emphasize breakthroughs in a number of areas, including regulation of virulence, and the importance of *R. equi* as a model organism to understand further aspects of intracellular bacterial parasitism.

016

PATHOGENESIS OF MYCOPLASMOSIS

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Mycoplasmas are thought of as minimal organisms, but they establish infection in a variety of sites in the body and persist for extended periods. Although considered mucosal pathogens, many can also invade and localise in a variety of sites, and some are dedicated parasites of erythrocytes. While they were initially found to lack most of the transcriptional regulators recognised in other bacteria, recent studies have established that they do possess complex regulatory systems. The different pathogenic mycoplasmas of domestic animals possess distinct and complex mechanisms for generating antigenic, and possibly functional, diversity in key cell surface proteins, and also produce a diverse range of proteins that may have both nutritional and pathogenetic roles during infection. The diversity of mechanisms involved in pathogenicity reflects the complex evolutionary history of each of these organisms, and their close relationship with a small range of hosts. While such diversity makes it challenging to generalise, there is one major common feature of mycoplasmoses, a profuse lymphocytic infiltration of tissues at the site of infection. This response appears to be induced by the lipoproteins that are a major component of the cell surface. Mycoplasma lipoproteins have a distinct structure, as they are diacylated, rather than triacylated like other bacterial lipoproteins, and are potent ligands of TLR-2. Thus, while each mycoplasma species appears to have a range of mechanisms for persisting, and in some cases invading, its host, local intense stimulation of cytokine release appears to be central pathway in pathogenesis.

017

ROLE OF THE *BURKHOLDERIA PSEUDOMALLEI* TYPE III SECRETION SYSTEM CLUSTER 3 (TTSS3) IN INTRACELLULAR SURVIVAL, ESCAPE FROM HOST AUTOPHAGY AND VIRULENCE

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Burkholderia pseudomallei is a Gram-negative pathogen endemic in southeast Asia and northern Australia. It is the causative agent of the disease melioidosis, an often fatal disease of humans and many other animal species. *B. pseudomallei* is internalised by phagocytic cells but can escape from the phagosome into the host cytoplasm, where it replicates and moves within and between cells by actin-mediated motility. Autophagy is a cellular degradation system that also forms part of the host innate immune defence against intracellular pathogens. When *B. pseudomallei* was used to infect the macrophage-like cell line RAW 264.7 cellular autophagy was induced, but only a small subset of the bacteria was observed to co-localize with the autophagy marker protein LC3. Thus, wild-type *B. pseudomallei* is highly resistant to uptake and killing by host autophagy. However, when autophagy was chemically induced by treatment with rapamycin, bacterial co-localization with LC3 was significantly increased and bacterial survival reduced. The *B. pseudomallei* Type III secretion system cluster-3 (T3SS3) is known to play a role in intracellular survival, but its importance in avoidance of host autophagy has not been fully elucidated. In this study, we investigated the role of several T3SS3 proteins including BipD (translocator), BsaS (putative ATP synthase), BopA (putative effector) and BapA (putative effector) in the evasion of autophagy. The *bipD, bsaS* and *bopA* mutants showed a higher level of co-localization with LC3 than either the wild-type strain or the *bapA* mutant. These data suggest that a functional T3SS3 and the effector protein BopA are critical for evasion of autophagy, but that the effector BapA is not involved in evasion of autophagy. Interestingly, both the *bopA* and *bapA* mutants showed reduced virulence in a mouse melioidosis model.

018

THE SECRETION, BUT NOT THE SYNTHESIS, OF CATECHOLATE SIDEROPHORES CONTRIBUTES TO THE VIRULENCE OF AVIAN EXTRA-INTESTINAL PATHOGENIC ESCHERICHIA COLI

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Extra-intestinal pathogenic *Escherichia coli* (ExPEC) synthesize and secrete siderophores to sequester ferric iron from host ferroproteins in tissues and blood, where iron availability is limited. Enterobactin and its glucosylated variants, the salmochelins, are catecholate siderophores commonly produced by ExPEC and *Salmonella* spp. Previous studies demonstrated that enterobactin is exported by the EntS transporter, whereas certain forms of salmochelins are secreted by IroC. Using a chicken sepsis model, we investigated the importance of export and synthesis of catecholate siderophores for virulence of an avian ExPEC strain producing the siderophores enterobactin, salmochelins, and aerobactin. The export of all species of catecholate siderophores by strains was also assessed by liquid chromatography/mass spectrometry. Loss of *entS* impaired enterobactin and salmochelin MGE secretion, whereas loss of *iroC* impaired secretion of salmochelins DGE (S4), and TGE. Complementation restored siderophore secretion to wild-type levels. In single-strain infections, Δ*entS* and Δ*entS*Δ*iroC* mutants were attenuated in blood and tissues, whereas the Δ*iroC* mutant was only attenuated in the blood. By contrast, a Δ*entD* mutant producing only aerobactin retained full virulence, whereas a Δ *entD*Δ*iuc* strain was completely attenuated. In co-infection experiments with the wild-type strain Δ *entS* and Δ *iroC* mutants were highly attenuated, suggesting that loss of the export capacity of endogenous catecholate siderophores was largely responsible for attenuation. In accordance with these findings, loss of *entS* and *iroC* also resulted in increased secretion and intracellular accumulation of catecholate siderophore monomers. Abrogation of catecholate siderophore synthesis in the Δ*entS*Δ*iroC* strain restored virulence. Overall, results establish that EntS and IroC mediate specific export of different types of catecholate siderophores. Further, the role of these exporters in ExPEC during infection is contingent on cathecholate siderophore synthesis, which in itself is not required when other siderophores such as the aerobactin system are functional.

019

INCREASED PHO REGULON ACTIVATION CORRELATES WITH DECREASED VIRULENCE OF AVIAN PATHOGENIC *ESCHERICHIA COLI*

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Avian pathogenic *Escherichia coli* (APEC) strains are associated with respiratory infections and septicemia in poultry (1,2). The Pho regulon is jointly controlled by the two-component regulatory system PhoBR and by the phosphate-specific transport (Pst) system (3,4). It is well recognized that inactivation of the Pst system, constitutive expression of the Pho regulon and bacterial virulence are linked (4). In APEC strain χ 7122, a deletion of the *pstCAB* genes was shown to reduce virulence in a chicken infection model and to affect multiple virulence attributes (5,6,7). To determine the involvement of the PhoBR regulon and the Pst system in the pathogenesis of the APEC O78 strain χ7122, different *phoBR* and *pst* mutant strains were tested for *in vivo* and *in vitro* virulence traits. Mutations resulting in constitutive activation of the Pho regulon affected the in vitro virulence attributes. In addition, production of type 1 fimbriae was also impaired in these strains. Using a chicken competitive infection model, all PhoB constitutive mutants were out-competed by the wild-type parent, including strains containing a functional Pst system. In addition, cumulative inactivation of the Pst system and the PhoB regulator resulted in a restoration of virulence. In addition, loss of the PhoB regulator alone did not affect virulence in the chicken infection model. Interestingly, the level of attenuation of the mutant strains correlated directly with the level of activation of the Pho regulon. Overall, results indicate that activation of the Pho regulon rather than phosphate transport by the Pst system plays a major role in the attenuation of APEC

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020

NEW INSIGHTS INTO THE PATHOGENESIS OF *CLOSTRIDIUM PERFRINGENS* TYPE B, C AND D DISEASES

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Clostridium perfringens type B, C, and D isolates cause disease in sheep, pigs, goats, cattle and other animals. In addition to expressing alpha toxin, Type B and C isolates always produce beta toxin, while type B and D isolates always make epsilon toxin. However, some type B-D isolates can express up to 5 different toxins, raising questions about which toxins are most important for the pathogenesis of type B-D isolates. Recent animal model studies are using isogenic mutants to determine the contributions of individual toxins to the virulence of type C or type D isolates. A functional VirS/VirR system was shown to be important for the *in vivo* virulence of type C isolates. Collectively, these new findings have significant implications for understanding type B-D-induced disease and may impact diagnosis and vaccine development. Genetically, both beta and epsilon toxins are encoded by large plasmids that exhibit some variability in size and gene carriage. However, some epsilon toxin plasmids show consistent relatedness to one another and to other *C. perfringens* toxin plasmids. In addition, most beta toxin plasmids and epsilon toxin plasmids carry the *tcp* locus, which has been shown to mediate conjugative transfer of the tetracycline resistance plasmid pCW3. Direct conjugative transfer of epsilon toxin plasmids has also been demonstrated. In addition to being localized to conjugative plasmids, the beta toxin and epsilon toxin genes are also closely associated with insertion sequences that can apparently mobilize these toxin genes. Therefore, the beta toxin and epsilon toxin genes are highly mobile, which might contribute to the establishment of disease by allowing transfer of these toxin genes, to type A isolates resident in the normal gastrointestinal flora of animals.

021

NEW INSIGHTS INTO THE PATHOGENESIS OF OF MAN AND PIGS

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Clostridium perfringens is an important extracellular pathogen causing a variety of diseases in animals and humans by secreting potent exotoxins. *C. perfringens* type C isolates have long been known to cause a fatal, segmental, necro-haemorrhagic enteritis in animals and humans. Epidemiological and experimental data showed, that beta-toxin (CPB) is the main virulence factor of pathogenic type C strains. Typical lesions are acute, segmental intestinal necrosis and haemorrhage. Histopathologically, these lesions are accompanied by microvascular thrombosis in affected intestinal segments. In immunohistochemical studies on spontaneously affected piglets and human patients we demonstrated endothelial localization of CPB. We therefore hypothesized that CPB contributes to local vascular necrosis by damaging endothelial cells directly. To investigate whether CPB disrupts the endothelial barrier *in vitro,* we exposed primary porcine aortic endothelial cells as well as human endothelial cells to *C. perfringens* type C culture supernatants and recombinant CPB (rCPB). *C. perfringens* type C culture supernatants and rCPB, induced marked cytopathic and cytotoxic effects in endothelial cells. Exposure to very low concentrations of CPB rapidly caused disruption of the actin cytoskeleton and cell border retraction in endothelial cells. This lead to a disruption of the endothelial monolayer. Cytopathic effects were followed by necrotic cell death. We furthermore demonstrated that CPB acts via multimeric pore formation at the plasma membrane of endothelial cells. In summary our results suggest, that a disruption of endothelial cells by CPB contributes to the rapid and fulminant tissue necrosis which occurs in *C. perfringens* type C enteritis in animals and humans.

022

THE DETERMINATIVE ROLE OF RTX TOXINS IN HOST SPECIFICITY OF PATHOGENIC *PASTEURELLACEAE*

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RTX toxins are bacterial pore forming toxins that are particularly abundant among pathogenic species of *Pasteurellaceae* where they play a major role in virulence. RTX toxins of several primary pathogens of the family of *Pasteurellaceae* are directly involved in causing necrotic lesions of the target organs. Many RTX toxins are mainly known as haemolysins due to their capacity to lyse erythrocytes *in vitro*, an effect that is non-specific, but serves as a useful marker for bacterial diagnostics and as an easily measurable signal in certain experimental *in vitro* setups. More recent results show that the specific targets of most RTX toxins are leukocytes, where RTX toxins bind to the corresponding β-subunit (CD18) of β2 integrins and then cause a cytotoxic effect. After uptake, at sub-lytic concentrations, certain RTX toxins are addressed, upon uptake by the target cell, to mitochondria and induce apoptosis. For several RTX toxins the binding to CD18 was shown to be host specific and seems to be the basis determining the host range of a given RTX toxin. Observations on very closely related species of the *Pasteurellaceae* family such as *Actinobacillus suis*, a strict porcine pathogen affecting particularly suckling pigs and *Actinobacillus equuli* subsp. *haemolytica* that causes Pyosepticaemia of the new-born foal (sleepy foal disease), revealed that they express different RTX toxins, named ApxI/II and Aqx respectively. These RTX toxins show specific cytotoxicity towards porcine and equine leukocytes respectively. Furthermore the ApxI and Aqx toxins of these species, expressed as recombinant toxins in an isogenetic background of *Escherichia coli*, showed specific cytotoxicity to leukocytes of their respective hosts. These data indicate the determinative role of RTX toxins in host specificity of pathogenic species of *Pasteurellaceae*.

023

DISTRIBUTION, EXPRESSION AND SECRETION OF THE RTX-TOXIN GTXA AMONG MEMBERS OF THE GENUS *GALLIBACTERIUM*

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Gallibacterium anatis is a frequent cause of salpingitis and peritonitis in egg-laying chickens globally. The RTX-toxin (repeat in toxin) GtxA is responsible for *G. anatis'* haemolytic and leukotoxic activity and is expexted to be a key virulence factor. The haemolytic activity of individual *G. anatis* strains and strains of other *Gallibacterium* species vary considerably likely influencing their pathogenic potential. The aim of this study was to investigate the background for these variations by (i) examining *gtxA*'s distribution and sequence conservation (ii) identifying the secretion system responsible for the export of GtxA and determine its distribution and (iii) examining expression differences among *gtxA*-positive strains by evaluating *gtxA*-mRNA levels and GtxA protein levels. The *gtxA* gene was present in strains belonging to the three haemolytic *Gallibacterium* species; *G. anatis*, *G.* genomospecies 1 and *G.* genomospecies 2. *gtxA* was present in both nonhaemolytic (biovar anatis) and haemolytic (biovar haemolytica) *G. anatis* strains. However, only strains of biovar haemolytica produced detectable levels of GtxA. We identified a type I secretion system locus (*gtxEBD*) responsible for the export of GtxA. Its presence correlated with *gtxA*'s, thus, the biovar anatis strains did not lack a secretion system. Targeted mutagenesis showed that at least one biovar anatis strain did produce functional GtxA. In just two of nine biovar anatis strains examined, *gtxA* was interrupted by a transposon insertion indicating that the nonhaemolytic phenotype of biovar anatis strains have individual explanations. Expression of GtxA varied among biovar haemolytica strains and the intra-species variation in haemolytic activity was partly explained by differences in expression levels. In conclusion, the presence of *gtxA*, *gtxEBD* and the production of GtxA determine the haemolytic activity of *Gallibacterium* strains.

024

NATURAL SELECTION IN THE CHICKEN HOST IDENTIFIES KDO KINASE RESIDUES ESSENTIAL FOR PHOSPHORYLATION OF *PASTEURELLA MULTOCIDA* LPS

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Pasteurella multocida, the causative agent of fowl cholera, expresses two LPS glycoforms simultaneously (glycoforms A and B) that differ only in their inner core structure. The key difference between the two structures is that the glycoform A inner core contains a single phosphorylated Kdo residue (lipid A-Kdo-P) with the phosphate group frequently substituted with phosphoethanolamine (PEtn), whereas the glycoform B inner core contains two Kdo residues (lipid A-Kdo-Kdo). For assembly of the inner core, KdkA is required for the phosphorylation of the single Kdo residue in glycoform A and two acceptor-specific heptosyl-1-transferases, HptA (glycoform A) and HptB (glycoform B) are required for the addition of the first heptose. Assembly of both glycoforms beyond the first heptose residue is performed by the same set of LPS transferases. Strains lacking a functional *hptA* gene are avirulent; they express full length glycoform B, plus a large amount of severely truncated glycoform A, which renders them vulnerable to the innate immune response in the host. However, in this study we found that the injecting the *hptA* mutant into chickens at high doses selected for virulent *hptA* mutants within the population that had lost the expression of the truncated glycoform A. Analysis of the genes essential for glycoform A assembly revealed that all the *in vivo*-derived *hptA* mutants analysed had second-site suppressor mutations within *kdkA*, encoding the Kdo kinase (KdkA) that adds a phosphate group to the glycoform A Kdo residue. The *kdkA* mutations identified included the single amino acid substitutions A112V, R123P, H168Y and D193N. Complementation of a defined *kdkA* mutant with wild-type *kdkA* restored expression of glycoform A to wild-type levels, whereas complementation with A112V, R123P, H168Y or D193N *kdkA* did not. This study demonstrates that the amino acids A112, R123, H168 or D193 are critical for the function of the KdkA kinase.

025

STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE EXTRACELLULAR PROTEASES OF *DICHELOBACTER NODOSUS*, THE CAUSATIVE AGENT OF OVINE FOOTROT

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Dichelobacter nodosus is the essential causative agent of ovine footrot, a highly contagious disease that is characterized by the separation of the hoof from the underlying tissue. Major virulence factors include type IV fimbriae and extracellular proteases. *D. nodosus* secretes three subtilisin-like proteases whose analysis forms the basis of diagnostic tests that differentiate between virulent and benign strains. We have mutated all three protease genes in *D. nodosus*; their analysis in a sheep virulence model revealed that one of the proteases, AprV2, was required for virulence. These studies challenge the previous hypothesis that the elastase activity of AprV2 is important for disease progression, since *aprV2* mutants were virulent when complemented with *aprB2*, which encodes a variant that has impaired elastase activity. We have determined the crystal structures of both AprV2 and AprB2 and characterized the biological activity of these enzymes. These data reveal that an unusual extended disulphide-tethered loop functions as an exosite that governs the ability of AprV2 to degrade insoluble extracellular matrix components. The disulphide bond and Tyr92, which was located at the exposed end of the loop, were functionally important. Bioinformatic analyses suggested that other bacterial pathogens, of both humans and animals, may have similar proteases and we postulate that this exosite mechanism may represent a more common biochemical process. In conclusion, we have used a integrated multidisciplinary combination of bacterial genetics, whole animal virulence trials in the original host, biochemical studies and comprehensive analysis of crystal structures, to provide the first definitive evidence that the extracellular secreted proteases produced by *D. nodosus* are required for virulence and to elucidate the molecular mechanism by which these protease bind to their natural substrates.

026

A TWO-COMPONENT SYSTEM MODULATES TWITCHING MOTILITY IN DICHELOBACTER NODOSUS

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Dichelobacter nodosus is a gram negative, anaerobic bacterium that is the principal causative agent of footrot in sheep. The major virulence factors of *D. nodosus* are type IV fimbriae and extracellular proteases. We have previously shown that the fimbriae are essential for twitching motility, protease secretion, and adhesion and that twitching motility is essential for virulence in sheep. Genome sequencing of a virulent isolate of *D. nodosus* identified a signal transduction system with similarity to the *Escherichia coli* CheA/CheY chemotaxis system. Bioinformatic analysis predicted that the sensor kinase, TwmS, contained C-terminal domains similar to CheA, while the N-terminal region had limited homology to methyl accepting chemotaxis proteins (MCPs). This analysis suggested that TwmS may function as a chemotaxis sensor as well as acting as a sensor histidine kinase. The cognate response regulator, TwmR, was predicted to have a single receiver domain and like CheY had no C-terminal DNA binding domain. Insertional inactivation of *twmR* led to a twitching motility defect in the resultant mutant. This defect was analysed by video microscopy, which showed that the twitching motility rate of the mutant was significantly lower than that of the wild-type and that the remaining movement was less directional. This difference in twitching motility was not due to the production of fewer fimbriae or to a slower growth rate. Complementation of the mutant with an intact copy of *twmR* restored the twitching motility phenotype to that of the wild-type. Finally, a bacterial two hybrid system was used to detect protein-protein interactions between TwmS and TwmR and between TwmS and TwmS. In conclusion, we have identified a novel signal transduction system in *D. nodosus* that modulates twitching motility, a major virulence factor in this important pathogen.

027

PLASMID-ENCODED GENES INVOLVED WITH THE RHAMNOSE BIOSYNTHETIC PATHWAY (*RFB* GENES) AND/OR GLYCOSYLATION CONTRIBUTE TO VIRULENCE IN *BRACHYSPIRA HYODYSENTERIAE*

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The anaerobic intestinal spirochaete *Brachyspira hyodysenteriae* is the aetiological agent of swine dysentery (SD). The pathogenesis of SD is incompletely understood, but chemotaxis and motility are important for spirochaetal colonization of the large intestine, while local colitis is thought to be mediated via one or more haemolytic toxins and/or the activity of the spirochaetal lipooligosaccharide (LOS). The aim of the current study was to investigate the presence of potential virulence-associated genes amongst *B. hyodysenteriae* strains. DNA microarrays containing 1,718 of the predicted 2,669 (64%) open reading frames of *B. hyodysenteriae* strain WA1 were designed and manufactured by Affymetrix, and were hybridised with DNA prepared from six virulent strains of *B. hyodysenteriae* and eight strains that have been reported to be avirulent or of low-virulence. The only consistent difference found in hybridisation profiles between these two sets of strains was in a block of six genes: these were located on the ~36 Kb plasmid of *B. hyodysenteriae* WA1, and were present in the virulent strains but not in the avirulent strains. These genes were predicted to be involved in rhamnose biosynthesis (*rfb* genes) and glycosylation, and hence to be associated with lipooligosaccharide assimilation and protein glycosylation. To help evaluate the potential influence of these genes on virulence, 24 pigs were experimentally challenged with a *B. hyodysenteriae* strain naturally lacking the plasmid and 12 were challenged with a strain possessing the plasmid. Eleven of the latter 12 pigs became colonized and developed SD compared to 13 of the 24 pigs challenged with the *B. hyodysenteriae* strain lacking the plasmid. This difference in incidence was significant ($P = 0.03$), and supports the likelihood that the plasmid-encoded genes contribute to virulence in *B. hyodysenteriae*.

028

NOW YOU SEE IT, NOW YOU DON'T – REGULATION OF CAPSULE AND PROTEIN EXPRESSION IN *STREPTOCOCCUS ZOOEPIDEMICUS*

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Streptococcus zooepidemicus (*S. equi* subsp. *zooepidemicus*), although normally a benign resident of the equine tonsillar complex, has the potential to become a highly virulent pathogen in the lower respiratory tract. Isolates from pneumonic lung are usually encapsulated but tonsillar isolates are not. Our recent studies have focused on proteins of Sz from cases of equine pneumonia, expression of which was co-regulated with hyaluronic acid synthesis and might therefore affect virulence. Two classes of isolates were recognized, SzT_{37} in which capsule expression was significantly upregulated at 37ºC but unstable, and SzT_{22} in which capsule expression was stable and significantly greater at 22ºC than 37ºC. Encapsulated (cap +) but not cap – of Sz grew in fresh horse blood. Surface expression of DnaK and alanyl-tRNA synthetase (AlaS) was upregulated in the (cap +) SzT_{37} group. Secreted proteins of 70, 64 and 58 kDa were also upregulated. The SzP protein of cap – SzT_{37} strains was smaller by 5kDa. Surface-exposed leucyl-tRNA synthetase (LeuS), enolase, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and arginine deiminase were upregulated in cap + SzT_{22} strains. These strains also secreted proteins of 125, 120, 70, 47, 37 and 34 kDa into culture supernatant. Both surface and secreted proteins of the SzT_{37} and T_{22} groups showed variable reactivity with convalescent horse sera. However, secreted enolase and a 115 kDa protein of unknown function were consistently immunoreactive in strains of both groups. In conclusion, encapsulation appears to be coregulated with numerous surface and secreted proteins, some of which are potentially involved in chaperone, cell division and plasminogen binding activities.

029

CELLULAR INTERACTIONS IN MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS INFECTION

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The study of host immune responses to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is complicated by a number of factors, including the protracted nature of the disease and the stealthy nature of the pathogen. Noted as one of the more fastidious mycobacteria, infection with MAP is often characterized by periods of subclinical infection extending for 3 to 5 years. Many animals will clear the infection during this period but it is almost impossible to distinguish by current methods animals that have cleared the infection from those that remain infected but are able to control the progression of disease. Escalation of paratuberculosis to a more clinical state, marked by diarrhea and weight loss, is thought be caused by immune dysfunction. Early measures of host immune responses to infection with mycobacterium, including MAP, have been dominated by a strong bias towards Th1-mediated IFN-γ production. Since IFN-γ is a key effector cytokine involved in the activation of T cells and macrophages, maturation of dendritic cells, upregulation of MHC I and II molecules, and production of reactive oxygen and nitrogen species by macrophages, it is purported to be not only an immune response variable but also a correlate of immune protection. However, it is unlikely that the paradigm of host immune responses in the early stages of infection and subsequent resistance to infection is quite so simplistic. Rather, a complex coordination of immune responses is more probable, with these responses shifting as the host transitions through the different stages of infection and disease (subclinical to clinical).

030

PATHOGENESIS OF LEPTOSPIROSIS: THE INFLUENCE OF GENOMICS

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Studies of the pathogenesis of leptospirosis in the pre-genomic era were hampered by the lack of genetic tools for manipulation of pathogenic *Leptospira* species. The availability of genome sequences for pathogenic and saprophytic *Leptospira* spp. has allowed comparative genomics approaches to identify genes unique to pathogens. However, genes of unknown function are over represented in this subset of pathogen-specific genes, suggesting that mechanisms of pathogenesis for *Leptospira* may differ from those in better characterised bacterial species. This notion is supported by studies with whole genome microarrays which showed that the majority of genes differentially regulated under simulated *in vivo* conditions, such as physiological temperature, osmolarity or iron concentration, were also of unknown or poorly defined function.

The development of a transposon mutagenesis system for pathogenic *Leptospira* has allowed the construction of a bank of defined mutants, while with direct genomic DNA sequencing has facilitated the identification of the disrupted gene. This approach has identified unequivocally for the first time a small number of leptospiral virulence factors, including lipopolysaccharide, flagella, heme oxygenase and an OmpA like protein.

031

MULTIDRUG EFFLUX PUMPS CONFER RESISTANCE OF *SALMONELLA* ENTERITIDIS TO EGG WHITE BY THE EXPORT OF ANTIMICROBIAL COMPONENTS THROUGH THE TOLC OUTER MEMBRANE CHANNEL

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Salmonella Enteritidis contamination of chicken eggs has been a major threat for human health since many years. Although the exact mechanism for the specific ability of the serotype Enteritidis to contaminate eggs is unknown, an enhanced survival of the bacteria in the hostile environment of the egg albumen, compared to other serovars, has been suggested. Using inoculations in egg white, we could indeed show that the serotype Enteritidis was superior in egg white survival compared to other serotypes. In order to identify bacterial factors that are responsible for this enhanced survival, an "*in vivo"* expression technology (IVET) screening was used. The *tolC* gene, encoding the outer membrane channel of the multidrug efflux pumps, was shown to be highly expressed in egg white. In order to confirm the role of this gene in egg white survival, a *tolC* deletion mutant strain was constructed. This mutant was no longer capable of surviving in egg albumen, while complementation reversed survival. The expression of the *tolC* promoter was evaluated using a reporter plasmid in which a transcriptional fusion between the *tolC* promoter and the reporter gene was present. An enhanced expression of the *tolC* promoter upon contact with egg albumen was shown. As multidrug efflux pumps have been shown to be associated with the efflux of antibiotics out of the bacteria, it is suggested that these pumps export antimicrobial molecules to survive in specific biological niches, such as the egg white. Chromatographic methods were used to identify egg antimicrobial proteins of the egg white that are exported by the efflux pumps through *tolC*. Whether this protection mechanism is specifically used by the serotype Enteritidis is currently under investigation.

032

THE ROLE OF NITRIC OXIDE IN LPS-INDUCED MUSCLE WASTING IN C2C12 MURINE MYOTUBES

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Nitric oxide (NO) has been recognized as a vital messenger molecule, which plays a crucial role in many physiological and pathological processes within the animal and human body (Culotta & Koshland, 1992) . Production of NO has been characterised for immune cells such as dendritic cells, NK cells etc (Bogdan, 2001) . Skeletal muscle has also been shown to produce cytokines amongst which TNF-α. LPS and TNF- α have been shown to induce the excretion of NO through the stimulation of NOS in skeletal muscle. Although, the precise role of NO in skeletal is still unknown it might play a pathophysiological role during sepsis. Recently NO has shown to decrease protein synthesis by regulating the phosphorylation of eukaryotic initiation factor eIF2α (Frost *et al.*, 2003) . In our experiment, we incubated C2C12 murine myotubes cells with LPS (1μg/ml) for various time periods (5 and 30mins, and 1, 3, and 18h). Conditioned media was collected and stored at -20°C. NO production was measured as μM nitrite using the standard Griess assay (Ding *et al.*, 1988; Sung *et al.*, 1991) . Our findings showed that LPS significantly increased NO production in C2C12 murine myotubes by 12% ($p<0.05$), 13% ($p<0.05$), and 19% ($p<0.01$) at 1h, 3h, and 18h time points respectively. Using the same model, LPS also significantly increased the rate of protein degradation and decreased protein synthesis.

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033

GALLIBACTERIUM ANATIS DOES NOT REQUIRE EXPRESSION OF A CAPSULE TO CAUSE LESIONS AND MORTALITY IN INTRAPERITONEALLY INFECTED CHICKENS

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In attempt to identify putative virulence factors in *Gallibacterium anatis* biovar *haemolytica* (12565-12), we localized a 16.9 kb locus predicted to be involved in capsule biosynthesis. The *cap* locus organization resembled that of the *E. coli* group 2 capsules, being divided into three regions containing genes predicted to be involved in synthesis, lipidation and export, respectively. Transmission electron microscopy of polycationic ferritin-labelled bacterial cells revealed a thin capsule. Transcriptional analysis of genes from region I and II was performed using reverse transcriptase-PCR and confirmed that the genes were expressed during late logarithmic growth phase *in vitro*.

To test the phenotypic significance of the capsule we constructed a capsule mutant by allelic exchange. There was no difference between the level of serum resistance observed for the wild type and the isogenic capsule mutant. The importance of the *Gallibacterium* capsule during infection of chickens was tested by inoculating chickens intraperitoneally with 10^8 CFU of either the wild-type or the acapsular mutant.

Surprisingly, the acapsular mutant was still able to cause disease. Indeed, four out of eight animals infected with the mutant strain died within 48 hours, while only two out of eight animals died after having been inoculated with the wildtype strain. Interestingly, the mutant caused more severe lesions than the wild-type strain including oophoritis, salpingitis and peritonitis. Therefore, capsule is not essential for the virulence of *Gallibacterium anatis* in chickens.

034

HOST DEFENCE PEPTIDES OF FARM ANIMALS: FUNCTIONS AND APPLICATIONS

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Effector molecules of the innate immune system are present on and within all surfaces of the organism that are in contact with the outside world. These molecules comprise host defence peptides (HDPs) and proteins like collectins that either kill microbes directly or enable killing of microbes by phagocytic cells. This broad-spectrum defence system is evolutionary very ancient, and many species rely solely on this system. However, in vertebrates the system remained important in spite of the superposition of a very sophisticated adaptive immune system. Evidence is accruing that these effector molecules are responsible for warding off many threats of infection, without eliciting an inflammatory response, an important feature of the system that prevents tissue damage. In vertebrates effector molecules have additional functions, such as modulation of the adaptive immune response. We identified and characterized several HDPs and collectins from chickens and pigs. The significance and possible applications of some of these molecules will be discussed.

035

THE MOLECULAR FUNCTION AND EVOLUTION OF MEMBRANE ATTACK COMPLEX / PERFORIN-LIKE (MACPF) PROTEINS

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The Membrane Attack Complex / Perforin-like (MACPF) proteins perforin and complement C9 are mammalian pore forming molecules associated with the immune response against viruses and pathogenic bacteria respectively. Further, recent studies suggest that perforin may be important for tumour surveillance and in eliminating pre-cancerous cells.

Recently, we showed that MACPF proteins are distantly homologous to cholesterol dependent cytolysins (CDCs), an important family of bacterial pore forming toxins. Based upon these data it is suggested that, like CDCs, MACPF proteins may firstly oligomerise on the surface of the membrane to form a pre-pore form. Followed pre-pore formation it is suggested that a concerted conformational change permits two clusters of a -helices to unwind and insert into the membrane as amphipathic b -strands. Here, using structural, biophysical and mutagenesis studies we test this hypothesis and investigate how perforin as well as related MACPF-domain-containing proteins form membrane inserted pores. We also investigate how certain mutations in MACPF proteins result in dysfunction and disease. In particular, it is suggested that many perforin mutations may interfere with proper perforin folding whereas other mutations may directly interfere with the mechanism of membrane insertion. Finally, we discuss the evolution of the MACPF/CDC family particularly with respect to a number of important bacterial pathogens that cause disease in animals.

036

ALTERATION OF THE PIG DISTAL GUT MICROBIOTA BY TYLOSIN AS AN ANTIBIOTIC GROWTH PROMOTER

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Antibiotics have been used extensively as growth promoters (AGPs) in agricultural animal production. The specific mechanism of action for AGPs is unknown. Following demonstrations that antibiotics do not have growth-promoting effects in germ-free animals, studies of the mechanism for growth promotion have focused on interactions between the antibiotics and the gut microbiota. We hypothesized that the effects of AGPs are mediated by influencing compositional changes of the pig gut microbiota. Chronic administration of AGPs also have been used to control infectious diseases. Fecal samples from Tylosin (40mg/kg) treated (n=10) and Tylosin-non-treated pigs (n=10) were collected 5 times at 3-week intervals starting when the pigs were 8 weeks of age. The sequences of 16S rRNA were generated using 454 pyrosequencing. Sequences were quality assessed, aligned, and manually curated. Phylogenetic assessment was conducted using RDP classifier, and richness and diversity indices were generated using Mothur. Sequencing generated over a million sequence reads. The pig gut bacterial communities of both groups were dominated by Firmicutes and Bacterioidetes accounting for >80% of total sequences, and showed highly diverse community structure (Shannon > 4.3, and Simpson 1-D >0.9). Most of the sequences (>95% of the total sequences) were shared by pigs in the two treatment groups. Components of the classes Actinobacteria, Clostridia, Fibrobacter, and Erysipelotrichi were different between the groups. To determine if Tylosin had an affect on the carriage of one infectious agent, Salmonella enterica, fecal samples were cultured. The prevalence and concentration of Salmonella enterica found in pigs treated with Tylosin was the same as non-treated pigs. Generalized inflammation is another measure of infectious diseases. However there was no difference in generalized inflammation as measured by C-reactive protein. These results provide us with a reference of the pig distal gut microbiota profile and fundamental knowledge for later studies to design new strategic approaches to replace AGPs in the future, such as probiotics or prebiotics.

037

NEW PROSPECTS ON THE AERODIGESTIVE IMMUNITY FOR MUCOSAL VACCINE DEVELOPMENT

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The mucosal immune system (MIS) associated with the aero-digestive tract (ADT) is capable of inducing a prompt and robust antigen-specific mucosal immune response at the entry of pathogen in order to prevent invasion of the infectious agent. Further, the administration of vaccine via the ADT associated mucosa resulted in the induction of systemic immunity, leading to the double layers of protection at mucosal and systemic comportments. The delivery of vaccine antigen to the MIS is thus logical and known to be effective and non-invasive for the induction of antigen-specific mucosal and systemic immune responses against emerging and re-emerging infectious microorganisms. In order to achieve our common goal for the development of most attractive, effective and safe mucosal vaccine delivery system, our laboratory has been developing two novel antigen delivery vehicles, rice-based vaccine (MucoRice) and nanogel-based vaccine (Chaperon vaccine). MucoRice, a seed of transgenic rice plant expressing vaccine antigen, is a cold-chain- and needle-free vaccine which offers long-term stability of vaccine antigen more than 3 years without any refrigeration storage and resistance to digestive enzyme. These unique characteristics qualify MucoRice system as a new generation of oral vaccine production, preservation, and delivery system. Oral vaccination of MucoRice thus resulted in the induction of antigen-specific protective immunity in both mucosal and systemic compartments without any major adverse events. Further, nanogel-based vaccine (or Chaperon Vaccine) holds vaccine antigen in the nanoparticles formed by cationic cholesteryl group-bearing pullulan (CHP) and functions as an antificial chperon for the delivery of native form of vaccine antigen to the MIS for the induction of antigen-specific immune response. The adaptation of chaperonging nanogel technology led to the creation of an adjuvant- and needle-free nasal vaccine. Nanogel-based vaccine is effective in the delivery of vaccine antigen to the upper respiratory MIS including nasophalynx-associated lymphoid tissue and nasal epithelium leading to the induction of protective immunity in both mucosal and systemic compartments. These new generation mucosal antigen delivery vehicles will lead to the innovative vaccination strategy against emerging and re-emerging infectious diseases.

038

CLOSTRIDIUM PERFRINGENS VACCINES TO PROTECT CHICKENS AGAINST NECROTIC ENTERITIS

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Clostridium perfringens is an anaerobic, spore-forming, gram-positive, bacterial pathogen that causes a variety of diseases in humans and animals. In chickens it causes necrotic enteritis, a disease which is estimated to cost the global poultry industry $2 billion/annum in prevention, control and production losses. In most production animals clostridial infections have been well controlled with conventional toxoid vaccines. However, the development of a highly protective conventional vaccine for use in chickens has proven difficult. To address the need for a necrotic enteritis vaccine we are investigating two complementary approaches. Firstly, we are investigating the use of the secreted toxin, NetB, the recently identified major virulence factor found in pathogenic strains of *C. perfringens* associated with necrotic enteritis, as a protective vaccine antigen. Secondly, we are taking a "reverse vaccinology" approach to identify antigens. We have used complete genome information from a chicken derived strain of *C. perfringens* to predict the full complement of proteins that are likely to be seen by the host immune system. A high-throughput pipeline has been developed which facilitates rapid PCR amplification, cloning, expression and protein purification and this has enabled us to screen a large number of potential antigens for protective efficacy in a disease induction model. A number of antigens have provided good levels of protection when delivered as simple subunit vaccines. Further investigation, using whole genome information from a range of *C. perfringens* isolates, has shown the level of antigen conservation across strains and has helped focus our efforts on particular antigens.

039

DEVELOPMENT AND EVALUATION OF GAMMA IRRADIATED TOXOID VACCINE OF *SALMONELLA ENTERICA* VAR TYPHIMURIUM

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Salmonella enterotoxin (Stn) of *Salmonella enterica* serovar Typhimurium (DT 193) was inactivated with 40kGy of gamma (γ) rays. The gamma irradiated toxoids were then used to formulate a single effective vaccine against non-typhoidal salmonellosis which is very challenging due to the presence of hundreds of serovars of *Salmonella*. The gamma irradiated toxoid vaccine (ITST) containing 100µg protein/ml was administered subcutaneously into 3 weeks old poultry birds. A booster dose was administered on the 14th day post primary vaccination. Birds in the control groups received PBS instead. All the birds were challenged on the 28th day post-primary vaccination with homologous (*S.* Typhimurium) and heterologous (*S.* Gallinarum) challenges, intraperitoneally and orally (10^8 organisms / ml). The immune responses of the immunized birds were monitored every day. ITST afforded 100 percent protection and cross-protection to the vaccinated birds against both homologous and heterologous challenges. The overall protective index (PI) was found to be 95.83. On the other hand, 100 percent mortality was observed in the unvaccinated birds after challenge. The mean (± SE) serum antibody titre for ITST peaked (3.143 ± 0.031) in 6th week post primary vaccination which slowly declined to reach 2.602 ± 0.045 in the 10th week. After assessing all the tests results, it was concluded that gamma irradiated toxoid vaccine (ITST) prepared from *Salmonella* enterotoxin of *Salmonella enterica* serovar Typhimurium (DT 193) was very effective in protecting poultry birds against experimental salmonellosis by homologous as well as heterologous serovars of *Salmonella*.

040

COLONISATION OF THE CHICKEN INTESTINE BY SALMONELLA ENTERITIDIS: ANALYSIS OF *IN VIVO* PROTEINS AND POTENTIAL FOR VACCINATION

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Salmonella are important causes of human food poisoning, poultry are the main source. Although, *S.* Typhimurium and *S.* Enteritidis produce little systemic disease in adult chickens, in colonizing the alimentary tract they contaminate the carcasses and enter human food chain. Salmonellosis costs the EU 500-900 million Euros annually. Salmonellosis in food animals is a major target for reduction of human infection by the EU. The major serovars in terms of public health consequences are *S.* Typhimurium and *S.* Enteritidis, which together caused 75% of all cases in Europe in 2002. The caeca are the main sites of chicken gut colonization. However, the mechanism of colonization is not clear. Preventing initial colonization is important in reducing infection. In this study, newly-hatched chickens were infected with *S.* Enteritidis, the bacteria were harvested from the caeca, proteins were extracted by sonication and analysed utilizing 1-D gel electrophoresis, followed by identification of proteins by mass spectrometry. The immunogenicity of *S.* Enteritidis proteins harvested from chicken and those from broth culture were compared as orally inoculated vaccine candidates in chickens. The results demonstrated that extracted proteins have a stronger vaccination effect against *S.* Enteritidis colonisation in chickens than formalin killed bacteria. In particular, proteins from *in vitro* preparations had much greater effect than proteins from *in vivo* material. In addition, *S.* Enteritidis mutants were created to a selection of the proteins identified from *in vivo* and *in vitro* preparations. The capability of these mutants will be assessed in chicken colonization to detect the best candidate for a rationale live vaccine.

041

TRANSCRIPTIONAL ANALYSIS OF *SALMONELLA* ENTERITIDIS P4 COLONISATION IN THE CHICKEN

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The recent association between *S.* Enteritidis P4 and poultry products has caused a great deal of concern from adverse publicity and with resulting national and international requirements to control the major food-poisoning *Salmonella* serotypes at the breeder and layer levels in order to ensure that poultry products are *Salmonella*-free. The exact mechanism whereby these serotypes are able to colonise the intestine of chickens is still exactly unknown.

Indeed, there is increasing evidence that colonisation is not solely a metabolic function but that some form of physical association with cells or an organ in the gut is involved. Thus, invasion and fimbrial genes required for colonisation were identified suggesting physical contact was required.

An alternative approach would be to analyse the patterns of gene expression by microarray analysis at the site of colonisation (caeca). This has been done for a number of niches and is now being applied to intra-cellular infection but has not so far been applied to the intestine.

The S. Enteritidis transcriptome during the colonization of the caeca of one day chicks was characterised by Agilent microarray. The Microarray results were evaluated by real-time PCR with 95% compatibility. Fumarate respiratory and osmotic response genes were selected from the up-regulated genes and were mutated and tested in the lab for their inhibitory effect and for competitive growth under anaerobic and osmotic environment showing variable responses.

There is considerable scope for improvement in inactivated vaccines through a more rational approach and the work presented in this project could form the basis to such a vaccine.

042

DEVELOPMENT OF NEW VACCINE STRATEGIES AGAINST *PASTEURELLA MULTOCIDA*

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Pasteurella multocida serogroup A is the bacterial pathogen causing shipping fever in young dairy calves. Cattle samples found to be positive for *P. multocida* were predominantly serotype A:3. This study focuses on strategies to develop novel vaccines against *P. multocida* P-1062 serotype A:3 isolated from the lung of a calf affected with shipping fever. Three DNA vaccines were constructed using *ompH* gene encoding outer membrane protein H and *plpE* gene coding for *Pasteurella* lipoprotein E from *P. multocida* strain P-1062. One of these DNA vaccines was constructed using *ompH* gene without a signal sequence. The other two were N terminal fragment of *plpE* without a signal sequence (*plpE*N) and C terminal fragment (*plpE*C). The genes were amplified via PCR and cloned in pGEMT vector. Nucleotide sequences were analyzed and submitted to GenBank. The genes were then cloned in pCMV vector and expressed under CMV promoter. Prior to vaccination of mice, expression of *ompH*, *plpE*N and *plpE*C with a *gfp* gene at 3' in HEK-293 cells were visualized under fluorescence confocal microscope. Two protein based vaccines composed of OmpH-PlpEN and OmpH-PlpEC were also obtained. Fusions of these genes were expressed in pET28a vector and His-tagged proteins were purified using nickel based columns. Purity of the proteins were checked via SDS-PAGE. Six BALB/c mice per group were vaccinated with these DNA and protein based vaccines and blood samples were collected. Humoral and cellular responses raised against these vaccines are being assayed by ELISA technique using the sera collected from vaccinated animals.

043

PROGRESS TOWARDS A VACCINE AGAINST EXTRAINTESTINAL PATHOGENIC *ESCHERICHIA COLI*

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Escherichia coli is a common colonizer of the human gastrointestinal tract. However, some isolates have the potential to also cause disease. Pathogenic *E. coli* can be divided into three major subgroups depending on their pathogenic traits: commensals or nonpathogenic; pathogenic causing intestinal infections, and extraintestinal pathogenic *E. coli* (ExPEC). The ExPEC group includes human and animal pathogens causing urinary tract infections (UPEC), and others causing neonatal meningitis (NMEC) and septicemia. The development of an efficacious ExPEC vaccine would have a significant public health and economic impact, considering the increasing antibiotic resistance among ExPEC strains and the associated mortality, morbidity and lost productivity. The attempts to develop a broadly protective and safe vaccine against ExPEC have not been successful due to the large antigenic and genetic variability of these strains. Today, multiple genomic sequences of *E. coli* have been determined providing an opportunity to use a "reverse vaccinology" approach to identify novel vaccine candidates. Since most of the genomes available are from uropathogenic strains, we have decided to sequence a neonatal meningitis-associated K1 *E. coli*, IHE3034, and compare this to available genome sequences of other ExPEC strains and a few nonpathogenic *E. coli*. By using a subtractive reverse vaccinology approach we identified 230 antigens present in ExPEC but absent (or present with low similarity) in nonpathogenic strains. Nine antigens were protective in a mouse sepsis challenge model. The gene encoding the most protective antigen was detected in most of the *E. coli* isolates, highly conserved in sequence and found to be exported by a type II secretion system, which seems to be non-functional in nonpathogenic strains. Interestingly, some of the antigens identified are conserved not only in ExPEC strains but also in pathogenic intestinal *E. coli*, suggesting that they may be useful for a broadly cross-reactive *E. coli* vaccine.

POSTER PRESENTATIONS

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FACTORS INVOLVED IN BIOFILM FORMATION BY *STREPTOCOCCUS UBERIS*

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Mastitis, an inflammation of the mammary gland, remains a major challenge to the worldwide dairy industry despite the widespread implementation of mastitis control strategies. *Escherichia coli* and *Streptococcus uberis* are now the two most common causes of bovine mastitis and are an increasing problem in low somatic cell count herds. We speculate that one of the reasons behind the increased prevalence of *S. uberis* infections is its ability to form biofilms. The objectives of this study were to determine the ability of *S. uberis* to produce biofilm. Twenty-nine isolates of *S. uberis* from dairy cattle (10 clinical, 10 subclinical and 9 low cell counts) were examined by the microtitier plate assay using Todd Hewitt broth (THB) as the basic medium. THB was supplemented with EDDA, lactoferrin, β-lactoglobulin , casein, sucrose, or lactose. Moreover, milk was added to THB (raw, heat treated, skim milk, with and without filtration to remove indigenous flora) in different percentages; 12.5%, 25% or 50%, to test the effect of milk compounds and indigenous flora on biofilm production. A known biofilm producer, *Staphylococcus epidermidis* RP62A , was used as a positive control. Most isolates produced very small amounts of biofilm; the amount produced was not affected by iron limitation or by supplementing the medium with β-lactoglobulin , casein, sucrose or lactose. Biofilm production by most isolates was enhanced in the presence of whole milk and skim milk in a dose-dependent manner, although there were differences between the behaviour of individual isolates. In general Biofilm production was greatest in the presence of raw milk and least with skim milk. Removal of indigenous flora by filtration reduced the amount of biofilm. The results suggested that the indigenous flora of milk might interact with *S. uberis* and enhance biofilm production. Given the economic impact of mastitis on the dairy industry, continued research into understanding the factors that influence the ability of *S. uberis* to colonize and maintain infections is critical for appropriate treatment.

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ISOLATION OF *TATUMELLA PTYSEOS* FROM BEEF IN IBADAN, NIGERIA

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Two hundred meat samples randomly collected from Bodija meat market in Ibadan, South Western Nigeria were culturally examined. Four Isolates resembling members of the enterobacteraceae by colony characteristics, cell morphology and gram staining reaction were further examined on MacConkey, 5% sheep blood agar plates and Simmon's citrate agar slope. The four isolates were biochemically characterised using microbact^{TM} GNB, 24E kit (Oxoid, Basingstoke, UK). Other biochemical tests performed include oxidase, catalase, nitrate reduction, esculin hydrolysis and phenylalanine deaminase tests. Motility test was performed using the hanging drop method at 25^0C and 37^0C. Four isolates from 200 meat samples analysed were identified as *Tatumella ptyseos* using an online Advanced Bacterial Identification Software (ABIS). The susceptibility of the four isolates to antimicrobial agents was tested using antibiotic tests discs (Oxoid, UK). All the four isolates grew well under aerobic conditions with no haemolysis on the sheep blood agar. Lactose was not fermented on MacConkey agar. On sheep blood agar, colonies were 0.5 - 1mm in diameter, smooth with entire edge after 24h incubation. The four isolates were gram negative bacilli, oxidase negative, Catalase positve, Simmon citrate positive at 25^0C but not at 37^0C and phenylalanine deaminase positive. Acid was not produced from mannitol, xylose, lactose, adonitol, raffinose, malonate, inositol, sorbitol and rhamnose. The four isolates were motile at 25^0C but not at 37^0C. The four isolates were sensitive to gentamicin, oxytetracycline, ciprofloxacin, norfloxacin, penicillin, enrofloxacin and amoxycillin and resistant to sulphamethoxazole. The four isolates were homogenous with regards to their cultural and biochemical characteristics, cell morphology and antimicrobial test results, suggesting a single species and strain. There are little or no information on previous isolation of this organism from meat (beef) in Nigeria. The identification of *T. Ptyseos* in fresh meat (beef) in Nigeria as well as other food sources around the world and its complication in human infections should be of interest to scientists, especially the pathogenic potential of this organism.

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MUCOSAL AND SYSTEMIC IMMUNOGENICITY OF FUSION OF SE18.9 TO SEM OF *STREPTOCOCCUS EQUI*

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Equine strangles caused by *Streptococcus equi* (Se) continues to be prevalent in horse populations throughout the world. Vaccines are important for control but are either of low efficacy or have safety concerns. An improved strangles vaccine is therefore, a high priority. There is evidence that protective immunity to Se involves a tonsillar mucosal adaptive immune response.

Recently discovered in our laboratory, protein Se18.9 of Se has been shown to be a potent adhesin for tonsillar epithelium suggesting use as a carrier for delivery antigens to elicit protective mucosal immune responses.The antiphagocytic SeM is a major virulence factor and protective antigen of Se. Recombinant plasmids encoding Se18.9 and a fragment comprising 161-346 aa of SeM were constructed. The selected SeM fragment contains the region with the greatest number of epitopes reactive with serum and mucosal antibodies from convalescent horses. Substantial elevation of mucosal and serum antibodies against SeM and Se18.9 were detected following intranasal immunization of ponies with recSeM$_{161-346}$-Se18.9 and recSe18.9-SeM$_{161-346}$. No responses to SeM or Se18.9 was detected in ponies immunized with recSeM$_{161-346}$. Following commingling challenge with a virulent strain of Se, ponies immunized with recSeM$_{161-346}$-Se18.9 showed a substantial reduction in clinical manifestation of strangles compared with control ponies.

Our studies have therefore shown that fusion of a polypeptide to the N or C terminus of Se18.9 does not alter its mucosal binding capability or its immunogenicity and that of a vectored peptide.

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CROSS-SPECIES INFECTION AMONG RODENTS AND FARM ANIMALS BY *BRACHYSPIRA* SPP.

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Brachyspira spp. were isolated from rodents caught on four pig farms, three chicken farms, a city pond, and from pigs from two of the pig farms, and chickens from two of the chicken farms. Isolates from rodents and farm animals with identical phenotypic species characteristics were compared by randomly amplified polymorphic DNA (RAPD), and pulsed-field gel electrophoresis (PFGE). In addition, two isolates of *B. hyodysenteriae* from rats were studied by PFGE, and compared to previously typed isolates of *B. hyodysenteriae* from pigs and mallards. Identical RAPD and PFGE banding patterns were found among isolates of *B. pilosicoli, B. intermedia* and *B. murdochii* from pigs and rodents caught on the same farms, and of *B. murdochi* from chickens and rodents on one farm, suggesting cross-species infection at farm level. One isolate of *B. hyodysenteriae* collected from rat faeces on a pig farm showed unique RAPD/PFGE banding patterns. This farm had no history or clinical signs of swine dysentery. Surprisingly, the other isolate of *B. hyodysenteriae* from a rat caught at a city pond, showed a banding pattern similar to those of field isolates from previously sampled pigs. Necropsy and histological examination of rodents didn't reveal any signs of pathological changes. In conclusion, the results of this study indicate that rodents and pigs, and to a lesser degree rodents and chickens, on farms regularly carry apparently identical clones of *Brachyspira* spp., together with rodent-specific clones. The findings of *B. hyodysenteriae*, seemingly apathogenic to rats, may be useful in comparative studies of pathogenicity mechanisms in *Brachyspira* spp.

EVALUATION OF THE CORRELATION BETWEEN THE OCCURRENCE OF THE YMOA GENE AND PRODUCTION OF THE ENTEROTOXINS YST BY *YERSINIA ENTEROCOLITICA* USING MULTIPLEX PCR AND INFANT MICE ASSAY

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The purpose of the study is to evaluate the correlation between the occurrence of the *ymoA* gene and production of the enterotoxins Yst by *Yersinia enterocolitica* using multiplex PCR and infant mice assay. All the strains under study had the *ystA* gene, which indicates that they belong to the strains commonly regarded as pathogenic. Amplification of a fragment of *ymoA* gene was also detected in all the strains under study based on the presence of the 330 bp band. No 180 and 284 bp bands, indicating adequately the presence of the *ystB* and *ystC* gene amplicons were also obtained. In the study directed at evaluating the ability to produce enterotoxin *in vitro* with a test on infant mice, there were 95 *Yersinia enterocolitica* strains isolated from fattening pigs from farms with large herds – 39 strains were from a warm farming, and 56 from a cold farming. A positive bioassay result was stated in 14 cases, a doubtful result in 33 cases, the remaining 48 strains did not induce the production of enterotoxins. All *Yersinia enterocolitica* strains isolated in our study belonged to the biotypes and serotypes considered as pathogenic; they possessed the sequences characteristic for genes encoding the virulence markers in the form of enterotoxins Yst and YmoA protein, which testifies to their potential pathogenicity for humans. However, the dependence between the occurrence of the *ymoA* gene and the production of the enterotoxins Yst by *Yersinia enterocolitica*, determined by infant mice assay, was not shown, since all the studied strains possessed that gene. This report suggests that *ymoA* gene is present in all *Yersinia enterocolitica* strains, irrespective of their potential pathogenicity, whereas the lack of a silencing influence on other genes, including *yst genes* responsible for the production of enterotoxins, might probably result from a mutation occurring in its boundaries.

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CLOSTRIDIUM DIFFICILE SURVEY IN ITALIAN PIGGERIES AND MOLECULAR CHARACTERIZATION OF ISOLATES

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Little is known about the epidemiology of *C. difficile* (CD) within the animal population in Italy and the relationship between strains of animal origin and those related with human CD-infection (CDI). The objectives of our work were:

· to investigate the diffusion of CD and its toxins in Italian piggeries.

· to perform molecular characterization of swine isolates.

To these purposes 121 samples (79 faecal samples, 30 intestinal contents and 12 rectal swabs) were collected in 31 different farms from pigs with an history of diarrhoea. All samples were stratified according to the animal growth phase in: suckling, post-weaning, growing and fattening. Each sample was cultured in a selective medium for CD and the isolates were identified both by a commercial biochemical kit and by a species-specific PCR. The presence of toxin A, B and binary toxin encoding genes was subsequently revealed by a multiplex PCR (2,3). All isolates were then rybotyped (1) and metronidazole and vancomycin MICs were also determined by E-test strips.

CD was recovered from 30/121 samples (24.8%) collected in 11/31 different farms and the highest prevalence was detected in suckling piglets (52.63%). 29/30 strains (96.6%) carried the toxin coding genes whereas only 1/30 (3.4%) was non-toxigenic. The binary toxin genes were found in 14/30 strains (46.6%). 2/30 strains belonged to PCR-ribotype 020, 12/30 to the PCR-ribotype 078 and the other 15 belonged to two other ribotypes but not ascribable to the epidemic European CD strains used as a controls. None of the isolates resulted resistant to the first-choice antibiotics against human CDI. In summary our results demonstrate that CD is widespread in Italian piggeries and that, as previously reported in other studies, the strains belong mostly to PCR-ribotype 078.

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FIS IS A CRITICAL REGULATOR OF VIRULENCE GENE EXPRESSION IN *PASTEURELLA MULTOCIDA*

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Pasteurella multocida causes many economically important diseases in animals, including fowl cholera in birds. Most fowl cholera-causing isolates of *P. multocida* express a capsular polysaccharide composed of hyaluronic acid which is critical for virulence. There have been numerous reports indicating that during laboratory passage *P. multocida* may spontaneously lose the ability to produce capsule and become avirulent. However, the mechanism by which this occurs has not been determined. In this study, we characterized three independent spontaneously-arising acapsular strains. Quantitative RT-PCR showed that all strains had significantly reduced transcription of the capsule biosynthetic genes, but DNA sequence analysis identified no mutations within the *cap* biosynthetic locus including the promoter region. We used whole-genome sequencing of a capsulated strain and its acapsular derivative to show that the acapsular strain contained a mutation within *fis*, predicted to encode a transcriptional regulator. Sequencing of *fis* from another two independently derived, spontaneously-arising acapsular strains showed that each also contained a mutation within *fis*. Complementation of the strains with an intact copy of *fis* returned capsule expression to all strains. Therefore, Fis is absolutely required for capsule expression in *P. multocida*. To extend these results we used electrophoretic mobility shift assays and deletion analysis to show that Fis directly binds to the DNA region 75-149 bp upstream of the capsule locus promoter. As Fis acts as a global regulator in other bacterial species, we used DNA microarrays to compare the transcriptional profile of a *fis* mutant with the wild-type strain during exponential growth. Approximately 30 genes were down-regulated in the mutant showing that Fis is involved in the regulation of numerous *P. multocida* genes. These included genes encoding the known *P. multocida* virulence factor Pfh_B2 and the cross protective surface antigen PlpE.

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DETECTION AND DIVERSITY OF A PUTATIVE NOVEL HETEROGENEOUS POLYMORPHIC PROLINE-GLYCINE REPEAT (PGR) PROTEIN IN THE FOOTROT PATHOGEN *DICHELOBACTER NODOSUS*

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Dichelobacter nodosus, a Gram negative anaerobic bacterium, is the essential causative agent of footrot in sheep. Depending on the clinical presentation in the field, footrot is described as benign or virulent; *D. nodosus* strains have also been classified as benign or virulent based on laboratory tests, but this designation is not always consistent with clinical disease. Bioinformatic analysis of the *D. nodosus* VCS1703A genome and comparative genome hybridization identified a gene encoding a large, repetitive secreted protein (DNO_0690), designated here as Pgr for proline-glycine repeat, that was present in virulent strains but absent in benign strains. The aim of this study was to determine the diversity of the *pgr* gene in *D. nodosus* isolates from different geographic locations. The *pgr* gene was present in all isolates of *D. nodosus* that were examined and based on analysis of their sequence; two variants, *pgrA* and *pgrB* were observed . In *pgrA*, there were two coding tandem repeat regions, R1 and R2, with variable numbers of repeats in each region. These regions were absent from *pgrB*. Both variants were present in strains from Australia, Sweden and the UK, however, only *pgrB* was detected in isolates from Western Australia. The *pgrA* gene was detected in *D. nodosus* from tissue samples from two flocks in the UK with virulent footrot, but only *pgrB* was found in a flock with no virulent or benign footrot for >10 years. Bioinformatic analysis of the PgrA protein suggested that it contained a cell surface anchor motif that might be involved in attachment to the host in the invasion process. Our results suggest that the *pgr* gene may be a useful molecular marker for epidemiological studies.

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MOLECULAR INSIGHTS INTO THE INNATE IMMUNE RESPONSE OF BOVINE ENDOMETRIAL CELLS TO THE ZOONOTIC ABORTIFACIENT PATHOGEN *LEPTOSPIRA*

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Leptospirosis, caused by the abortifacient zoonotic pathogen *Leptospira*, is responsible for economic loss in the cattle industry and is also emerging as a globally important disease among the human population. Limited research has been performed on the mechanism by which *Leptospira* interact with bovine endometrium. This study investigates modulation of TLR2 and TLR4 by *Leptospira hardjo bovis* strain hardjo bovis, and the effect of pregnancy and stress upon this, using a bovine caruncular epithelial cell line (BCECT1). Results from semi-quantitative polymerase chain reaction (PCR) showed the mRNA expression of TLR1-4, 6 and 9, oestrogen and progesterone receptor expression by the BCECT1 cell line. Quantitative PCR showed that LPS increases the TLR2 and TLR4 mRNA expression of the BCECT1 cell line, and that progesterone and cortisol reduces the increase seen with LPS challenge. Live and heat-killed *Leptospira* also modulated TLR expression. The effect of *Leptospira* infection on proinflamatory cytokine and prostaglandin production will be shown.

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ESCHERICHIA COLI STB ENTEROTOXIN INTERNALIZATION IS CELL TYPE DEPENDENT

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Previous studies have shown that *Escherichia coli* STb enterotoxin is internalized by intestinal epithelial cells suggesting that internalization is required for STb pathogenesis. Using flow cytometry, we studied internalization of STb labelled with fluorescein isothiocyanate in porcine intestinal epithelial IPEC-J2 and control murine fibroblast NIH-3T3 cell lines. In contrast to the selective pronase resistance of STb in NIH-3T3 cells at 37°C but not at 4°C, indicative of toxin internalization, most of STb toxin was pronase sensitive at both temperatures in IPEC-J2 cells. Inhibitors of cytoskeleton rearrangement showed that actin reorganization is required for STb internalization by NIH-3T3 cells. Nevertheless, inhibition of endocytosis of lipid rafts, known to contain sulfatide, the STb receptor, with methyl-β-cyclodextrin or genistein did not preclude toxin association with either cell line. Sulfatide is present in the plasma membrane of both cell lines and anti-sulfatide antibodies are internalized by both cell lines over time at 37°C. STb internalization is therefore differentially regulated in various cell types. Interestingly, porcine epithelial cells showed reduced uptake relative to control NIH-3T3 cells. This suggests that toxin uptake may not necessarily be related to biological activity in porcine host cells. Factors other than sulfatide may be involved in regulating STb internalization in porcine intestinal epithelium.

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THE MULTIMERIC PORE FORMING STRUCTURE OF THE EPSILON TOXIN FROM *CLOSTRIDIUM PERFRINGENS*

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The epsilon toxin is one of the major toxins produced by *C. perfringens*, particularly by toxinotypes B and D of the bacterium. The crystal structure of epsilon prototoxin has been determined to 2.6 Å resolution by X-ray crystallography and revealed a high structural similarity with aerolysin from *Aeromonas hydrophila* (Cole *et al.*, 2004), which is considered as the prototype of the small beta pore-forming toxins. The epsilon toxin has a mass of 32.5 kDa and is secreted by the bacterium as an inactive prototoxin, which has to be processed first for its activation by proteases which are either provided by the host, as trypsin or chymotrypsin, or by the bacterium itself, as the λ-protease (Bhown & Habeerb, 1977, Minami *et al.*, 1997). Cleavage of the C-terminal part of the protein is absolutely necessary, as several studies showed that recombinant proteins without an unprocessed C-terminus were unable to oligomerise and form a functional pore complex (Miyata *et al*., 2001). So far, the crystal structure of the activated toxin or the assembled pore complex have not been determined. In order to do so, the epsilon toxin was overexpressed and purified in its activated form. The protein was analysed by mass spectrometry and PAGE and preliminary structural data has been obtained. Site directed mutagenesis studies of functionally important residues for its biological activities are underway.

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ANTIBACTERIAL EFFECTS OF *THYMUS VULGARIS, ECHINACEA PALLIDA* AND *EUCALYPTI FOLIUM* ON *E. COLI* AND *SALMONELLA* SPP. ISOLATED FROM CHICKENS

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Nowadays, the use of essential oils of plants as food preservative and antimicrobial agents are increasing possibly due to having a low level of toxicity and broad spectrum of activities. *Thymus vulgaris, Echinacea pallida* and *Eucalypti folium* have been used as medicinal plants in various parts of the world.

The commercial extracts of *Thymus vulgaris, Echinacea pallida* and *Eucalypti folium* (both extract and essence) were used. Bacteria were isolated from chickens and characterized. Their antimicrobial activities were determined against 34 *E. coli* and 30 *Salmonella* species using impregnated filter paper disk diffusion method. Minimum inhibitory concentrations (MIC) of the products were also determined using serial dilution technique.

E. coli and *Salmonella* reflected the highest and the least sensitivities to thymus and eucalyptus extracts, respectively. The disk diffusion method showed that the zones of inhibition for *E. coli* ranged from zero to 14 mm. The widest diameter (19mm) belonged to the thymus extract. The serial dilution method illustrated that 1/64 dilution is the equivalent MIC value for both *E. coli* and *Salmonella*.

Generally, the results of the present study indicate that the above plant products exert low to moderate antibacterial potency against the test microorganisms

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ABORTION DUE TO *MANNHEIMIA HAEMOLYTICA* IN A COW

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The causes of infectious abortions in cattle are numerous, and opportunistic bacteria represent a large percentage among all the microorganism involved. *Pasteurella spp*. are infrequently implicated in bovine abortion, and according to literature, *P. multocida* is the only species which has been reported to be associated with this pathological process in cattle.

A nine month old bovine fetus was presented for bacteriological examination. Following the routine culture methods and according to the results *Mannheimia haemolytica* was considered to be the direct cause of the abortion. Susceptibility test was performed for isolated bacteria with some antibiotic disks. The results showed that this bacterium is sensitive to Difloxacin (DIF25), Chloramphenicol (C30), Trimethoprim Sulfamethoxazole (SXT), Erythromycin (E15), Tetracycline (Te30), and Tylosin (Ty30). To our knowledge, this seems to be the first report of an abortion in cattle due to *M. haemolytica* in Iran

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BACTERIAL MASTITIS IN SHEEP AND GOATS AND ITS PROBABLE RELATIONSHIP WITH BACTERIAL FLORA OF THE MOUTH OF *LAUDAKIA NUPTA*, A SMALL REPTILE, IN SOUTH OF IRAN

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Mastitis is one of the more common health problems affecting sheep and goats. Severe cases can result in death of the ewe, but more often it takes its toll in the form of treatment costs, premature culling, and reduced performance of lambs and kids.

Purpose of this study was to investigate the bacterial agents of mastitis in sheep and goats flocks in Darab city, Fars province, south of Iran. The investigation was also extended to the probable relationship of the mastitic agents and bacterial flora of the mouth of *Laudakia nupta*, a small reptile which is abundant in the region. Sheep and goats owners often say that the teat canal of ewes and goats are bitten by a small reptile. The bite can cause infection leading to severe inflammation, pus and even sloughing the teat canal.

Seventy milk samples from ewes and goats and 34 swab samples from the mouth of the reptiles were investigated for aerobic and facultative anaerobic bacteria. Single colonies were identified at species level according to standard methods. Staphylococci (17, %26), streptococci (9, %14.5), salmonellae (6, %9.6) and corynebacteria (5, %8) were the predominant isolated bacteria. *S. aureus* was one of the most common bacterium isolated from both milk and reptile samples. several samples from both milk and reptiles had no growth.

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FUNCTIONAL ANALYSIS OF THE C-TERMINAL DOMAIN OF THE EXTRACELLULAR PROTEASE APRV5 FROM *DICHELOBACTER NODOSUS*

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Dichelobacter nodosus is the principal causative agent of ovine footrot. The virulence factors of *D. nodosus* include type IV fimbriae and extracellular proteases. Virulent isolates of *D. nodosus* secrete three subtilisn-like serine proteases: AprV2, AprV5 and BprV. These enzymes are each synthesized as a precursor molecule, including a signal or pre-peptide, a pro-peptide and a C-terminal domain, each of which is processed to the mature active form. The function of the C-terminal domain of these proteases and the mechanism of protease processing and secretion are unknown. It has been shown that AprV5 contributes to the majority of the protease activity secreted by *D. nodosus*. To understand the role of the C-terminal domain of AprV5, we constructed a series of C-terminal-truncated deletions in *D. nodosus* by allelic exchange. Analysis of these strains showed that the C-terminal domain of AprV5 is required for total protease activity. We also demonstrated that AprV5 is involved in processing AprV2 and BprV to their mature forms and that the C-terminal domain of AprV5 is required for this function. In addition, we obtained evidence that the C-terminal domain cleavage sites of these proteases could not be accurately predicted by bioinformatic analysis. These studies have increased our understanding of how these virulence-associated proteases interact and are secreted.

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USE OF TRANSPOSON MUTAGENESIS AND MICROARRAY ANALYSIS TO IDENTIFY GENES ASSOCIATED WITH BIOFILM FORMATION IN *ACTINOBACILLUS PLEUROPNEUMONIAE*

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Actinobacillus pleuropneumoniae is the Gram-negative bacterium responsible for porcine pleuropneumonia. This respiratory infection is highly contagious and characterized by high morbidity and mortality rates. In some cases, *A. pleuropneumoniae* infection can develop into a chronic disease. Pigs suffering from a chronic infection are considered to be carriers and these carriers likely play an important role in transmission. In several bacterial infections it is known that persistent chronic infections are characterized by the presence of a biofilm. Furthermore, it was recently demonstrated that clinical isolates and reference strains of *A. pleuropneumoniae* can form biofilms. Therefore, it was hypothesized that *A. pleuropneumoniae* form biofilms during the infection process. The objective of this study was to identify genes associated with biofilm formation. Two approaches were used to accomplish the objective. The first approach used transposon mutagenesis analysis of *A. pleuropneumoniae* serotype 1 strain S4074, the subsequent transposon library was screened to identify mutants with increased or decreased ability to form biofilms in 96-well polystyrene plates. In the second approach, the transcript profiles of 6 hour biofilm cells was compared to the transcript profiles of 6 hour planktonic cells using an *A. pleuropneumoniae*-specific microarray. The transposon mutagenesis analysis identified 78 mutants that exhibited abnormal biofilm formation, at least 32 unique genes were identified. Most genes identified in the enhanced-biofilm mutants encoded proteins related to the cell-envelope, whereas most genes identified in the biofilm-negative mutants encoded proteins related to transport and metabolism. Microarray analysis flagged 49 up-regulated genes and 68 down-regulated genes in the 6 hour biofilm. The down-regulated genes were mostly associated with energy metabolism, whereas the up-regulated genes were mostly associated with transporters. Interestingly, 5 genes were identified in both analyses. In conclusion, both approaches allowed the identification of new genes associated with biofilm formation in *A. pleuropneumoniae*.

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CLINICALLY ASYMPTOMATIC ENDEMIC *CHLAMYDIA PECORUM* INFECTIONS REDUCE MILK PRODUCTION AND QUALITY IN DAIRY COWS VIA INFLAMMATORY LIVER INJURY

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Infections with chlamydiae, particularly *C. pecorum*, are ubiquitous in cattle worldwide, with seroprevalences approaching 100% (1). The vast majority of *Chlamydia* spp. infections in cattle are not associated with obvious clinical disease (2,3). We have previously shown that vaccination against *C. pecorum* and *C. abortus* temporarily eliminates clinically inapparent bovine mastitis associated with chlamydial infection in cattle (4), but mechanisms by which these *Chlamydia* spp. infections cause mastitis and milk production losses are unclear. Our study investigated the effects of asymptomatic chlamydial infections on bovine milk and serum parameters. All cows had high concentrations of IgM serum antibodies against *C. pecorum*, indicating endemic and recurring asymptomatic infections. Low numbers of *C. pecorum* were detected in 12% of the vaginal swabs, but only in 0.7% of quarter milk and in zero mammary tissue samples. Thus, mastitis associated with chlamydial infection primarily results from systemic effects of mucosal infections (5) rather than from local effects of direct udder colonization. In principal component analysis, two indices composed of anti-*C. pecorum* IgM levels, serum cholesterol, albumin, and globulin, separated all cases into 3 clusters. All clusters had similar frequencies of chlamydial detection, but clusters 1 and 2 had significantly lower incidences of bacterial mastitis than cluster 3. Cluster-1 cows also had the highest chlamydial antibody levels and optimum parameters for liver health (high serum cholesterol and albumin, low globulin; 6,7), produced most milk with lowest SCC, and importantly had the highest fertility as indicated by calving interval. Cluster-2 cows had significantly lower chlamydial antibodies, and lower metabolic health, milk production, and fertility. Cluster-3 cows had significantly lower chlamydial antibodies than cluster-1 cows, and had the lowest liver health, production, and fertility. Therefore, high immunity to chlamydiae associates highly significantly with improved health and performance in dairy cows.

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PCR-RFLP ANALYSIS OF *BORDETELLA BRONCHISEPTICA* STRAINS ORIGINATED FROM DIFFERENT HOSTS ON *FLA*A GENE

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Bordetella bronchiseptica is a widespread Gram negative bacterium occurring in different mammal species. It is known to play a role in the aetiology of infectious atrophic rhinitis of swine, in canine kennel cough, respiratory syndromes of cats and rabbits, and sporadic human cases have also been reported. Motility is important in approaching a susceptible host and flagellin, the monomeric component of flagella, is a potent pro-inflammatory factor of *B. bronchiseptica*. The BvgAS system of *B. bronchiseptica* regulates reciprocally the flagellum gene transcription and the expression of virulence factors. Study of the flagellin gene(s) provides an alternative genotyping approach and is an incidental marker of host-adaptation. Eighty three strains from different geographical regions and from different hosts were studied on the 1165 base pairs of *fla*A with PCR-RFLP method. It resulted in eight variations (designated A to H) using three restriction endonucleases (*Hinc*II, *Bgl*I and *Msp*I). Most of the strains belonged to three major types (A, B, and C). Homogeneity of Hungarian isolates originated from dogs (type A) and guinea pigs (type C) was established, and only one strain from pig showed different fragments from the other pig isolates. Diversity and uniqueness of human isolates (type F) and turkey originated strains (type E and G) was also noticed. Signs of host adaptation were observed in distinct geographical regions. Prevalent types were established by PCR-RFLP analysis among foreign and Hungarian strains originated from pigs and dogs. Strains isolated from pigs had the same RE-type (B) at a rate of 89% while strains isolated from dogs had the same RE-type (A) at a rate of 71%. Isolates from rabbits showed RE-types A and B in equal portions.

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IMMUNOPROTEOMIC ANALYSIS OF CELL ENVELOPE PROTEINS OF *BRUCELLA CANIS* FOR VACCINE DEVELOPMENT

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Brucella canis is the specific agent of canine brucellosis. Canine brucellosis is a contagious disease with venereal and oral modes of transmission that results in late abortion in females, epididymides and prostatitis in males. For the control of brucellosis in animals, live vaccines such as *B. abortus* RB51 and *B. melitensis* Rev1 have been using in animals, however there are not available vaccines for canine brucellosis. For the development of vaccine for canine brucellosis, this study investigated cell envelope proteins of *B. canis* that show immunodominant activity using 2DE and western blot which was performed with antisera obtained from naturally infected dogs. A total of 12 proteins were identified by using MALDI-TOF MS and these proteins seem to be used for developing of a novel vaccine against *B. canis* infection.

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INTRA-SPECIES VARIATION IN *ACTINOBACILLUS PLEUROPNEUMONIAE* – TRANSCRIPTIONAL RESPONSE TO IRON LIMITATION IN SEROTYPES WITH DIFFERENT VIRULENCE POTENTIAL

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Background: Comparative analysis of gene expression among serotypes within a species may provide valuable information of important differences in related genomes. For the pig lung pathogen *Actinobacillus pleuropneumoniae* (Ap), 15 serotypes with a considerable variation in virulence potential have been identified [8,5,6,7]. This difference is only partly explained by the difference in RTX toxin genes in their genomes [4,1]. Iron acquisition *in vivo* is an important bacterial function during infection. In this study, gene expression in response to iron restriction *in vitro* in six Ap serotypes of variable virulence was studied, applying a NimbleGen microarray targeting the genomes of all the included serotypes.

Results: In total, 45 and 67 genes were significantly ($p < 0.0001$) up- or down-regulated, respectively, in response to iron limitation. 12 of these genes also displayed significant serotype related response to iron limitation including three co-regulated, putative haemoglobin-haptoglobin binding proteins which have recently been described in Ap [3] and share homology with the HmbR haemoglobin receptor of *Neisseria meningitidis* (Nm), which contributes to Nm survival in rats [9]. Except for the moderately virulent serotype 6, the expression of this gene cluster was at the highest in the most virulent serotypes, 1 and 5.

Conclusion: Comparative analysis of gene expression among 6 different serotypes of Ap identified a common set of genes involved in iron regulation. The results support previous observations concerning the identification of new potential iron acquisition systems in Ap [3,2], showing that this bacterium has evolved several strategies for scavenging the limited iron resources of the host. The conjugated effect of iron-depletion and serotype proved to be modest, indicating at least *in vitro* that serotypes of both medium and high virulence are reacting almost identical to iron restriction. One notable exception, however, is the haemoglobin-haptoglobin binding gene cluster, which merits further investigation.

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MODULATORY EFFECTS OF PROBIOTIC LACTIC ACID BACTERIA ON VIRULENCE AND PATHOGENICITY OF VTEC AND *SALMONELLA TYPHIMURIUM*

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Probiotics are defined as live microorganisms that confer a benefit to the host when administered in adequate amounts. Although the concept of probiotics has been widely accepted, the mode of action and the molecular and cellular mechanisms underlying the probiotic effects remain largely to be elucidated.
The modulatory effects of probiotics on virulence of pathogenic *E. coli* have been investigated using DNA microarray technology and real-time PCR. Co-culture of VTEC with sub-lethal doses of *Lactobacillus rhamnosus* resulted in down-regulation of toxin genes and genes coding for potential virulence associated factors such as genes encoding the expression of flagella, motility and chemotaxis.
In addition, anti-inflammatory effects of probiotics were investigated using in vitro cell culture model. Probiotic lactobacilli and bifidobacteria showed modulatory effect on *Salmonella typhimurium*–induced inflammatory host response and inhibited production of chemokine interleukin-8. In conclusion, our study suggests that probiotics possess anti-inflammatory properties and modulate virulence gene expression in pathogenic *E. coli.*

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DETECTION OF *HELICOBACTER HEILMANNII* IN THE GASTROINTESTINAL TRACT OF STRAY CATS

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The main *Helicobacter* species identified in the stomach and oral secretions of cats are *H. heilmannii* and *H. felis* but presence and importance of them in other parts of alimentary tract are largely unknown. The aim of current study was detection of *H. heilmannii* in different parts of gastrointestinal tract of stray cats by polymerase chain reaction. Tissues from six cats were obtained and genus-specific PCR identified 34/36 (94.4%) of the samples as *Helicobacter* spp.-positive. *H. heilmannii* species-specific PCR revealed 4/6 (66.6%), 1/6 (16.6%), 0/6, 2/6 (33.3%), 0/6 and 0/6 infection in gastric, gall bladder, pancreas, duodenum, jejunum and colon specimens, respectively. It seems that *H. heilmannii* is mainly colonized in upper GI tract but not in the large intestine. Currently, efforts are being conducted in our lab to detect other species

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SALMONELLA ENTERITIDIS UNIVERSAL STRESS PROTEIN (USP) GENE EXPRESSION IS STIMULATED BY EGG WHITE AND SUPPORTS OVIDUCT COLONIZATION AND EGG CONTAMINATION IN LAYING HENS

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Hen eggs contaminated with *Salmonella* Enteritidis have been a major source of human food-borne salmonellosis in the last 25 years. Despite the huge importance for public health, it is not yet resolved why this particular serotype is highly capable to contaminate eggs. Using an *in vivo* expression technology (IVET) approach, genes involved in cell wall integrity, regulation of fimbrial operons, stress responses and motility were identified as highly expressed in the oviduct tissue. Using this expression screening method, the universal stress protein A and B genes (*uspA*, *uspB*) were identified to be highly expressed in the oviduct tissue as well as in eggs, derived from different animals. Using a lux-dependant reporter system, it was shown that egg albumen, which is the product of the magnum compartment of the oviduct, induced usp expression. A deletion mutant was defective in oviduct colonization, although gut and spleen colonization was similar as compared to the wild type strain. An intravenous infection experiment showed a 80% decrease in the number of contaminated eggs after inoculation with the usp mutant as compared to the wild type strain. Usp genes are known to be expressed as a consequence of environmental stress conditions and seem to contribute to stress resistance. We is hypothesize that the usp genes could cause the *Salmonella* bacteria to reside in a non-replicating persistent state in the antibacterial oviduct and egg environment.

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REACTIVITY OF HEAT-STABLE LEPTOSPIRA ANTIGENIC PREPARATION USED IN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF LEPTOSPIRAL ANTIBODIES IN SWINE SERUM

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Serology plays an important role in laboratory diagnosis of leptospirosis. Apart of most often used microscopic agglutination test (MAT), enzyme-linked immunosorbent assay (ELISA) seems to be useful especially in screenings of animal herds. The ELISA set used in our laboratory for detection of antibodies against selected Leptospira serogroups in swine serum samples contains heat-stable antigenic preparation composed from cultures of Leptospira interrogans serovars Icterohaemorrhagiae and Pomona and L. borgpetersenii serovar Sejroe.

The aim of the present study was to identify and analyze ELISA heat-stable antigen fractions playing role in reaction with leptospiral antibodies indicated in swine serum.

Reactivity of three-component antigenic preparation was compared in immunoblotting with reactivity of six heat-stable antigenic preparations made from following single serovars: L. interrogans serovars Icterohaemorrhagiae, Pomona, Canicola, L. borgpetersenii serovars Sejroe, Tarassovi and L. kirschneri serovar Grippotyphosa. All antigenic preparations were submitted to SDS-PAGE and transferred to nitrocellulose membrane using semidry system. After the transfer, membrane was incubated with diluted swine serum containing antibodies specific for one of six mentioned above Leptospira serovars.

For the three-component antigenic preparation and antigens prepared from single serovars the immunoblot revealed reaction of sera with fractions of 20 - 26 kDa region and around 14 kDa region. Antigenic fraction 20 - 26 kDa was visible in each reaction of three-component antigen with serum specific for particular single serovar and on paths with single serovar antigens specific for serum used in the reaction. Antigenic fractions around 14 kDa were visible on all paths (three-component antigen and single antigens preparations) in reactions with majority of used sera.

Investigated heat-stable Leptospira antigenic preparation contains fractions demonstrating serogroup and species specificity. Fraction 20 - 26 kDa plays role in reactions with indicated in swine sera serogroup specific antibodies, whereas the fraction around 14 kDa represents rather species specific antigens.

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STUDY OF ANTIBACTERIAL EFFECTS OF SOUR AND SWEET POMEGRANATE PEEL EXTRACTS

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Nowadays, uncontrolled and continuous use of antibiotics may cause emergence of microbial resistance among pathogenic agents. Therefore, the use of new synthetic and natural antimicrobial compounds is inevitable. One source of natural compounds in this respect comes from herbs. The purpose of this study was to examine the antibacterial effects of peels extracts from sour and sweet pomegranate. Methanolic extracts of sour and sweet pomegranate peels and aqueous solution of tetracycline and chloramphenicol were prepared. Antibiogram tests using disk diffusion technique and serial dilution method was performed on ten pathogenic bacteria (*staphylococcus aureus, Enterococcus faecalis, Bacilus cereus, Clostridium perfringens, Listeria monocytogenes, E. coli O157: H7, Pasturella multocida, Psudomonas aeruginosa, Salmonella typhimurium, Yersinia enterocolitica)* isolated from animals, and relative MIC and MBC values were also determined for the above compounds. The greatest zone of inhibition induced by the action of herbal extracts was obtained for *staphylococcus aureus* (about 25 mm) and the smallest zone of inhibition was obtained for *Pasteurella multocida* (about 9 mm). In addition, the lowest MIC and MBC values of above herbal extract were obtained for *Staphylococcus aureus* (7.8 and 62.5 mg/ml, respectively). Results of serial dilution tests indicate that bactericidal effect of sour pomegranate peel extract exerts a bacteriostatic action against bacteria. The antibacterial effect was more against gram positive bacteria. Effects of these extracts were considerably lower than those for tetracycline and chloramphenicol. In conclusion, methanolic extracts of pomegranate peels exhibit releatively good bacteriostatic and bactericidal effects

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DETECTION OF *FUSOBACTERIUM NECROPHORUM* IN LAME CATTLE

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Lameness in dairy cows has been attributed to many causes including poor hoof structure, herd and dairy parlour management, microorganisms, the environment, climate and nutrition. Various microorganisms including *Fusobacterium necrophorum* are associated with lameness internationally.

Fifteen hoof scrapings from lame cows were collected from 8 commercial dairy farms around Shiraz, south east Iran.

Enriched blood agar was used for culture of *F. necrophorum.* As a consequence of the difficulty in isolating and culturing, a PCR technique was used to detect the organism.

None of the colonies on enriched blood agar was identified as *F. necrophorum*. Four (26.6%) out of the 15 hoof scrapings examined were tested positive for the presence of the *lktA* gene (402 bp) of *F. necrophorum.*Culture can not be considered as the gold standard method for isolating *F. necrophorum.* Molecular detection is suggested as an alternative method.

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DEVELOPMENT OF A MULTIPLEX PCR FOR DIFFERENTIATION OF *MYCOBACTERIUM TUBERCULOSIS* COMPLEX AND NONTUBERCULOUS MYCOBACTERIA

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Tuberculosis due to *Mycobacterium bovis* (*M.bovis*) in bovine and deer was identified as an important disease in Korea and the prevalence has increased in recent years. In the past 5 years, there has been rapid outbreak of bovine and deer tuberculosis in Korea .

Nontuberculous mycobacteria (NTM) are emerging pathogens causing opportunistic infections in humans and animals. And, also, NTM often confounds the interpretation of skin testing, resulting in false-positive reactions.

We developed a multiplex PCR (m PCR) targeting the *cfp* 32 and *hsp*65 gene for detection and differentiation of *Mycobacterium tuberculosis* complex (MTC) and NTM. The results were validated by DNA sequencing of the species –specific internal transcribed spacer (ITS) and 12.7-kb fragment region and were matched completely. A total of 332 isolates of *Mycobacterium* spp. originating from cattle and deer were collected from 2007 to 2010, with 98 and 234 isolates identified as MTC and NTM, respectively.

All MTC isolates was identified as *M. bovis*, and among the NTM, isolates of *M. avium* complex *are* most frequent, followed by , *Mycobacterium fortuitum* complex, *M. smegmatis* , *M. septicum, M.chelonae, M. aurum, M. kansasi* and *M. phlei.*

This m-PCR with DNA sequencing may be very useful as routine test for identification and differentiation of *Mycobacteria* spp. and is required for monitoring the spread of *M. bovis* and NTM in bovine and deer.

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EVALUATION OF A FLUORESCENCE POLARIZATION ASSAY FOR THE DIAGNOSIS OF *SALMONELLA* GROUP D INFECTION

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Introduction: Fluorescence polarization immunoassay (FPA) was developed for the diagnosis of bovine brucellosis by Nielsen et al [1] . The FPA is a homogeneous assay, very simple, available in the field, requires no washing and reduces human error with high sensitivity and specificity. In this study, we developed and evaluated the FPA for the diagnosis of *Salmonella* somatic group D infection like *S.* Enteritidis in chicken sera.

Materials and Methods: For the FPA to be used for the diagnosis of *Salmonella* group D, the *O* -polysaccharide (OPS) of *S. enteritidis* is labelled with fluorescein isothiocyanate (FITC) and used as the tracer. This tracer is added to diluted serum and a measure of the antibody content is obtained in about 2 minutes for serum using a fluorescence polarisation analyser. Sera from infected herds were used to compare the indirect ELISA (IELISA, Bioline). Sera were obtained from chicken, and were diagnosed by the rapid serum plate agglutination test and IELISA. Among these sera, positive reaction sera and negative sera in IELISA were used to analyze the ROC curve and determine the relative sensitivity of the FPA.

Results: The analysis of the ROC curve with the IELISA positive reaction sera and negative sera suggested the optimal cut-off value of 89 mP. The FPA was evaluated as 98.7% relative sensitivity and 99.8% specificity at the 89 mP cut-off value and the Kappa value was 0.98.

Conclusions: The present study suggests that the FPA is a rapid and accurate method and may be useful for the diagnosis of salmonellosis in farms, slaughter houses and livestock markets.

Acknowledgement: This work was supported by Korea Research Foundation Grant (KRF 2010-0002624).

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MAPPING SWEDISH OUTBREAKS OF *S. EQUI* INFECTION (STRANGLES) IN HORSES USING THE SEM GENE

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Strangles is a highly contagious upper airway disease in horses caused by the pathogenic bacterium *Streptococcus equi* subsp *equi* (*S. equi*). Clinical signs range from mild disease to life threatening illness and include fever, purulent nasal discharge, swollen lymph nodes of the head and neck, coughing and anorexia. An outbreak of strangles is usually also of substantial economic importance to the horse owners. Different strains of *S. equi* are highly homogenous and may therefore be difficult to subtype. Subtyping of strains is important to determine sources of outbreaks and to limit further spreading of the disease. SeM is a *S. equi* specific virulence factor variable at the N-terminus and sequencing of this gene has been shown to be a useful tool in tracing sources of strangles outbreaks (Kelly, C. *et al* 2006, Waller, A. S. *et al* 2007, Anzai, T. *et al* 2005). The SeM gene was sequenced for 36 *S. equi* isolates from eight strangles outbreaks in Sweden during 2009 and 2010 and compared to previously characterized SeM alleles in the online database http://pubmlst.org/szooepidemicus/seM/. The SeM sequence was identical within each outbreak, and several outbreaks clustered together. The results will be further discussed.

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(2) Waller, A. S. et al 2007

(3) Anzai, T. et al 2005

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COMPARATIVE ANALYSIS OF *SALMONELLA ENTERICA* SENFTENBERG FROM HUMANS AND ANIMALS

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Salmonella species are known worldwide as a major cause of disease in humans and animals. In this study, we assessed the relationship between *Salmonella senftenberg* isolates of human and animal origin (bovine, porcine and avian) for their molecular profiles using pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and antimicrobial susceptibility using the National Antimicrobial Resistance Monitoring System (NARMS). Included in the study is our own sequenced *S. senftenberg* strain isolated from a North Dakota turkey and found to possess a *Yersinia* HPI located on a 200kb plasmid.

Results from the data demonstrated that the PFGE provided greater differentiation of *S. senftenberg* strains compared to MLST alone. MLST analysis found that the majority of isolates were of ST 14 with a small number identified as ST 185. PFGE analysis separated the isolates into three primary clusters at approximately 75% similarity. Of interest was our sequenced strain which was found to cluster along with isolates of humans and turkeys. Antimicrobial susceptibility analysis found that the majority of isolates from humans were pan susceptible to all agents against which they were tested compared with the animal isolates which displayed multidrug resistance with most isolates displaying resistance to 4-6 antimicrobials.

Data from the study suggests that there is dissemination a small number of clones of *S. senftenberg* among food production animals and humans in the US.

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DETECTION OF *CAMPYLOBACTER FETUS* AND *TRITRICHOMONAS FOETUS* IN SOUTHERN AFRICAN CATTLE USING SPECIES-SPECIFIC PCR

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Tritrichomonosis and campylobacteriosis are venereally-transmitted diseases that are caused by parasitic protozoan *Tritrichomonas foetus* and *Campylobacter fetus* bacterium respectively. These sexually transmitted diseases continue to pose economic losses in Asia, America and southern Africa due to infertility and abortion. As numerous cattle are asymptomatic, accurate detection of the infected animals is important for curbing the proliferation *of C. fetus* and *T. foetus* among herds through effective management of the diseases. This scenario highlights the necessity for rapid and accurate diagnosis of *C. foetus* and *T. foetus*. Diagnosis of *C. fetus* using conventional microbiological techniques is challenging due to low discriminatory power of biochemical tests and reduced viability of the microorganisms. Likewise, *T. foetus* may be mistaken for similar trichomonadid protozoa. To avoid misdiagnosis, species-specific PCR can be used for diagnosis of these venereal diseases. The aim of this retrospective study was to apply an established species-specific PCR technique for detection of *C. fetus* and *T. foetus* among cattle in Southern African countries. The sheath washings and sheath scrappings were submitted to the PCR laboratory of the Agricultural Research Council-Onderstepoort Veterinary Institute from January 2010 to May 2010 inclusive for testing. Extraction of template DNA was performed using the Silica-Guanidium thiocyanate method. Amplification of template DNA was done using species-specific PCR for *C. fetus* and *T. foetus* and reference strains were included alongside DNA from field samples. The PCR amplicons were analysed by electrophoresis through 1.5% agarose gels, followed by observation under ultraviolet light. Thirty-one of the 962 tests (3.4%) for diagnosis of *T. foetus* were positive, whilst 13 of 931 tests (1.8%) for *C. fetus* were positive. The use of PCR for diagnosis of *T. foetus* and *C. fetus* was fast and accurate.

Key words: Species-specific PCR, 16S rRNA, diagnosis, venereal disease, *Campylobacter fetus*, *Trichomonas fetus*

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DIGESTIVE RESPONSE OF YOUNG RABBITS TO AN EXPERIMENTAL REPRODUCTION OF COLIBACILLOSIS ACCORDING TO TWO FEEDING STRATEGIES

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Enteric diseases frequently occur in rabbits around weaning leading to extensive use of antibiotics. Feed restriction is a strategy routinely used to protect rabbit health during rearing. The objectives were (1) to study the impact of enteropathogens (EPEC) on the balance of the rabbit gut microflora and (2) to study the effect of feed restriction on the infection level.

Rabbits (Hycole strain) were divided into 4 groups (n = 45/group) considering two factors at two levels. The first factor was the infection and the second the intake level (*ad libitum vs*. restricted at -25% of the voluntary feed intake). The infection was carried out at 31 days old (3 days post weaning) by oral inoculation of *Escherichia coli* 0128:C6. The excretion of total *E. coli* was quantified, ileal and ceacal bacterial communities were monitored by a fingerprint method (CE-SSCP), and ileal morphometry was also evaluated.

Diarrheas were observed between 3-6 days post inoculation (pi), the higher number of morbid animals was recorded 4 days pi. The peak of excretion was detected 6 days pi. The higher effect of the infection on bacterial community structure was detected 5-7 days pi. The infection had a significant impact on ileal bacterial community, differences (t-test) between profiles (uninfected *vs*. infected) were 38%. Feed restriction had a weak but significant impact (7%) on ceacal infected bacterial community. No effect of feed restriction on the intestinal microbial structure was detected in healthy rabbits. At 6 days post infection, the *villus* height was decreased by 25% in the infected group whereas no parameter of ileal morphometry was modified by feed intake level. The resilience of the ileal bacterial community at 12 days pi was faster with a feed restriction.

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A SIMPLE *IN VITRO* MODEL FOR MONITORING CAECAL MICROBIOTA ALTERATIONS DURING THE DEVELOPMENT OF CARBOHYDRATE-INDUCED EQUINE LAMINITIS

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Laminitis is the most serious disease of the equine foot, often necessitating destruction of the animal. Despite the long held recognition of the importance of laminitis, numerous gaps still persist in our knowledge of the pathophysiological mechanisms of this disease. One of the few aspects of carbohydrate-induced laminitis generally accepted is that hindgut bacterial fluctuations, occurring in response to consumption of excess dietary carbohydrates, are intrinsically linked to the development of foot pathology. There is now substantial evidence implicating the rapid proliferation and/or death en masse of hindgut streptococci as the precipitating event in the laminitis process. This project aimed to develop a simple, *in vitro* system for monitoring changes in bacterial populations of the equine caecum which could be used to supplement live animals in early stage laminitis trials.

Equine caecal fluid was incubated in bioreactors in the presence/absence of oligofructose and samples were collected over a 16h period. Samples were collected 2 hourly and analysed by denaturing gradient gel electrophoresis (DGGE), quantitative real-time PCR and fluorescence *in situ* hybridisation. Analyses indicated that bacterial diversity was maintained in the control bioreactor throughout the time course of the experiment and changes in the microbial diversity and abundance in the bioreactor supplemented with oligofructose approximated those observed in the caecal fluid of horses during the development of laminitis.

This *in vitro* system may be useful to analyse the efficacy of a variety of antimicrobial compounds to prevent changes in bacterial populations of the equine caecum previously identified as having a putative role in the laminitis process. It will not ultimately negate the need for animal trials; however, it provides a simple, economical and more humane means of performing the initial experiments for initial screening of antimicrobial compounds with potential for further investigation.

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IDENTIFICATION OF HOST PROTEINS THAT INTERACT WITH LIPL32, THE MAJOR OUTER MEMBRANE PROTEIN OF PAHTOGENIC LEPTOSPIRA, BY PHAGE DISPLAY TECHNOLOGY

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Leptospirosis is a worldwide zoonotic disease. The causative agent of leptospirosis is a spirochetal bacterium called Leptospira interrogans. Rodent species are the major reservoir hosts that can excrete leptospires into urine leading to contaminated environment. After gaining entry into hosts mostly via skin breaks or mucosa, the organism disseminates through the bloodstream to target organs causing a wide range of clinical manifestations in susceptible mammalian hosts. The crucial step of infection requires host-pathogen interactions. LipL32, the major outer membrane protein (OMP) of pathogenic Leptospira, is conserved among pathogenic leptospires, immunogenic, and expressed in target organs during acute infection in animal models. Therefore, it may play a key role in in host-microbe interactions. To identify host proteins that interact with LipL32, phage display technology was employed in our study. Recombinant LipL32 was produced in E.coli-based system and purified by affinity chromatography and gel filtration before used as a target molecule for biopanning with random heptapeptide phage library to enrich for phages expressed peptides with high affinity to LipL32. After three rounds of panning, 44 plaques of eluted phage were subjected to pyrosequencing. Six different nucleotide sequences were obtained and converted to corresponding peptide sequences and subsequently used to search for matched protein in the database. Putative proteins with potential binding to LipL32 are proteins known to be expressed on the surface of target cells of pathogenic Leptospira such as scavenger receptor class F, chloride channel assembly 2, laminin alpha-5, coronin 2A, prostaglandin E receptor 1, and glycoprotein VI. However, true interactions of LipL32 with these host proteins and their role in the pathogenesis of leptospirosis requires further investigations.

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EPIDEMIOLOGY OF *PASTEURELLA MULTOCIDA* IN CALF REARING UNITS

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Pasteurella multocida (PM) is a common finding in calf enzootic pneumonia. On the other hand, the bacterium can be readily isolated from nasal and pharyngeal swabs from healthy calves. Little is known about virulence genes of PM associated with bovine respiratory disease. Epidemiological research of PM-associated respiratory disease has been scant.

We examined 40 herds with signs of respiratory disease in 38 all-in-all-out calf rearing farms. In each herd tracheobronchial lavage samples from 10 non-medicated calves were taken during the first visit and again 3-4 weeks later. Samples were cultured and PM isolates identified using standard bacteriological techniques. Selected PM strains were subjected to pulsed-field gel electrophoresis using *Sal*I restriction.

In the first sampling PM was detected in 70% of the herds and in 34% of the samples, and during the second sampling in 85% of the herds and in 47% of the samples, respectively. We examined more closely from 23 different herds 63 paired PM strains from calves that were PM positive in both samples. Of these calves, 47 had the same *Sal*I type on both occasions and 16 had different types. At herd level, in 11 herds all isolated PM strains were identical between calves and during both sampling times, and in 12 herds 2 to 3 different genotypes circulated. No identical genotypes were seen between herds. Virulence gene profiles of the PM strains will be discussed.

In conclusion, the PM isolates associated with respiratory disease are genetically very heterogeneous. However, certain PM genotypes may persist in calves and are predominantly prevalent in herds. This points out that some PM strains may be more infective and pathogenic than others.

IS NATURAL EXPOSURE TO *ANAPLASMA PHAGOCYTOPHILUM* IN THE HORSE ASSOCIATED WITH CHRONIC CLINICAL DISEASE?

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Anaplasma phagocytophilum can persist in horses for up to 3 months post infection; albeit without clinical abnormalities (Franzén et al, 2009). Clinical questions remain as to whether horses can have persistence of clinical problems long after initial infection by AP. This study recruited horses during tick seasons that clinicians suspected as being related to persistent effects of anaplasma infection, based on being seropositive (> 1:40 IF) to AP.

All horses underwent a panel of clinical and diagnostic tests. Laboratory analysis included serology against *Borrelia burgdorferi*. Serology against AP and PCR for the presence of the DNA in blood of each horse was performed as previously published (Franzén et al, 2006).

Results:

Table 1: Clinical and laboratory findings of 7 horses with suspected "chronic anaplasmosis".

Breed/ Age/gender	Presenting Clinical problem	Case: Serology: PCR	Control horse: Serology: PCR	Final clinical diagnosis
16 y mare	Mild multiple limb lameness	>1:640/neg	Neg/neg	Lameness: undefined
5 y Mare	Lethargy/lame several joints	1:80/neg	Neg/neg	IAD*
14 y gelding	Lethargy/lameness	1:40/neg	1:40/neg	Lameness: undefined
17 y mare	Lethargy	1:160/neg	1:160 /neg	Mild anemia
12 y gelding	Problems riding / tires readily	1:160/neg	Neg/neg	IAD/ lameness
11 y gelding	Limb swelling/ fetlock lameness	Neg/neg	Neg/neg	Tricuspid insufficiency/ IAD
8 y mare	Stumbling/easily tires	1:160/neg	1:40/neg	IAD

*IAD= "Inflammatory Airway Disease" based on Couëtil et al 2007

We found no evidence that the organism persisted in these suspect horses following apparent exposure and seroconversion to AP. Moreover, in 4/7 horses other medical diagnoses were made that could help explain signs for which the horses were presented; in particular signs such as lethargy or tiredness.

If indeed persistence is related to clinical problems in the horse, far more suspect animals must be similarly studied since the presenting complaints for such horses can have a wide range of underlying causes.

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MICROARRAY-BASED DETECTION OF *SALMONELLA* ENTERITIDIS GENES INVOLVED IN REPRODUCTIVE TRACT COLONIZATION

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Although chickens can become infected with a variety of *Salmonella* serovars, *Salmonella* Enteritidis is found in 90% of *Salmonella* contaminated eggs. A possible explanation for this phenomenon is that the serovar Enteritidis is better adapted to the chicken reproductive tract. Previous studies clearly showed that *S.* Enteritidis is more capable of surviving in the chicken reproductive tract than other serotypes.

To identify genes of *Salmonella* Enteritidis that are essential to colonize the oviduct, a microarray-based negative selection screening, using a transposon library, was performed. This library was inoculated for three consecutive passages on primary oviduct tubular gland cells *in vitro* and ligated oviduct loops in anesthetized hens. Mutants with a disruption in a gene that is important for reproductive tract colonization were no longer able to survive and thus no longer present in the mutant pool after the selection procedure. gDNA was isolated from the mutants that were recovered from the oviduct cells, and used for RNA transcription from the T7 promoter that was located on the transposon. This RNA was labelled and hybridized against full gDNA on a cDNA microarray to identify genes that were no longer present after the selection procedure compared to the initial library, i.e. required to colonize the oviduct cells.

The data not only confirm the role of SPI1 (invasion) and SPI2 (intracellular survival) in the colonization of the oviduct, but also document the involvement of other genomic islands in colonization of this organ. These include fimbrial genes and genes belonging to the so called regions of difference, that are specific to a subset of serotypes, including Enteritidis. Detection of *Salmonella* Enteritidis specific genes involved in oviduct colonization could lead to the identification of virulence factors that play a role in the predilection of this serotype to contaminate eggs.

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CLOSTRIDIUM DIFFICILE IN PIGLETS IN FINLAND

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Introduction: In recent years *Clostridium difficile* has emerged as a major cause of neonatal diarrhoea in piglets in many parts of the world. This is the first time that the occurrence of *C. difficile* in pigs has been investigated in Finland.

Materials and methods: A total of 98 samples were investigated between September 2009 and May 2010. Seventy eight of the samples were from pigs that were submitted for necropsy. Twenty were faecal samples from pigs with diarrhoea. A total of 44 pig farms were included.

The samples were plated on selective medium for isolation of *C. difficile* by using FAA-agarbase supplemented with cefoxitine and cycloserine (48 hours at 37°C). One to three presumptive colonies of each sample were isolated and further confirmed by Gram staining and API 20A test system. An ImmunoCard Toxins A&B rapid toxin test (Meridian) was used for detecting the presence of *C. difficile* toxins A and B. Intestinal samples of each autopsied pigs were subjected to routine histological examination (HE staining).

Results: Pigs of different ages were investigated. *C.difficile* was not detected in pigs over 2 weeks old. 35 samples were from unweaned piglets from 19 farms. *C. difficile* was detected in 10/35 (29 %) samples (9 samples from piglets less than one week old and 1 from a 2 week old piglet) from 7/19 farms (37 %). All isolates were positive by the toxin test . In the histological examination inflammation in the large intestine was detected.

Conclusions: The results obtained here show for the first time the presence of *C.difficile* in Finnish pig farms and that it can be found in piglets with diarrhoea. Further research needs to be undertaken also in Finland to evaluate the role of *C. difficile* as a primary pathogen in neonatal diarrhoea in piglets.

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PREVALENCE OF ANIMALS BRUCELLOSIS IN THE WILAYA OF MOSTAGANEM, ALGERIA

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To assess the prevalence of animals brucellosis in Wilaya of Mostaganem, a serological survey was conducted between Janury and June 2007. A total of 2572 animals sera were analysed. 1607 sera from 264 dairy farms in different districts of the Wilaya have been reviewed by the rose Bengale test (RBT) as a screening test and complement fixation method for confirmation of positives animals. A single overall prevalence of 0.43% and an overall herd prevalence of 2.8% were obtened. Of 965 sera collected from slaughterhouses, the RBT was applied. The overall seroprevalence of about 2%, 0% and 3.92% respectively were found in cattle, sheep and goats. the results show the dispersion of brucellosis in some districts of the Wilaya, whith a very high herd infection rate. Morever, inadequate or no screening in animals allows the persistence of infection in farms and 100% of brucellosis cases in cattle observed were due to newly introduced animals. These results show that active collaboration between different partners must be implanted for better management of animals health and ovoid a risk to human.

Keywords:animals,brucellosis, complement fixation method, rose Bengale test, Mostaganem, Algeria.

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CONJUGATIVE TRANSFER OF THE NETB TOXIN PLASMID IN *CLOSTRIDIUM PERFRINGENS*

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Necrotic enteritis in chickens causes significant economic losses worldwide. Disease pathogenesis involves NetB, a pore-forming toxin produced by avian necrotic enteritis strains of *Clostridium perfringens*. Our objective was to determine the location and mobility of the *netB* structural gene. We used a derivative of the tetracycline resistant necrotic enteritis strain EHE-NE18, in which *netB* is insertionally inactivated by the thiamphenicol resistance gene, *catP*. Both tetracycline and thiamphenicol resistance could be transferred either together, or separately, to a recipient strain in plate matings. The transconjugants could act as donors in subsequent matings, which demonstrated that the tetracycline resistance determinant and the *netB* gene were present on separate conjugative elements. Large plasmids were isolated from the transconjugants and analysed by next generation sequencing. Sequence analysis of the 49 kb tetracycline resistance plasmid showed that it was very similar to the prototype tetracycline resistance plasmid pCW3 from *C. perfringens*. Analysis of *netB* plasmid preparations revealed that there were two very similar plasmids present, each carrying a 40kb region that included the plasmid replication and transfer regions. One plasmid (82 kb) contained the *netB* gene and other potential virulence genes and was shown to be conjugative. The second plasmid (69 kb) carried the same conjugation locus and also carried the *cbp2* gene, which encodes a different pore-forming toxin, β2 toxin. In conclusion, we have shown that the gene for NetB toxin is encoded on a conjugative plasmid and that strain EHE-NE18 harbours three large plasmids, each with 40kb of shared DNA that includes a common conjugation locus.

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ANTIBODY RESPONSE TO A *TREPONEMA PHAGEDENIS*-LIKE RECOMBINANT PROTEIN IN CATTLE WITH DIGITAL DERMATITIS

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Digital dermatitis (DD) is a contagious claw disease causing lameness in cattle, most commonly seen in intensive dairy production. Besides being an animal welfare problem, economic losses due to reduced milk production and weight loss are associated with DD. There is strong circumstantial evidence that *Treponema* spp. are central in the etiology of DD and the *Treponema* phylotype closely related to *T. phagedenis* (Tpl) has been indicated in several studies to be a key agent in the pathogenesis. Diagnosis of DD is performed by visual examination of the claw and due to the character of the infection (poly-microbial including fastidious bacteria) conventional diagnostic methods such as culturing and PCR are not used. A humoral immune response against *Treponema* spp. has been demonstrated in cattle with DD and a serologic test for identification of healthy herds would be of use to control the disease. In this study, an immunogenic protein (PrrA) was identified by selection of a Tpl phage display library against antibodies from a rabbit immunized with live bacteria. This protein was recombinantly produced and used as antigen in an indirect ELISA. Monoclonal and polyclonal horse-radish peroxidase conjugated anti-bovine IgG antibodies were evaluated as secondary antibodies. Analysis of both serum and milk samples from cattle with and without DD showed high levels of antibodies against PrrA in the majority of samples from cattle with DD. The best discrimination was achieved with the monoclonal secondary antibodies. Further testing of a larger number of individuals and herds will show if this antigen would be useful in an ELISA, alone or in combination with additional antigens. Such a test could also be used for epidemiological studies both on herd and national level and in a control program to declare herds as free from DD.

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DEVELOPMENT OF A MULTIPLE LOCI VNTR ANALYSIS (MLVA) METHOD FOR TYPING *DICHELOBACTER NODOSUS*, THE CAUSAL AGENT OF FOOTROT IN SHEEP

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Variable number tandem repeats (VNTR) are repeat nucleotide sequences arranged in tandem that can vary in copy number. They are present in both prokaryotic and eukaryotic genomes and are located in coding and non-coding regions. Studies of VNTRs within intragenic regions of pathogenic bacteria suggest changes in copy number of tandem repeats may aid virulence and also play a role in adaptation to different environmental conditions. Multiple-loci VNTR analysis (MLVA) is a method of bacterial strain typing using polymorphic tandem repeats. MLVA has proved to be very successful in the epidemiological typing of pathogenic bacteria.

The aim of this study is to develop a MLVA method for typing *D. nodosus*, the causal agent of ovine footrot. The tandem repeats finder program (http://.bu.edu/trf/trf.html) was used to identify VNTRs within the *D. nodosus* genome strain (VCS1703A). From the 61 VNTRs located, 23 were selected for further investigation based on the consensus sequence of the repeat being ≥5bp and the copy number >2. The 23 VNTR regions were analysed in 35 isolates. Of the 23 VNTRs selected 18 were discarded for use in a MLVA assay, the reasons included: repeat regions were not polymorphic, unable to amplify the region with PCR, PCR amplification produced multiple products, the repeat region was located in VAP/VRL regions (not present in all *D. nodosus* strains). The results of this study have provided a panel of 5 polymorphic VNTR regions (MLVA5) which have the potential to be used as a typing system in *D. nodosus*. The MLVA5 will be used for *D. nodosus* typing in future epidemiological studies. Additional studies will analyse isolates from more diverse geographical regions and *D. nodosus* isolated from other species e.g. cattle and goats. This will enable us to determine potential reservoirs of infection and transmission routes of *D. nodosus* infection in sheep.

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PCR, CYTOLOGY, RUT: WHICH IS THE METHOD OF CHOICE FOR DETECTION OF *HELICOBACTER* SPP. IN FELINE GASTRIC MUCOSA?

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This study was designed to find out the best method for diagnosis of non-*pylori Helicobacter* infection in gastric mucosa of cats. Forty-three adult stray cats which were clinically healthy, were included in this study. During endoscopy, a total of three pairs of biopsy specimens were obtained from the antrum and body. One pair was used for the RUT test and monitored for color change for up to 24 hours at 37°C. The second pair was used for cytological evaluation and the third for PCR. According to cytology and rapid urease tests, 25/43 (58%) and 29/43 (67.5%) of the gastric specimens were mild to moderately positive for *Helicobacter* spp., respectively ($P < 0.05$). Genus-specific PCR identified 29/43 (67.5%) of the subjects as *Helicobacter* spp.-positive in the gastric mucosa. Accordingly, the sensitivity and specificity of rapid urease test and cytology were assessed against PCR and determined to be 93%, 86% and 83%, 93%, respectively. PCR is the most accurate method for detection of *Helicobacter* spp. in the gastric biopsies and should be performed for all specimens that are found negative by other tests .

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ANTIBACTERIAL ACTIVITY OF PHYTOGENIC SUBSTANCES AGAINST SWINE PATHOGENS

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Infections in swine with pathogenic bacteria such as *Escherichia coli* can have severe consequences like diarrhea in weaners which subsequently can lead to high mortality. Furthermore, infections with salmonellae, streptococci and clostridia affect the performance of sows, piglets and weaners. Phytogenic substances are promising to counteract these pathogenic bacteria and serve as potential alternative to antibiotics. Therefore, nine phytogenic substances were tested *in vitro* against *Escherichia coli* F4, *Salmonella typhimurium*, *Streptococcus suis* and *Clostridium perfringens* toxin type C in a broth microdilution assay. In the assay, bacteria cultures with defined microbial count were incubated together with different concentrations of the test substances for 24 h. The change in optical density of the bacteria culture led to a quantitative result, indicated as the MIC_{50} value. The lowest MIC_{50} values were reached for totarol against *S. suis* (15.6 mg/l) and *C. perfringens* (20 to 39 mg/l) as well as the two oleoresins of *Curcuma longa* and *Capsicum annum* against *S. suis* (each 78 mg/l). High differences between the strains were observed for an ethanolic extract of *Sophora flavescens* that did not inhibit the Gram negative strains, *E. coli* and *S. typhimurium* (both MIC_{50} values >2500 mg/l), but the Gram positive strains, *S. suis* (MIC_{50} value 625 mg/l) and *C. perfringens* (MIC_{50} value 313 to 625 mg/l). Thymol and an ethanolic extract of *Berberis aristata* showed moderate inhibition of all four strains (MIC_{50} values from 313 to 1250 mg/l and 78 to 2500 mg/l, respectively). Comparing the four strains, *S. suis* was the most sensitive strain whereas *E. coli* F4 showed the highest MIC_{50} values. In summary, several tested phytogenic substances showed high antibacterial activity and could therefore be used to counteract infections with swine pathogenic bacteria.

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CHARACTERIZATION OF *TREPONEMA PEDIS* ISOLATES FROM NECROTIC SKIN ULCERS IN PIGS

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Ear necrosis and shoulder ulcers in pigs are both a welfare problem and ethical issues. Shoulder ulcers also cause economical losses for the producer due to a shorter life span of affected sows. Since the early 1900s, spirochetes have been observed microscopically in the ulcers but have previously not been cultured and therefore not characterized. In this study, isolates from ear necrosis, shoulder ulcers and gingiva, identified by 16S rRNA gene sequencing as *Treponema pedis*, were used (Pringle et al., 2009, Pringle and Fellström, 2010). The isolates were characterized by biochemical tests, including api®ZYM, and by testing the antimicrobial susceptibility to tiamulin, valnemulin, tylosin, tylvalosin, lincomycin, and doxycycline using broth dilution. In addition genetic fingerprinting through randomly amplified polymorphic DNA (RAPD) analysis was performed. The enzyme production and antimicrobial susceptibility patterns were similar for the isolates, however the RAPD fingerprints were discriminatory. The api®ZYM results differed from the enzyme activity of the *T. pedis* type strain T3552B^T isolated from a bovine digital dermatitis lesion (Evans et al., 2009). The MICs of all tested antimicrobial agents were low. *Treponema* spp. cause diseases with lesions in the skin and mucus membranes in several animal species including humans. No such disease has been described in pigs and in consideration of our findings further studies are planned to investigate the occurrence and importance of *Treponema* spp. in porcine necrotic skin ulcers.

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EFFICIENT OVIDUCT COLONIZATION AND EGG WHITE SURVIVAL STRATEGIES OF *SALMONELLA* ENTERITIDIS AS A POSSIBLE EXPLANATION FOR THE EGG-ASSOCIATED PANDEMIC

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Salmonella Enteritidis is the predominant serovar associated with egg-borne salmonellosis in humans. Apparently this serotype possesses particular characteristics that increase its chance to contaminate eggs. To identify the traits that enable the serovar Enteritidis to more efficiently contaminate eggs, different steps in the pathogenesis were modelled and the behaviour of different strains from multiple serotypes was tested. Briefly, egg contamination can be the result of external (on the shell) and internal egg contamination. Internal egg contamination has been shown to be the consequence of oviduct colonization and subsequent incorporation of Salmonella in the forming egg. Survival in the antimicrobial egg white compartment is essential to cause human salmonellosis. After an intravenous infection of laying hens, it was observed that the ability of serotype Enteritidis strains to colonize the reproductive organs was significantly higher compared with the *Salmonella* Heidelberg, *Salmonella* Virchow and *Salmonella* Hadar strains, while the latter could colonize the gut, liver and spleen to the same level as the Enteritidis strains. Using more than 90 strains from multiple serovars, it was shown that strains belonging to the serovar Enteritidis survived much better in egg white compared to all other serovars tested. It is thus proposed that *Salmonella* Enteritidis has strategies that permit an efficient colonization of the oviduct and survival in the antimicrobial egg white compartment. Instead of being more virulent or invasive and thus more capable to establish infection as compared to other serotypes, *Salmonella* Enteritidis tends to suppress virulence, and to shift its metabolic state towards maintenance and thus persistence in harsh environments, such as the oviduct tissue and the egg.

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CLOSTRIDIUM PERFRINGENS GENES WITH IMPLICATIONS FOR BOTH VIRULENCE AND COLONISATION DURING NECROTIC ENTERITIS

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Necrotic enteritis is a disease of serious concern to the world poultry industry in terms of both animal welfare and its impact upon poultry production. The disorder is caused by the anaerobic bacterium *Clostridium perfringens*, which, although in healthy chickens is a normal part of the enteric population, under certain conditions can dramatically proliferate leading to disease. The identification of genes that are found to be preferentially associated with strains of *C. perfringens* known to cause disease will help us to better understand this disease. Analysis of an in-house genomic sequence of a *C. perfringens* strain isolated from a bird suffering from necrotic enteritis coupled with wider PCR screenings of other *C. perfringens* strains identified several genes strongly associated with disease causing isolates. Two of these genes subsequently were mutated in a virulent strain background. The mutants displayed markedly different phenotypes compared to the wild-type strain; including reduced ability to bind immobilised extracellular matrix elements, significant ($p<0.05$) reductions in virulence in a chicken disease induction model and reduced colonisation *in vivo* as measured by caecal titres and cloacal swabs. These results suggest that these genes may play an important role in virulence and will help us to better understand the pathogenesis of this important disease.

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SEROPREVALENCE OF LEPTOSPIRA SPECIES IN PIGS IN POLAND. INCREASING IMPORTANCE OF SEJROE SEROGROUP IN ETIOLOGY AND PATHOGENESIS OF LEPTOSPIROSIS IN SWINE?

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Leptospirosis is a worldwide zoonotic disease caused by pathogenic spirochetes of the genus Leptospira. Swine are significant reservoir of this pathogen and important source of Leptospira infection for man. Serological screenings help effectively control epidemiological situation in swine herds and prevent transmission of Leptospira from animals to man.
The purpose of this study was to investigate by serological methods the prevalence of infections caused by selected Leptospira serogroups in swine population in Poland.

A total of 13999 swine serum samples were examined. The samples, collected in years 2008 - 2009, came from 280 counties situated in 16 provinces of Poland. All sera were examined by enzyme-linked immunosorbent assay (ELISA) using heat-stable antigenic preparation. Samples positive or doubtful in ELISA were investigated by microscopic agglutination test (MAT) with use of serovar Icterohaemorrhagiae, Pomona, Canicola, Sejroe, Tarassovi and Grippotyphosa. Serum dilution (including added antigen) used during preliminary examination was 1 : 100.

Of the collected sera examined by ELISA 127 (0.91 %) samples were positive, 164 (1.17 %) - doubtful and 13708 - negative. Among ELISA-positive and doubtful sera 128 samples (coming from all examined provinces) were recognised in MAT as positive. Among MAT-positive samples 56 (43.75 %) sera demonstrated titres with serovar Sejroe, 45 (35.16 %) - with serovar Pomona, 16 (12.50 %) - with Icterohaemorrhagiae, 5 (3.91 %) with Tarassovi and each 3 samples (2 x 2.34 %) - with serovar Canicola and Grippotyphosa. Among mentioned samples 6 sera demonstrated titres each with two serovars.

Described screening indicates Sejroe as most prevalent pathogenic Leptosppira serogroup infecting swine herds in Poland. Domination of this serogroup was observed in some last years. Unnoticeable or often slightly demonstrated clinical symptoms in swine presenting antibodies specific for Sejroe serogroup suggest the need of wide investigation concerning the role of the infections with mentioned Leptospira serogroup in swine pathology.

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OVINE PEDOMICS- THE FIRST STUDY OF THE OVINE FOOT 16S RRNA BASED MICROBIOME

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We report the first study of the ovine bacterial microbiome of the interdigital skin based on 16S rRNA deep sequencing by pyrosequencing and conventional cloning-Sanger sequencing approach. Sheep with clinical conditions described as healthy (H), with interdigital dermatitis (ID) and with footrot (FR) from three farms with different disease history were studied. Phylogenetic analyses revealed that the ovine foot bacterial community varied significantly between farms and clinical conditions. The diversity within OTUs for the three conditions was greatest for ID compared to H and FR. Actinobacteria, Bacteriodetes, Firmicutes and Proteobacteria were the most abundant phylum distributed in 25 genera. *Peptostreptococcus*, *Corynebacterium* and *Macrococcus* were significant different populations associated to H, ID and FR respectively. Sequences corresponding to *Dichelobacter nodosus*, the causal agent of ovine footrot, were absent from all libraries possible due to mismatches in the 16S rRNA forward primer (27F). An alternative qPCR platform was developed based on *rpoD* which allowed detection of *D. nodosus* in all clinical samples. Sheep from farms with no prior history of footrot had feet colonised by *D. nodosus*. However, all animals diagnosed with ID had significantly higher (10^4-10^9 cells/g tissue) *D. nodosus* cell than those with H and FR status. The latter had values at or near 10^3 cells/g tissue corresponding the detection threshold. In conclusion, differences in the structure and in the phylogenetic origin of the bacterial community were observed and shared core populations in all clinical conditions were identified.

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OVINE FOOTROT: DETECTION AND QUANTIFICATION OF *DICHELOBACTER NODOSUS* AND *FUSOBACTERIUM NECROPHORUM* USING REAL-TIME PCR AND FLUORESCENCE *IN SITU* HYBRIDIZATION TECHNIQUES

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Footrot is a highly infectious and debilitating disease of sheep and is estimated to cost the UK agricultural industry £24m p.a. The current consensus is that the onset of disease occurs when the interdigital skin becomes damaged allowing superficial colonization of the epidermis by *Fusobacterium necrophorum* , a ubiquitous soil and faecal anaerobe, which results in inflammation and necrosis (interdigital dermatitis). This permits other organisms to become established, including *Dichelobacter nodosus*, another obligate anaerobe with proteolytic capabilities. The synergistic action of both organisms can ultimately result in the separation of the hoof horn from the sensitive underlying tissue (footrot), causing lameness.

The fusobacteria have been implicated as key components in a number of human and animal diseases. We were therefore interested in the interactions between these two anaerobes and their roles in disease pathogenesis. The aim was to employ a multi-disciplinary approach, combining molecular microbiology with epidemiology to enhance our current understanding of disease progression. Real-time PCR was used to quantify the numbers of both organisms in a longitudinal data set. Fluorescence *in situ* hybridization was then used to examine the spatial distribution and prevalence of footrot-associated organisms in tissue samples from sheep presenting with a range of clinical conditions. The results indicate that the presence of *Dichelobacter nodosus* correlates with interdigital dermatitis severity in a dose-dependent manner, and that colonization with *Fusobacterium necrophorum* appears to be a subsequent process. However, once footrot develops, levels of *Dichelobacter nodosus* detectable in the surface lesion decrease, but *Fusobacterium necrophorum* levels remain high. The results thus far call into question the current understanding of ovine footrot pathogenesis.

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