A viable quantitative approach (v-qPCR) for detecting Arcobacter species

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Welcome to CHRO 2015!

It gives us great pleasure in welcoming you to the 18th International Conference on *Campylobacter, Helicobacter,* and Related Organisms – CHRO 2015 – being held in the beautiful town of Rotorua in New Zealand!

Rotorua is located in the Bay Of Plenty region and offers unique experiences amidst breathtaking landscapes. It has a long (over 200 years!) history of welcoming visitors and its geothermal activity offers the chance to enjoy warm, relaxing natural spa pools, as well as some truly beautiful scenery!

Rotorua is the heartland of New Zealand Māori culture, and you will find many opportunities to engage with and enjoy the warm hospitality and traditions of the Māori. Side-by-side with our rich cultural heritage are all the modern facilities one would expect from a modern, progressive nation – typified perhaps by the Conference location at the Energy Events Centre, a purpose-built facility that is conveniently located just a short walk away from most of the major hotels that partnered with us to offer special conference rates.

From the honour (and surprise!) of winning the right to host the conference back in 2013, developing CHRO 2015 has been an unforgettable journey. We thank all of our sponsors, the city of Rotorua, our plenary speakers, international advisors and most of all YOU for helping to make CHRO 2015 a very special occasion indeed.

We hope you enjoy the conference – and all that New Zealand offers in conjunction with it.

Convenor

Stephen On (Institute of Environmental Science and Research

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Nigel French	Chair, Massey University
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Jacqui Keenan	Christchurch Medical School / University of Otago
Peter van der Logt	Ministry for Primary Industries

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Daniel Power	Thermo Fisher Scientific / NZ Microbiology Society Executive Officer, Committee member 2013-2014)

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Marc	Wösten	Netherlands

Plenary Speakers

Peter	Greenberg	USA
Bill	Hanage	USA
GwangPyo	Ko	South Korea
Ichizo	Kobayashi	Japan
Mike	Konkel	USA
Ernst	Kuipers	Netherlands
Sarah	O'Brien	UK
Richard	Peek	USA
Hanne	Rosenquist	Denmark
Christine	Szymanski	Canada
Brendan	Wren	England

PLENARY SPEAKERS - Biographies

E. Peter Greenberg

Dr. Greenberg holds a BA in Biology from Western Washington University, a MS in Microbiology from the University of Iowa and a PhD in Microbiology from the University of Massachusetts. After his postdoctoral at Harvard University Dr. Greenberg was on the faculty at Cornell University and then the University of Iowa College of Medicine before moving to the University of Washington in 2005. Dr. Greenberg is an elected member of the National Academy of Sciences, the American Academy of Arts and Sciences, the American Association for the Advancement of Sciences and the American Academy of Microbiology.

The research in Dr. Greenberg's laboratory is focused on the emerging field of sociomicrobiology. He is principally concerned with three aspects of sociomicrobiology:

I. The biochemistry and molecular biology of environmental sensing and response in bacteria with a particular emphasis on a form of chemical communication between bacteria termed quorum sensing.
II. The mechanisms by which bacteria switch from a nomadic existence to a sessile biofilm lifestyle and the mechanisms underlying the ability of biofilm bacteria to survive the action of antibiotics.
III. The ways in which clonal populations of bacteria can discriminate themselves from other clonal populations.

All of these phenomena are of importance in pathogenesis. Dr. Greenberg has concentrated much of his effort on Pseudomonas aeruginosa, an opportunistic pathogenic bacterium that can cause both acute and persistent biofilm infections.

William Hanage

Dr William (Bill) Hanage (PhD) is an Associate Professor in the Department of Epidemiology, Harvard School of Public Health's Center for Communicable Disease Dynamics in Boston, USA. He was a Postdoctoral researcher at the University of Oxford and Imperial College London, before being awarded a Royal Society University Research Fellowship. Prior to joining the Center for Communicable Disease Dynamics, he was a Reader in the department of Infectious Disease Epidemiology at Imperial College London.

Dr. Hanage has worked extensively developing multilocus sequence typing (MLST; www.mlst.net) and analysis (MLSA) for the study of bacterial pathogens and species, as well as means of analyzing data developed using this method. He has worked on both bacterial and viral pathogens, and is particularly interested in using an evolutionary framework such as methods derived from population genetics to inform epidemiology.

He has acted as an advisor to Glaxo SmithKline and the World Health Organisation. He is the winner of the 2012 Fleming Prize for research in Microbiology and the recipient of a 2012 ICAAC Young Investigator Award from the American Society for Microbiology. His work on pathogen evolution was recognized with the 2012 Fleming Prize from the Society for General Microbiology. Recently, Hanage has been among the pioneers of genomic epidemiology for bacterial pathogens. He exemplifies the combination of theoretical and experimental skills that will be fundamental to the future of infectious disease epidemiology, describes nominator Marc Lipsitch, Harvard School of Public Health.

GwangPyo Ko

Dr. Ko has successfully established and conducted an internationally recognized and highly regarded academic research program in the field of microbiology, metagenomics, and translational research. Dr. Ko received Ph.D. degree from Harvard University, and did post-doctoral training at the University of North Carolina at Chapel Hill. He was a faculty at University of Texas HSC at Houston prior to joining to Seoul National University in 2005.

He has been very successful in obtaining national and international funding for his research, including large program/project grants and more specific projects focused on critical contemporary research needs in human health associated with microbiome. Dr. Ko's group developed and identified the microbiological biomarkers for metabolic or other chronic diseases using over 2,000 Korean twins through a comparison of human microbiota using next-generation sequencing (NGS) and other novel techniques.

The success of his research accomplishments is reflected in his record of scholarly publications in the peer-reviewed scientific literature, with more than 70 peer-reviewed articles in high-impact journals.

Ichizo Kobayashi

Professor Ichizo Kobayashi (PhD) is a Professor in the Department of Computational Biology and Medical Sciences within the Graduate School of Frontier Sciences, and in the Institute of Medical Science at the University of Tokyo in Japan. His research centres around the concept of the genome being a community of genes with a focus on the unique role that restriction-modification systems play in bacterial epigenetics and evolution, most notably in H. pylori.

A gene for a restriction enzyme, which damages DNA at a specific sequence, is linked with a gene for a cognate modification enzyme, which methylates the same sequence to protect it from the damage. Restriction-modification (RM) gene complexes will attack invading DNA that has not been properly modified and thus may serve as a tool of defense for bacterial cells. However, some RM systems behave as discrete units of life, or mobile genetic elements, just as viruses and transposons. They move between genomes and rearrange them. They can even multiply. Movement and replacement of their target recognition domains frequently change their sequence specificity and consequently remodel bacterial methylomes. Each of these methylome states may have a unique gene expression pattern with a unique set of phenotypes and may provide units of adaptive evolution.

Professor Kobayashi's research has involved the development and use of bioinformatic, genomic, molecular-genetic and microbiological tools. CHRO 2015 is delighted to have Professor Kobayashi present on his research in genome/ epigenome dynamics and evolution in H. pylori, and of the implications of his research on infection control

Michael Konkel

Professor Michael Konkel (PhD) is Professor in the School of Molecular Biosciences at Washington State University. His research interests include the molecular basis of Campylobacter jejuni-host cell interactions; specifically the functional characterisation of C. jejuni virulence proteins and the use of in vitro and in vivo models to validate the importance of these proteins in disease.

Extensively published, with over 85 peer-reviewed scientific articles to his name and editorship of key reference works in the field, CHRO 2015 looks forward to welcoming Professor Konkel and hearing of his latest research in the context of virulence mechanisms in Campylobacter.

Ernst Kuipers

Professor Ernst Kuipers (MD, PhD) is Professor of medicine and Chief Executive Officer of the Erasmus University Medical Center Rotterdam, the Netherlands. Professor Kuipers has a longstanding and distinguished history in the field of Helicobacter pylori, gastritis, and esophageal and gastric cancer. In 1994, he participated in a WHO meeting on H. pylori and gastric cancer. In 1995, he completed his thesis on the interrelation between H. pylori, chronic gastritis and gastric cancer, which was subsequently awarded the Dutch Gastrointestinal Research Award in 1996. In 2006, he was made a fellow of the American Gastroenterology Association.

Professor Kuipers' current major research interests focus on the etiology, diagnosis and prevention of early neoplastic lesions of the gastrointestinal tract, and he chairs the Rotterdam Colon Cancer Screening group.

Sarah O'Brien

Professor Sarah O'Brien (MD) is a Professor in the Institute of Infection and Global Health at the University of Liverpool in England. Her research interests include gastrointestinal infection, zoonoses, food-related infection and food safety. She qualified in Medicine in 1986 at Newcastle University and undertook Higher Specialist Training in Public Health Medicine in Oxford and Newcastle-upon Tyne. Professor O'Brien has held Consultant positions in Health Protection in Birmingham, Glasgow and London before joining the University of Manchester in 2004, and subsequently the University of Liverpool in 2011.

Professor O'Brien's research has considered various aspects of disease epidemiology and notably for campylobacteriosis, including temperature and seasonality, patient demographics, molecular typing data and methods of food production.

Richard Peek

Professor Richard Peek (MD) is the Mina Cobb Wallace Professor of Medicine and Cancer Biology, Director of the Division of Gastroenterology, Hepatology & Nutrition and Director of the NIH-funded Digestive Disease Research Center at Vanderbilt University Medical Center. His research speciality is the pathogenesis of H. pylori infection and gastric cancer.

The overarching theme for Professor Peek's research has been delineation of the molecular signaling events initiated by bacterial:epithelial cell contact that regulate phenotypes related to carcinogenesis. Detailed studies of interactions between H. pylori and intact gastric epithelium have been limited by issues of in vivo accessibility or dedifferentiation in cell culture. Therefore, one aim of his recent research has been to develop and utilize a novel replenishable ex vivo three-dimensional system to identify constituents that mediate host-H. pylori interactions with carcinogenic potential. Gastroids are three-dimensional organ-like structures that provide unique opportunities to study host-H. pylori interactions in a pre-clinical model. His group found that gastroids developed into a self-organizing differentiation axis and that H. pylori increased proliferation in a CagA- and β-catenin-dependent manner.

Professor Peek has also recently shown that iron depletion augments the virulence of H. pylori via increased assembly of the cag pathogenicity island and that aberrant activation of NOD1 by H. pylori promotes the development of gastric cancer.

Hanne Rosenquist

Dr Rosenquist is the former Head of the Danish Zoonosis Centre (DZC), a Danish Government organisation whose task is to survey zoonoses and zoonotic agents in Denmark and to contribute to their prevention and control. The DZC publishes an annual report on zoonoses in Denmark and an annual report on antimicrobial resistance and consumption in Denmark (DANMAP). Dr Rosenquist has recently moved to a position as senior academic officer in the Danish Veterinary and Food Administration.

The focus of Dr Rosenquist's research is primarily the control of Campylobacter in broilers and broiler meat, including the understanding of the epidemiology of Campylobacter and methods to reduce numbers of Campylobacter. She also works on developing source attribution metrics for Campylobacter and gives scientifically-based advice to Danish and international food authorities. For example, Dr Rosenquist is key adviser for the national surveillance of Campylobacter in food and the Danish action plans to control Campylobacter. Dr Rosenquist has been member of working groups in EFSA and Codex that concerns Campylobacter, and is involved in national as well as international research projects in this area.

The operational and research work of the DZC is held in high regard internationally in the quality of its efforts to coordinate at a National level efforts to reduce the burden of zoonotic disease

Christine Szymanski

Christine Szymanski (PhD) is a Professor and AITF iCORE Strategic Chair in Bacterial Glycomics at the University of Alberta in Canada. She is a Principal Investigator in the Alberta Glycomics Centre and is also the "Glycans in Microbe-Host Interactions" Subgroup Leader and Steering Committee Member in the Consortium for Functional Glycomics. She is CEO of VaxAlta, a company specialising in the production of effective carbohydrate-based vaccines against important animal and zoonotic pathogens.

Professor Szymanski's main research focus is in examining host-pathogen interactions in order to understand and exploit virulence mechanisms used by bacteria colonizing mucosal surfaces. Her research group has been characterizing campylobacter glycosylation systems for almost 20 years, including the first identified bacterial N-linked protein glycosylation pathway and the extremely variable capsular polysaccharides, that have been demonstrated to play important roles in chicken colonization, bacteriophage evasion and diarrheal disease.

Her groundbreaking research in comparative glycomics has resulted in the production of a vaccine to protect poultry against colonisation with Campylobacter.

Bredan Wren

Brendan Wren studied for a PhD in Physical Chemistry and published seminal papers on the effect of ionizing radiation on DNA. He then changed subject discipline and took a post-doctoral position at St Bartholomew's Hospital, London. During this time he was the first to publish molecular cloning studies on Clostridium difficile, Campylobacter jejuni and Helicobacter pylori.

In 1999 he moved to the London School of Hygiene and Tropical Medicine and was awarded a chair in Microbial Pathogenesis. His primary research interest includes the molecular characterization of bacterial virulence determinants and the evolution of virulence. Throughout his research career he has been involved in developing innovative technologies to study bacteria. These include mutagenesis approaches, microarray-based methods and phylogenetic analyses. He had direct involvement in the Campylobacter jejuni, Clostridium difficile, Yersinia enterocolitica, Burkholderia pseudomallei,Yersinia pestis and Francisella tularensis genome projects, and is currently involved with similar efforts for major Helicobacter species in collaboration with the Sanger center.

His research group exploits a range of post genome research strategies to gain a comprehensive understanding of how these pathogens function and how they interact with their respective hosts.

i. Glycosylation in bacterial pathogens and the application of Protein Glycan Coupling Technology for the production of recombinant glycovaccines in E. coli
ii. The inhibition of glycosyltransferases for antibiotic development (glycobiotics)
iii. The development of alternative antibiotic approaches including biofilm disruption agents, phage/lysin therapy and bacteriotherapy.

Ministry for Primary Industries
Manatū Ahu Matua

The Ministry for Primary Industries welcomes participants to the CHRO 2015 conference

The Ministry for Primary Industries is focused on the success of the primary industries and growing and protecting New Zealand.

We do this by ensuring the food we produce is safe, improving sector productivity, increasing sustainable resource use, helping to maximise export opportunities for our primary industries, and protecting New Zealand from biological risk.

As the government agency responsible for food safety, MPI sets regulatory requirements to ensure that all food produced in New Zealand is safe and suitable.

We have a strong focus on evidence-based regulation and continually strive to provide robust scientific and risk assessment advice to risk managers so that control measures are successful in continually reducing the burden of foodborne illness. In the case of campylobacteriosis, we have had considerable success in reducing foodborne human illness.

New Zealand has a systematic process which includes a multitude of activities by industry, third parties and MPI to ensure that control measures are being implemented. This means any food safety concerns are identified early by appropriate surveillance, and traceability and recall systems are key parts of any response to a food safety issue.

MPI is proud to be the principal sponsor of this year's international CHRO 2015 conference held here in New Zealand for the first time ever. We hope you have a great conference!

GROWING AND PROTECTING
NEW ZEALAND

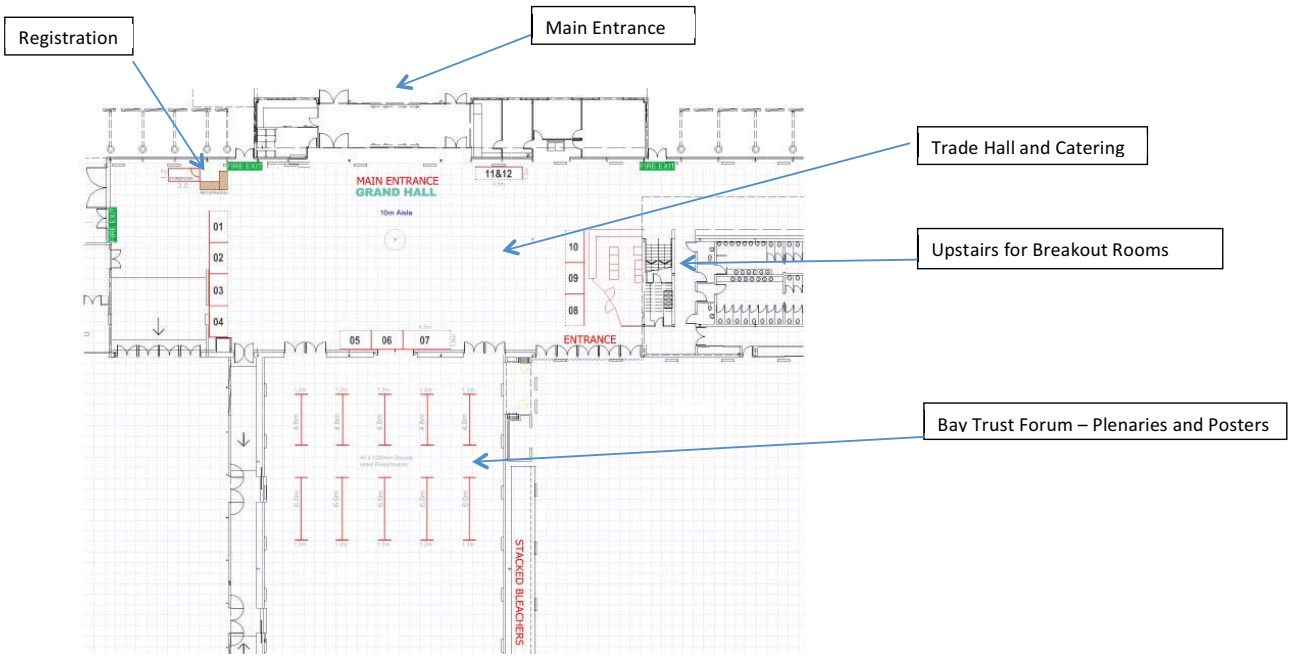
Programme

(Abstract titles abbreviated – refer to abstract book for details)

SUNDAY 1st NOVEMBER

1830 – WELCOME RECEPTION, MITAI MAORI VILLAGE (meet at Energy Events Centre by 1745 for bus pick up)

Energy Events Centre – Room locations



MONDAY 2nd NOVEMBER

ROOM	Bay Trust Forum		
0900-0920	**WELCOME TO NEW ZEALAND, ROTORUA AND CHRO 2015 – Mayor of Rotorua, CEO of ESR, CHRO 2015 Convenor**		
0920-1000	**OPENING LECTURE – Professor Brendan Wren, UK: The compare and contrast of Helicobacter pylori/Campylobacter jejuni pathogenesis** Chair: Nigel French		
1000-1040	**PLENARY LECTURE – Professor Rick Peek, USA: Gastric cancer, stem cells, microbial oncoproteins** Chair: Jacqui Keenan		
1040-1110	**MORNING TEA**		
ROOM	**Bay Trust Forum**	**Skellerup**	**Opus**
1110-1230	**Descriptive and Analytical Epidemiology Chair Norval Strachan/ Jackie Benschop**	**Taxonomy and emerging species Chair: Maria Figueras / Stephen On**	**Helicobacter pathogenesis and evolution Chair: Rick Peek / Ichizo Kobayashi**
	Keynote: O001 Havelaar, selection bias from immune and asymptomatics in c-c studies	**Keynote: O006 Duim, genomic classification of C fetus**	**Keynote: O011 Varga, Hp cag gene expression suppresses inflammation**
	O002 Nichols, Campy seasonality in Europe, comparison with weather data	O007 Haydon, Helicobacter spp from NZ sheep diagnostic challenges	O012 Deen, Hp in macrophages
	O003 Bouwknegt, Campy in EU and proton pump inhibitor use, extension of Dutch study	O008 Kirk, C concisus isolation from various locations	O013 Suarez, Hp molecular evolution
	O004 Geissler, Cj incidence, outbreaks and AMR in US foodnet data	O009 Huq, C concisus genome	O014 Blair, Hp cell shape proteins
	O005 Kornreich, Campy and other pathogens in stools from children <3 months in Belgium	O010 Cornelius, comparative genomics of C concisus	O015 Draper - H pylori: one genome to rule them all?
1230-1330	**LUNCH**		
ROOM	**Bay Trust Forum**		
1330-1410	**PLENARY LECTURE – Professor Mike Konkel, USA: Modulation of Campylobacter gene expression during human infection** Chair: Stuart Thompson		
ROOM	**Bay Trust Forum**	**Skellerup**	**Opus**
1415-1530	**Molecular Epidemiology - 1 Chair: Rick Meinersmann / Angela Cornelius**	**Host pathogen interactions Chair: Mike Konkel / Taghrid Istivan**	**Guillain Barré syndrome Chair: Craig Parker / Michael Baker**

	Keynote: O016 Taboada, Cj and Cc in Alberta, comparative genomic fingerprinting	**Keynote: O021 Bayliss, Cj persistence in chickens**	**Keynote: O026 Baker, GBS decline in NZ**
	O017 Macdonald, comparative genomic fingerprinting for Campy attribution in Canada	O022 Bojanic, wax moth infection model for C	O027 Heikema, LOS allele genotyping for GBS
	O018 Dolamore, outbreak investigation using MBiT	O023 Korolik, chemotaxis receptor Cj	O028 Biggs, pathogenomics of invasive and GBS-related Cj
	O019 Patrick, FoodNet: epi from culture independent tests	O024 Pascoe, GWAS of campy carriage in Peruvian children	O029 Islam, LOS in GBS cases and controls
	O020 Duarte, MLST human and broiler strains Belgium	O025 Nielsen, IgG response in diahorreal C concisus patients	
1530-1600	**AFTERNOON TEA**		
ROOM	**Bay Trust Forum**	**Skellerup**	**Opus**
1600-1740	**Genomic and Analytical Epidemiology Chair: Ed Taboada / Bill Hanage**	**Campylobacter pathogenesis Chair: Brendan Wren / Hazel Mitchell**	**Genetic and proteomic responses for adaptation - Campylobacter Chair: Marc Wosten / Amy Wedley**
	Keynote: O030 Cody, wild bird types as a source of human disease	**Keynote: O036 Kaakoush, C concisus pathogenicity**	**Keynote: O042 Kelly, oxygen mediated gene regulation and protein expression in Cj**
	O031 Hetman, WGS model for genomic epidemiology	O037 Madden, poultry Cj and Cc hcp gene enhances virulence	O043 Sacher Phage-encoded flagellar binding proteins affect growth
	O032 Nielsen, MLST of C concisus isolates from patients in Denmark	O038 Beerens, CRISPR-cas in Cj	O044 On, Campy has a proteomic memory for survival?
	O033 Martiny, epi of AMR campy strains in Belgium	O039 Heikema, binding of Cj to sialoadhesin-expressing cells	O045 Aidley, significance of phase variation rates
	O034 Marder, Exposures associated with Campylobacter infection in young children-USA	O040 Jensen, C. jejuni survival mechanisms in house flies	O046 Butler, first discovery MORN protein
	O035 Parker, MLST types in livestock and birds.	O041 Istivan, C concisus invasion characteristics	O047 Wu, Cj outer membrane protein mutations enhances virulence
1740	**End of day**		
1800	**Evening social function: Traditional Campylobacter vs Helicobacter Football match – SPORTSDROME, EEC**		

TUESDAY 3rd NOVEMBER

0815-	**PLENARY LECTURE – Dr Bill Hanage, USA: New developments in genomic epidemiology** Chair: Sam Sheppard		
ROOM	**Bay Trust Forum**	**Skellerup**	**Opus**
	Comparative genomics and genomic epidemiology (sponsored by Applied Maths) **Chair: Alison Cody / Nigel French**	**Pathogenomics and sequelae** **Chair Victoria Korolik / Steffan Backert**	**Government and regulatory aspects** **Chair: Hanne Rosenquist / Judi Lee**
	Keynote: O048 Sheppard, C genome mapping	**Keynote: O053 Grover, Cj and IBD**	**Keynote: O057 Soboleva, raw milk - science and policy**
	O049 Zhang, MLST analysis of Cj strains of diverse origin in Eastern China	O054 Skarp, comparative genomics of invasive Cj-Finland	O058 Hansson, Swedish Campy monitoring in chickens
	O050 Barker, core genome MLST for Cj	O055 Istivan, C concisus in IBD	O059 Jacobs-Reitsma International Standard ISO/DIS 10272 for detection and enumeration
	O051 Pruckler, WGS for PulseNet and beyond	O056 Zhang, C concisus toxin -role in pathogenesis	O060 Van der Logt, notification based QMRA
	O052 Van Rensberg, Campylobacter genomes from a South African archive		O061 Youssef Marsi, National reference labs in developing countries
1040-1110	**MORNING TEA**		
ROOM	**Bay Trust Forum**	**Skellerup**	**Opus**
1110-1230	**Comparative 'omics: Campy** **Chair: Arnoud van Vliet / Patrick Biggs**	**Emerging species - focus on Arcobacter** **Chair Maria Figueras / Laure Kornreich**	**Helicobacter - pathogenesis and sequelae** **Chair: Steffan Backert / Jay Solnick**
	Keynote: O062 Yahara, "chromosome painting" of 200 genomes	**Keynote: O066 Figueras, Arcobacter culture**	**Keynote: O070 Solnick, Hp CagY in animal models**
	O063 Sheppard, C genome recombination	O067 Salas-Masso, v-q PCR for Arcobacter	O071 Keelan, virulence genes and severe disease in Arctic aboriginals
	O064 Vaughan, new method for population dynamics	O068 Webb, molecular epidemiology of A butzleri	O072 Armstrong, Hp gastric cancer and ulcers in Arizona
	O065 Stoakes, fliF and flagellar synthesis pseudorevertants	O069 Van den Abeele, Arcobacter AMR	O073 Baker, Hp and stomach cancer ethnicity
	O148 Lin, Cj1501c and conjugation with *Campylobacter jejuni*		
1230-1330	**LUNCH**		

ROOM	Bay Trust Forum		
1330-1410	**PLENARY LECTURE – Professor Ichizo Kobayashi, Japan: Genome/epigenome dynamics in the short- and long-term evolution of H. pylori** Chair: Arnoud van Vliet		
ROOM	**Skellerup**	**Bay Trust Forum**	**Opus**
1415-1530	**Attribution workshop (including 4 talks):**	**Advances in identification and subtyping Chair: Birgitte Duim / Brent Gilpin**	**Helicobacter - genomics and models Chair Jay Solnick / Terry Kwok**
	O074 Vieira, Foodnet MLST attribution for C in US	**Keynote: O078 Majcher, MALDI TOF for CHRO**	**Keynote: O082 Perez, gastric microbiome in people infected with Hp**
	O075 Marshall, dynamic source attribution NZ,	O079 Penny, C subtyping using MALDI-TOF	O083 Berthenet, comparative pathogenomics
	O076 Cody, lack of effect from food chain interventions on MLST patterns, UK	O080 Fukuda, gyrB PCR-RFLP	O084 Shaffer, Hp secretion systems
	O077 Pollari, source attribution in Ontario, multiple techniques including comparative genomic fingerprinting	O081 Hansson, EU NRLs	O085 Van Vliet, Hp genomics virulence
			O086 Kamiya, Hp infection models
1530-1600	**AFTERNOON TEA**		
ROOM	**Skellerup**	**Bay Trust Forum**	**Opus**
1600-	**Attribution workshop (continued)**	**Advances in detection methods Chair Wilma Jacobs-Reitsma / Stephen On**	**Environmental survival Chair David Kelly / Nicola Williams**
		Keynote: O087 Buchanan, rapid diagnostic markers	**Keynote: O092 Pascoe, genetics for biofilms and multihosts C**
		O088 Henry, MPN-PCR for C in water	O093 Wedley, factors influencing survival in soil
		O089 Habib, Enhancing Enumeration & Detection of Campy using Chromogenic-like Media	O094 Huq, oral C concisus biofilm formation
		O090 Lew-Tabor, C fetus: culture or PCR?	O095 Turonova, architecture of Cj biofilms
		O091 Hazeleger, LOD_{50} methods	O096 Bronowski, gene expression in VBNC
1740-1810	**CHRO 2017 presentations**		
1815-	**End of day / poster session / joint social function with NZ Microbiological Society**		

WEDNESDAY 4th NOVEMBER

JOINT PROGRAMME WITH THE NEW ZEALAND MICROBIOLOGICAL SOCIETY ALL SESSIONS TO BOTH CONFERENCES OPEN TO ALL!		
ROOM	**Bay Trust Forum**	
0815-0900	**PLENARY LECTURE – NZMS – Professor E. Peter Greenberg, USA – Sociomicrobiology** Chair: Sandy Moorhead	
0900-0945	**PLENARY LECTURE – CHRO - Professor Sarah O'Brien, UK - Campylobacter: epidemiology of an enigmatic organism** Chair: Rob Lake	
ROOM	**Bay Trust Forum**	**Skellerup**
1000-1040	**Control strategies for Campylobacter (sponsored by the UK Society for Applied Microbiology) Chair: Jaap Wagenaar / Rob Lake**	**Non-poultry sources of Campylobacter spp. Chair: Kelli Hiett / Tanya Soboleva**
	Keynote: O097 Sommer, effectiveness of on farm controls	**Keynote: O099 Mughini-Gras, Cj in wild birds and environmental water**
	O098 Rodgers, comparative efficacy of bootdip formulations	O100 Taboada, Cj in Canadian racoons: potential as vectors
1040-1110	**MORNING TEA**	
	Control strategies (continued)	**Non-poultry sources (continued)**
	O101 Van der Logt, chlorination in immersion chillers	O106 Penny, Campylobacter spp in shellfish in France
	O102 Seliwiorstow, slaughterhouse food safety management system	O107 Leblanc-Maridor, Cj on pig farms
	O103 Cerda-Cuellar, biosecurity on Spanish broiler farms trials	O108 Gilpin, MBiT and C from river water
	O104 de Zutter, impact of Cj poultry cutting process	O109 Grange, Cj in NZ wildlife
	O105 Pacholewicz, Campy and E.coli control points during broiler processing	O110 Weis, genomics of Campylobacter from crows in California
1230-1330	**LUNCH**	
ROOM	**Bay Trust Forum**	
1330-1410	**Plenary LECTURE - CHRO – Dr Hanne Rosenquist, Denmark - Government and regulatory efforts to control Campylobacter** Chair: Peter van der Logt	
ROOM	**Bay Trust Forum**	**Skellerup**

1415-1530	**Roundtable discussion on poultry control - international perspective** (including J. Lee: The Campylobacter Risk Management Strategy in New Zealand: O111; and N. Strachan: interventions and Campylobacter genotypes in British broiler farms: O149) **Facilitators: Stephen On, Peter van der Logt, Jaap Wagenaar** Contributions and insights from other participants from EU, NZ, US industry and project representatives	**Molecular Epidemiology -2 Chair: Bob Madden / Philip Carter**
		Keynote: O112 Van Vliet, Comparative genomics molecular epidemiology of Cc
		O113 Ramonaite, new MLST types and AMR amongst Cj isolates from Lithuanian children
		O114 Chaloner, porA genetic diversity in chicken isolates from UK
		O115 Wieczorek, Cj MLST types from chicken in Poland
1530-1600	**AFTERNOON TEA**	
ROOM	**Bay Trust Forum**	
1600-1645	**PLENARY LECTURE – NZMS – Professor GwangPyo Ko, Korea - Comparative phylogenetic and functional analyses of human microbiota in Korean twins** Chair: Amy Van Way Lovett	
ROOM	**Bay Trust Forum**	**Skellerup**
1650-1815	**Roundtable discussion on control - international perspective part 2: Non-poultry sources. Including 2 talks: Facilitators: Peter van der Logt, Tan-ya Soboleva**	**Gene regulation and metabolism Chair: Julien Ketley / David Kelly**
	O116. Armstrong, risk factors in Arizona	**Keynote: O117 Thompson, CsrA regulation of metabolic and pathogenesis-related genes**
	N. Williams - Campylobacter survival and domestic food safety considerations	O118 Kelly, electron transport chains, Cj
	- summaries from studies identifying other important sources; and where to from here	O119 Vegge, glucose metabolism of C
		O120 Butler, post translational modifications in Cj
		O121 van der Stel, regulation of ggt gene in Cj
1815	**End of day**	
1930-2330	**CHRO 2015 Gala Dinner / announcement of CHRO 2017 host**	

Thursday 5th November

ROOM	Bay Trust Forum	
0815-0900	**PLENARY LECTURE – Professor Ernst Kuipers, The Netherlands – Control and management of H. pylori - the state of the art** Chair: Jay Solnick	
ROOM	**Bay Trust Forum**	**Skellerup**
0910-1035	**Control strategies for Campylobacter - 2** **Chair: Ingrid Hansson / Peter van der Logt**	**H pylori diagnosis and control** **Chair: Guillermo Perez / Jacqui Keenan**
	Keynote: O122 Elviss, Campy on chicken in UK: control implications	**Keynote: O127 McDonald, screening programme for optimal cost utility**
	O123 Seliwiorstow, Cj process controls in poultry	O128 Roujeinikova, Structure and specificity of Hp aminopeptidase
	O124 Williams, air chilling parameters for Cj reduction on broilers	O129 Keelan, anti-Hp activity of liposomes
	O125 Medel, animal feed effects on colonisation and numbers: EU CAMPYBRO project results	O130 Rawdon, Helicobacter spp in sheep in NZ
	O126 Wedley, longitudinal study of Cj in a UK dairy farm	O131 Modak, inhibition of Hp by sulfonamide
1035-1110	**MORNING TEA**	
ROOM	**Bay Trust Forum**	**Skellerup**
1110-1230	**Antimicrobial Resistance (AMR)** **Chair: Michael Baker / Kelli Hiett**	**Vaccines Chair:** **Christine Szymanski / Brendan Wren**
	Keynote: O132 Cody, WGS to predict AMR of human Cj isolates	**Keynote: O137 Williams, immunology in chickens**
	O133 Vinueza, AMR of C in broilers Ecuador	O138 Armstrong, adapting Salmonella vaccines for C in poultry
	O134 Bloomfield, antibiotics and impact on strain evolution in persistent human infection	O139 Meunier, Campy avian vaccine
	O135 French - an emerging AMR clone of C. jejuni in NZ	O140 Nothaft, protein glycosylation and vaccine production
	O136 Nisar, C prevalence & AMR in various meat types in Pakistan	O141 Chaloner, Antibody: no effect on caecal colonization
1230-1315	**LUNCH**	

ROOM	Bay Trust Forum	Skellerup
1315-1405	**Control strategies for Campylobacter - 3** **Chair: Helle Sommer / Nigel French**	**Late breakers session**
	Keynote: O142 Medel, probiotic strategies on-farm: EU CAMPYBRO results	O145 Elgamoudi - Effects of D-Tryptophan on Campylobacter jejuni biofilm formation
	O143 Jorgensen, C on packaging in UK	O146 Line, aerobic isolation method
	O144 Baker, Lessons from New Zealand's prolonged campylobacteriosis epidemic	O147 Backert, Conservation and essentiality of HtrA in HP
ROOM	**Bay Trust Forum**	
1410-1500	**PLENARY LECTURE – Professor Christine Szymanski, Canada - The Campylobacter jejuni N-linked protein glycosylation pathway and its engineering to create an effective chicken vaccine** Chair: Stephen On	
1500	**CONFERENCE CLOSING AND PASSING OF THE CHRO TORCH**	

Plenary Speakers – Abstracts

Professor Brendan Wren
Monday 0920-1000

The compare and contrast of *Helicobacter pylori/Campylobacter jejuni pathogenesis*

Wren, B.[1]

London School of Hygiene and Tropical Medicine, London, U.K.

This presentation will compare and contrast the pathogenic mechanisms of *Campylobacter jejuni* and *Helicobacter pylori*. It will include the roles of dedicated virulence determinants and how these influence interactions with the host, and will include more recently described phenomena such as the role of biofilm formation and outer membrane vesicles in disease transmission and progression.

Professor Richard Peek
Monday 1000-1040

Gastric cancer, stem cells, microbial oncoproteins

Peek, R.M.[1]
Vanderbilt University School of Medicine

Epidemiological studies have determined that the attributable risk for gastric cancer conferred by *Helicobacter pylori* is approximately 75%. However, only a fraction of colonized persons ever develop neoplasia, and disease risk involves well-choreographed interactions between pathogen and host, which are dependent upon strain-specific bacterial factors, host genotypic traits, and/or environmental conditions.

One strain-specific virulence determinant that augments the risk for gastric cancer is the *cag* pathogenicity island, a secretion system that injects the bacterial oncoprotein CagA into host cells. The longevity of intracellular CagA is prolonged in gastric stem cells due to inhibition of autophagy. However, *H. pylori* does not simply exist as a monoculture within the human stomach but instead, is a resident of a distinct gastric microbial ecosystem. While *H. pylori* is the dominant species, the presence of other microorganisms provides a genetic repository which may facilitate the generation of novel traits that influence gastric carcinogenesis. Host polymorphisms within genes that regulate immunity and oncogenesis also heighten the risk for gastric cancer, in conjunction with *H. pylori* strain-specific constituents.

Further, environmental conditions such as iron deficiency and high salt intake can influence *H. pylori* phenotypes that lower the threshold for disease. Delineation of bacterial, host, and environmental mediators that augment gastric cancer risk has profound ramifications for both physicians and biomedical researchers as such findings will not only focus prevention approaches that target *H. pylori*-infected human populations at increased risk for stomach cancer, but will also provide mechanistic insights into inflammatory carcinomas that develop beyond the gastric niche.

Professor Mike Konkel
Monday 1330-1410

Campylobacter jejuni modulates gene expression in response to conditions encountered during an infection

Negretti NM[1], Clair GC[2], Nissen MS[1], Adkins JN[2], Gourley CR[1], Konkel ME[1*]
School of Molecular Biosciences, College of Veterinary Medicine, Washington State University, Pullman, Washington, USA, 99164-75201
Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA, USA[2].

Bile acids are known to alter the pathogenic behavior of gastrointestinal pathogens, including *Shigella* spp. and *Vibrio parahaemolyticus*. Previous work also indicates that culturing *Campylobacter jejuni* with bile acid at physiological concentration results in a global change in gene expression.

We hypothesize that culturing *C. jejuni* with the bile acid deoxycholate (DOC) will alter gene expression in a time-dependent manner. We will identify unique and/or differentially expressed genes using RNA extracted from four *C. jejuni* strains cultured in the presence and absence of DOC by RNA sequencing using an Ion Proton™ Sequencer. RNASeq data will be mapped to the appropriate genome and fold-changes statistically determined using standard software packages.

Preliminary results have revealed that *C. jejuni* demonstrate unique growth curves when cultured in nutrient broth versus nutrient broth supplemented with DOC. We also found that culturing *C. jejuni* in DOC-supplemented media results in new or enhanced synthesis of a number of proteins compared to bacteria cultured without DOC, as judged by [^{35}S]-methionine metabolic labeling coupled with SDS-PAGE and proteomic analysis. Work is in progress to identify the gene expression profiles of four *C. jejuni* strains cultured in the presence and absence of DOC and to perform comparative analyses to identify gene expression responses shared amongst strains. We propose these results will provide a foundation to identify genes expressed by *C. jejuni* in response to *in vivo*-like culture conditions.

Professor Bill Hanage
Tuesday 0820-0900

New developments in genomic epidemiology

Hanage, W.[1]
Harvard School of Public Health, Boston, USA

Helicobacter and *Campylobacter* are among those bacteria that are competent and undergo recombination, and in their case a great deal of it. Recombination impacts evolution in many ways, and can both generate great diversity yet also act as a unifying force, preventing the emergence of distinct divergent lineages. But recombination rates vary greatly even among very closely related taxa, and some lineages are more likely to share DNA than others. This can reflect ecological barriers to recombination and hence genomic data may be useful to infer niche structure, as has been proposed for lineages of *C. jejuni*.

In bacteria diversity is not only apparent in sequence variation, but also in genome content, and we have extended prior work modeling the core genome to include the accessory genome. Fitting to population genomic data from another recombinogenic pathogen, we show that the large-scale features of the data emerge as a consequence of observed recombination rates, without the need to invoke selection. However we also observe 'satellite species' which we propose can be understood as arising from overlapping ecological niches. The result is distinct clusters, held together by the occasional opportunities for recombination that arise from the niche structure. The coming era in which the genome sequences of infectious agents are routinely determined is exciting for medicine, and arguably even more so for ecologists and evolutionary biologists.

Professor Ichizo Kobayashi
Tuesday 1330-1410

Genome and epigenome evolution of *H. pylori*

Kobayashi, I.[1]
Department Computational Biology and Medical Sciences, Tokyo University, Japan[1]

Here I review genome/epigenome dynamics in the short- and long-term evolution of *H. pylori* as revealed by comparison of complete genome/methylomes. *H. pylori* genomes rapidly evolve through high mutation as well as high mutual homologous recombination. They show wide phylogeographic divergence. The evolutionary pathway suggested for cagA oncogene is Western type - Amerind type - East Asian type. Chromosome painting *in silico*, which detects transfer of sequence chunks through homologous recombination, revealed their fine population structure and admixture. *H. pylori* have a large number of sequence-specific DNA methyltransferase genes, with different strains carrying unique repertoires. Using single-molecule real-time (SMRT) sequencing technology in a Pac Bio machine, we studied methylated DNA bases throughout *H. pylori* genomes. The results demonstrated that these methyltransferases often change DNA sequence specificity through allelic recombination as well as domain movement. The movement of coding sequences of target recognition domains between genes and within a gene. Knocking out these specificity determinant genes led to unique changes in transcriptome and phenotype. These results are consistent with the concept of adaptive evolution driven by changes in the methylome. Most of these DNA methyltransferases are paired with a restriction endonuclease to form a restriction-modification system. One family of restriction enzymes present in Helicobacter and Campylobacter excises a base from their recognition sequence by DNA glycosylase activity and then cleaves the strand by AP lyase activity. This surprising finding reminds us of the demethylation by base excision in plant and animal epigenetics.

Professor E. Peter Greenburg
Wednesday 0815-0900

Quorum sensing in *Pseudomonas aeruginosa* - social cheaters and co-operators (NZMS plenary)

Greenberg, P.[1]
Department of Microbiology, University of Washington School of Medicine, Seattle, WA, USA[1]

In the bacterium *Pseudomonas aeruginosa* a transcription factor called LasR activates dozens of genes in response to the LasI-produced quorum-sensing signal 3OC12-HSL. The most overrepresented LasR-controlled genes code for synthesis of exoproducts. Exoproducts are common goods that are made by individuals and shared amongst the group. Members of quorum-sensing groups are thus thought of as cooperators. I have referred to them as socialists (tongue in cheek) in the title of my presentation. Quorum-sensing cooperators are susceptible to invasion by social cheaters. LasR mutants emerge in and co-exist with LasR-wild-type cells. The LasR mutants benefit from cooperator-derived common goods without incurring a production cost. I will discuss three questions related to this bacterial social activity: I. If social cheaters have a fitness advantage over cooperators, then how can cooperation be evolutionarily stable-Darwin's dilemma? I will describe one molecular mechanism that restrains cheating. II. Why do cooperators and cheaters reach an equilibrium and co-exist with each other in some situations? I will describe at a molecular level how cooperators police cheaters. III. Can we manipulate conditions to induce a breakdown in cooperation and a catastrophic population crash-a tragedy of the commons? I will show that this can occur when the cost of cooperation is increased or when policing is eliminated. I will discuss implications of this sociomicrobiological view of *P. aeruginosa* population ecology in the context of chronic infections.

Professor Sarah O'Brien

Wednesday 0900-0940

Campylobacter: epidemiology of an enigmatic organism

O'Brien SJ[1,2]

Department of Epidemiology and Population Health, Institute of Infection and Global Health, University of Liverpool

NIHR Health Protection Research Unit in Gastrointestinal Infections

Methods:

Several methods were employed including a prospective, population-based burden of illness study; a modelling study combining data from a systematic review, national public health surveillance systems, outbreaks of foodborne disease and the IID2 Study; and an economic modelling analysis using secondary data.

We have also formed an interdisciplinary ENIGMA Consortium to study *Campylobacter* transmission to humans in a holistic manner incorporating expertise from social and natural sciences.

Results:

The estimated incidence of *Campylobacter* cases in the community in the UK was 10 cases per 1,000 person-years of follow-up (95% CI: 6.3-15.8) i.e. around 500,000 cases annually. *Campylobacter* was the cause of 280,400 (95% CrI: 182,503–435,693) food-related cases and 38,860 (95% CrI: 27,160–55,610) general practice (GP) consultations. Estimated societal costs were around £50M (95% CI: £33M-£75M) and the cost per case was around £85. The cost associated with *Campylobacter*-related GBS hospitalisation was £1.26M (95% CI: £0.4M-£4.2M). The corresponding cost per GBS case was approximately £11,000.

Preliminary findings from the interdisciplinary ENIGMA Consortium will also be show-cased at the conference.

Conclusions:

Campylobacter remains a very important cause of acute gastroenteritis and strategies to control it are needed urgently.

Dr Hanne Rosenquist

Wednesday 1330-1410

Danish approaches to control *Campylobacter*

Rosenquist, H.[1]

Senior Academic Officer, Danish Veterinary and Food Administration[1]

Close collaboration between researchers, authorities and industry combined with political will and persistence have kept *Campylobacter* in Danish conventional broiler flocks and broiler meat relatively low compared with other European countries. Voluntary control initiatives started in late 1990s and action plans continue to aim for a reduced burden of this organism.

Monitoring, risk factor studies, intervention studies and risk assessments have guided the Danish management strategies. Monitoring has identified high risk slaughter processes and foods, quantified effects of action plans and informed risk assessments and source attribution. Risk factor studies have identified farm management operations leading to an increased risk of *Campylobacter*, and intervention studies have looked into biosecurity and physical and chemical decontamination of broiler meat. Furthermore, risk assessments have estimated the most effective control measures for Danish conditions and emphasized the importance of reducing the *Campylobacter* concentration. Risk models have been developed to work on a day-to-day basis in the case-by-case control of poultry meat batches and as a plant tool estimating individual plant performances.

The Danish approach includes initiatives in all steps of the broiler production chain; strict biosecurity, fly screens (experimental only), good slaughter hygiene, an industrial code of practice, audits, freezing to the extent possible (stopped), case-by-case control of meat batches, consumer information campaigns and education of school children.

That strict biosecurity works, also in countries with a warmer climate, was shown in the EU project CamCon. An overview of possible control options identified in CamCon and Danish research will be presented.

Professor GyangPyo Ko
Wednesday 1600-1645

Comparative phylogenetic and functional analyses of human microbiota in Korean twins

Ko, GP[1]
Center for Human and Environmental Microbiome, Graduate School of Public Health, Seoul National University[1]

Recently, there were dramatically increased interests on human microbiome research worldwide. Human microbiome has been considered as the second genome in addition to our own genome and played very crucial roles in maintaining human health. Human microbiota typically reside on the surface of epithelial cells and play various biological roles ranging from metabolism, immune development, mental health, and to organ development. Since 2008, we have determined the diversity of Korean microbiome and determined the genes and pathways of gut microbiome using Korean Twin Cohort. The specific aims of this presentation are 1) to determine and characterize the composition of human microbiome as related to clinical biomarkers, 2) to investigate the effects of host genetics and gut microbiota on metabolic syndrome. We find that the abundance of numbers of OTUs is strongly correlated with BMI, HOMA index, and triglyceride levels. Interestingly, among the gut microbes associated with MetS status, Actinobacteria to which the Bifidobacterium belong, had the highest heritability. Even after adjustment for MetS status, reduced abundances of Actinobacteria and Bifidobacterium were significantly linked to the minor allele of specific SNP, which is associated with triglyceride level and MetS. Our data suggest that specific OTUs in the gut may contain metabolic genes the characteristics of which differ according to host genetic background and/or diet. Our research will help us to understand the association between human microbiome and diseases and to improve human health by intervening both human microbiome and diets.

Professor Ernst Kuipers
Thursday 0815-09100

Control and managment of *H. Pylori* - the state of the art

Kuipers, E[1]
Erasmus University Medical Center, Rotterdam, The Netherlands[1]

The recognition of *Helicobacter pylori* as a human pathogen was one of the most important discoveries in gastroenterology in recent decades. It is the primary cause of a range of gastric conditions, in particular chronic gastritis, peptic ulcer, gastric cancer, and gastric MALT lymphoma. *H. pylori* eradication can in part cure and prevent these conditions.

Clinical therapy requires combination treatment. This is usually done by means of profound acid suppression and multiple antimicrobial drugs given for 7-14 days. Standard antimicrobials are bismuth compounds, amoxicillin, metronidazole, clarithromycin, and tetracyclin. Salvage antimicrobials are in particular levofloxacin, rifabutin, furazolidone, doxycycline, and nitrozoxanide. These are usually given in combinations of 3 (triple therapies) or 4 drugs (quadruple therapies). With respect to triple therapies, a range of studies have taught that double dose PPI, and longer treatment increase eradication rates, while probiotics may decrease side effects. Quadruple therapies are either bismuth-based (combined with 3 antimicrobials or a PPI plus two antimicrobials), or non-bismuth-based. The latter typically consist of a PPI with three antimicrobials. Depending on the dosing schedule, the latter are categorized as sequential, hybrid, and concomitant treatments.

With the widespread occurrence of antimicrobial resistance, treatment in most parts of the world is shifting towards quadruple regimens as primary treatment for *H. pylori* infection, with the aim to achieve a $\geq$ 90% eradication rate with the first treatment. The presentation will discuss the recent state-of-art in control and management of *H. pylori* infection and *H. pylori* -related clinical conditions, with focus on gastric neoplasia.

Professor Christine Szymanski
Thursday 1410-1500

The *Campylobacter jejuni N*-linked protein glycosylation pathway and its engineering to create an effective chicken vaccine

Nothaft, H.[1], Davis, B.[2], Lock, Y.Y.[2], Perez-Munoz, M.E.[3], Vinogradov, E.[4], Walter, J.[3,5], Coros, C.[2], and Szymanski, C.M.[1]
Alberta Glycomics Centre and Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada
Delta Genomics, Edmonton, AB, Canada.
Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada.
Institute for Biological Sciences, National Research Council, Ottawa, ON, Canada.
Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada.

Bacterial protein N-glycosylation was first described in *Campylobacter jejuni*. The oligosaccharyltranferase, PglB, transfers a conserved heptasaccharide to asparagine residues within the D/E-X_1-N-X_2-S/T sequon ($X_{1/2}\neq P$) of acceptor proteins and also releases free oligosaccharides into the periplasm. The *C. jejuni* protein glycosylation machinery also can be functionally transferred into *Escherichia coli* to engineer novel glycoconjugates.

Since *C. jejuni* is a common cause of human gastroenteritis and consumption of contaminated poultry is a major source for infection, elimination of *C. jejuni* from chickens would significantly reduce the incidence of campylobacteriosis in humans. We engineered the *C. jejuni* N-glycan heptasaccharide to be expressed on the surface of *E. coli* cells as a heptasaccharide-LPS fusion. Vaccination of chickens with live *E. coli* expressing the heptasaccharide fusions resulted in >8 log(10) reduction of *C. jejuni* colonization compared to the control groups. Heptasaccharide-specific IgY titres were highest in birds that received the live *E. coli* vaccine and this strain was cleared from the birds prior to *C. jejuni* challenge. Sequencing of the gut microbiome revealed that Campylobacter was readily detectable in positive control birds and was absent in negative control and vaccinated birds. Most importantly, vaccination did not change the composition of the microbiome. We propose that the attenuated strain of *E. coli* expressing the *C. jejuni*-heptasaccharide on its surface is an effective and low-cost vaccine significantly reducing *C. jejuni* chicken colonization and therefore its entry into the food chain.

1110-1230 Room A

O001

Impact of waning acquired immunity and asymptomatic infections on case-control studies for enteric pathogens

Havelaar, A.H.[1]; Swart, A.N.[2];

University of Florida, Gainesville, FL, USA[1]National Institute for Public Health and the Environment, Bilthoven, the Netherlands[2]

Background: Case-control studies of outbreaks and of sporadic cases are important sources of information to identify risk factors for campylobacteriosis and quantify the attributable proportion of illnesses. The underlying assumption in such studies is that the observed exposure distributions in cases and controls are reflective of the disease incidence rate ratios. However, such relationships may be biased by acquired immunity or asymptomatic infections, due to selecting controls that are not at risk of disease.

Objectives: We provide a theoretical framework to quantify the bias in case control studies due to waning acquired immunity and asymptomatic infections.

Methods: We build on an earlier developed mathematical models of the protective effects of temporary immunity on the dynamics of campylobacteriosis and dose-response relationships, and apply these models to reconstruct case-control studies under different assumptions.

Results: If all individuals in a population who are not diseased are eligible as controls, unbiased estimates of the incidence rate ratio will only result if all infected individuals become ill (no asymptomatic infections) and if diseased individuals directly revert to a fully susceptible state. Asymptomatic infections and acquired immunity bias the odds ratio to the null. Unbiased estimates can be observed if only susceptible individuals could be identified as eligible controls.

Conclusions: Not accounting for acquired immunity and asymptomatic infections in case-control studies of campylobacteriosis results in biased estimates of the odds-ratio. As a consequence, the proportion of illnesses attributable to risk factors is underestimated. Methods need to be developed to control for such selection biases.

O002

The seasonality of human Campylobacter infections in Europe

Nichols, G.L.[1]; Takkinen, J.[2]; Rossi, M.[3]; Joshi, A.[3]; Sudre, B.[2]; Lake, I.[4]; Lo Iacono, G.[1]; Djennad, A.[1]; Gomez Dias, O.[2]; Tavoschi, L.[2]; Van Bortel, W.[2]; Kovats, S.[5]; Vardoulakis, S.[1]; Fleming, L.E.[6]; Semenza, J.[2];

Public Health England[1]European Centre for Disease Prevention and Control[2]Consultant[3]University of East Anglia[4]London School of Hygiene and Tropical Medicine[5]University of Exeter[6]

Surveillance data on one million cases of Campylobacter from 24 EU Member States were extracted from the ECDC TESSy database for the period 2008 to 2012 and have been used to examine how seasonality of Campylobacter infection varies across Europe. Most countries show an annual seasonal increase in incidence in the spring/summer, with countries in northern latitudes having a later peak that is also more pronounced. Using Modis data on weather (rainfall, minimum, average and maximum land surface and air temperatures, sea level pressure, soil moisture, humidity) collected through the ECDC E3 Geoportal, weekly cases were linked to weather parameters at a national level (NUTS0) for all data and at the sub-regional (NUTS3) level for a percentage of the data. At NUTS0 there were R^2 values for 17 countries ranging from 0.40 to 0.81 using polynomial regression for land surface temperature 4 weeks before infection. For humidity 13 countries had R^2 values ranging from 0.36 to 0.84 for average humidity over the prior four weeks. Lag periods differed between countries; some individual country responses to weather were stronger than others and data linkage at NUTS3 level resulted in higher R^2 values than that at NUTS0. Results suggest that there is an indirect relationship between temperature/humidity and Campylobacter infection that remains unexplained by current paradigms. The collection of human disease and weather datasets from European countries that allow more geographically close data linkage between case, date and weather in the period prior to the onset of infection should facilitate a clearer understanding of the relationship between weather parameters and infection. This will improve the understanding of Campylobacter epidemiology and may contribute to better interventions and improved predictions of the impacts of climate change on Campylobacter across Europe.

O003

EU-wide study on the association between campylobacteriosis incidence and proton pump inhibitor-use

Bouwknegt, M[1]; Godman, B[2]; van Pelt, W[1];

Centre for Infectious Disease Control, RIVM, The Netherlands[1]Division of Clinical Pharmacology, Karolinska Institutet, Stockholm, Sweden[2]

Background: proton pump inhibitors (PPI) are associated with increased gastrointestinal infections, including campylobacteriosis, both as confounding and risk factor. In the Netherlands, we estimated that about 27% of campylobacteriosis cases at population level were due to PPI-use. In this study we examine such impact at the EU-level.

Methods: Data on reported campylobacteriosis cases (2007-2013) were obtained from the European Centre for Disease Prevention and Control's database. EU Member States (MS) were asked to voluntarily contribute data for the period 2001-2007. Utilization data in ambulatory care for orally administered PPI in defined daily doses (DDDs) were obtained per MS. Demographic data were obtained from Eurostat. Where possible, data were stratified according to four age-groups: 0-24, 25-49, 50-69 and ≥70. Chicken consumption was included to account for this major risk factor using FAO-data. Negative binomial regression is used to assess the association, supplemented with counterfactual analyses to assess the impact at EU-level.

Results: Increasing trends in reported incidence were observed in 13 MS in the period 2007-2013, decreasing trends in 2 MS and stable incidences in 11 MS. These trends provide useful variation in assessing the association between PPI-use. The data collection for the latter is ongoing and analyses will follow in the next months.

Conclusion: this study provides information on the impact of PPI-use on the campylobacteriosis incidence in Europe. If needed, rational pharmacotherapy to battle over prescription of PPIs (as this also leads to other concerns including osteoporosis) and targeted education on prevention of gastrointestinal disease may prove useful in lowering the campylobacteriosis incidence in Europe.

O004

Campylobacter infections, outbreaks, and antimicrobial resistance in the United States, 2004-2012

Geissler, A[1]; Bustos, F[1]; Patrick, ME[1]; Swanson, K[1]; Fullerton, K[1]; Bennett, C[1]; Walsh, K[1]; Mahon, BE[1];

U.S. Centers for Disease Control and Prevention[1]

Background: Campylobacteriosis is a leading cause of enteric illness in the United States and became nationally notifiable in 2015. Data describing patterns and trends are limited.

Objective: Describe the epidemiology of campylobacteriosis in the United States.

Methods: Data on laboratory-confirmed *Campylobacter* infections were summarized from the Nationally Notifiable Disease Surveillance System, Foodborne Diseases Active Surveillance Network, National Outbreak Reporting System, and National Antimicrobial Resistance Monitoring System.

Results: During 2004–2012, 303,518 *Campylobacte*r infections were reported; average annual incidence was 11.1 cases/100,000 population, with substantial variation by state (range: 3.2–48.0/100,000). Annual incidence increased from 10.5/100,000 in 2004–2009 to 12.2/100,000 in 2010–2012. Incidence was >2 fold greater among patients aged <5 years than overall annual rates; incidence among males was highest in all age groups. Among the 353 *Campylobacter* outbreaks reported (annual mean: 28 for 2004–2009; 62 for 2010–2012), 242 (69%) were foodborne, 32 (9%) waterborne, and 16 (5%) were due to animal contact. Dairy products were implicated in 69%, poultry in 15%, and produce in 7% of the 125 foodborne outbreaks attributed to a single food category. Among 5,863 isolates tested for antibiotic susceptibility, 4,793 (82%) were from domestically acquired infections. Comparing 2004 to 2012, resistance increased for ciprofloxacin (11.8% to 14.4%) and erythromycin (0.8% to 1.5%) among these isolates.

Conclusions: Campylobacteriosis incidence, outbreaks, and clinically relevant antimicrobial resistance patterns increased during 2004–2012. These findings underscore the need for national surveillance that includes standardized data collection to help elucidate risk factors, sources, and potential control measures.

O005

Epidemiology, clinical and microbiological features of Campylobacter infections in Belgian infants over the first three months of life.

Kornreich, LK[1]; Goetghebuer, TG[1]; Vlaes, LV[2]; Dediste, AD[3]; Martiny, DM[2]; Vandenberg, OV[2]; Levy, JL[1];
Department of Paediatrics, Saint-Pierre University Hospital, Brussels, Belgium[1]Department of Microbiology, iris-Lab, iris- Brussels Public Hospitals Network, Brussels, Belgium National Reference Centre for Campylobacter, iris-Lab, Iris-Brussels Public Hospital Network, Brussels,[2]Department of Microbiology, iris-Lab, iris- Brussels Public Hospitals Network, Brussels, Belgium[3]

Background: *Campylobacter* is a major cause of gastroenteritis in children younger than 1 year.

Objectives: To evaluate clinical presentation and predisposing/protective factors of *Campylobacter* infections in newborns aged less than 3 months old.

Methods: Bacterial culture of all stool samples from infants between 0-3 months old submitted to the department of Microbiology of Saint-Pierre University Hospital, Brussels, Belgium from 2007 to 2013 were analyzed. All patients' charts with a positive stool culture for bacterial enteropathogens were retrospectively reviewed. The charts of a control group of children matched to children with *Campylobacter* infection for age in days (about 10 days), gender and date of stool sampling with negative stool culture result were also reviewed.

Results: Over 2,047 stool specimens from 1,567 different patients, 13.1% had stools positive for potentially pathogenic bacteria. Among them, *Campylobacter* (67.8%) was the most commonly isolated followed by enteropathogenic *E. coli* (23.9%), *Salmonella* spp (7.9%) and *Aeromonas* spp (1.1%). Compared to infants with gastroenteritis caused by other bacterial pathogens, *Campylobacter* infection were more associated with macroscopic rectal bleeding (71% vs 9%, p<0.001), and frequently associated with co-infection with another enteric pathogens (13.6%). Compared to controls and to children with other bacterial diarrhea, *Campylobacter* infection was less often encountered in breastfed infants but more often required oral rehydration and antibiotics treatment.

Conclusions: Our study highlights that the presence of bloody diarrhea in infant's aged less than 3 months old is highly suggestive of *Campylobacter* enteritis. In these cases, rapid diagnostic tools allowing rapid targeted clinical management are warranted.

1110-1230 Room B

O006

Genomic classification and unique features of *Campylobacter fetus* subspecies

Duim, B[1]; van der Graaf- van Bloois, L[1]; Miller, W.G.[2]; Zomer, A[1]; Forbes, K[3]; Wagenaar, J.A.[1];
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Background: *Campylobacter fetus* (*C. fetus*) can cause disease in both humans and animals. *C. fetus* comprises the subspecies: *C. fetus* subspecies *fetus* (Cff) and *C. fetus* subspecies *venerealis* (Cfv) that differ in their primary habitats: Cff colonizes the genital- and intestinal-tracts of cattle, sheep and humans while Cfv is restricted to the bovine genital tract and is associated with fertility problems.

Objectives: Defining the genetic differences between the subspecies and, as a consequence, their niche preferences.

Methods: 36 *C. fetus* strains were classified phenotypically as Cff or Cfv using 1% glycine tolerance and H2S production. Genomes were obtained from Roche GS-FLX Titanium, Illumina MiSeq and PacBio RS data, and annotated; orthologous relations between genes were determined using Orthagogue. The Harvest suite was used for core-genome alignment and reconstruction single-nucleotide polymorphism (SNP) based phylogeny.

Results: The Cff and Cfv genomes were highly syntenic, and core-proteins were 99-100% similar. Core-genome and SNP phylogeny identified two clusters (A and B) and 284 differentiating SNPs. The clustering was not consistent with phenotypic classifications. Cluster A (Cfv) strains were all MLST ST 4, sap type A and contained transposases. Cluster B strains (Cff) belonged to four MLST STs, contained CRISPR-cas genes, a unique glycosylation region and were partly sap type B.

Conclusions: Comparative genomics identified genetic markers for consistent identification of *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*. Considerable variation was observed in the accessory genome, that provides the possibility to explore the presence of virulence genes and genes involved in niche adaptation.

O007

Diagnostic challenges facing the investigation of *Helicobacter* spp. with flexispira morphology associated abortion in sheep

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In New Zealand, since the early 1990's, there have been several large sheep abortion outbreaks in the southern South Island, often presenting with liver lesions comparable to campylobacter, but where endemic agents had been excluded. Investigations into the potential aetiological agent were largely inconclusive. In 2009 another large abortion outbreak occurred, where electron microscopy revealed slightly curved rods with a spiral periplasmic membrane within the biliary caniuli of diseased liver tissue. This ultrastructure was consistent with that of the bacterium *Flexispira rappini* (subsequently *Helicobacter* spp. with flexispira morphology), which represents at least 10 different taxa. In an attempt to definitively identify the causative agent, liver samples from aborted foetuses in 2011 were collected for culture and molecular testing at MIT. To maintain viability of this fastidious organism samples were kept in brain-heart infusion (BHI) broth with 20% glycerol and sent on dry-ice. Results confirmed the presence of *Helicobacter* spp. with flexispira morphology in aborted lambs from New Zealand. A survey was carried out during 2012 with testing done in parallel between MIT and MPI Animal Health Laboratory to cross-validate molecular findings. The 16S rRNA Helicobacter genus specific conventional PCR's were proven to be more sensitive than culture, and sequence analysis of the larger 1200 base amplicons were more informative. Findings demonstrate highest similarity to Flexispira rappini taxa 5 and 8. Conventional PCR and sequencing is laborious and costly, so the next challenge is to develop real-time PCR's for use in regional veterinary laboratories as part of routine abortion diagnostic workups.

O008

Isolation of *Campylobacter concisus* from different anatomic locations for genetic characterization and comparison

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Background: Isolates of *Campylobacter concisus* from patients with inflammatory bowel disease (IBD) as well as healthy individuals have shown markedly genetic heterogeneity of the species. It is plausible that this heterogeneity, observed between individuals, also exists within an individual. Genetic/functional investigation of *C. concisus* isolates from different anatomic locations in the same person could clarify this further.

Objectives: To isolate *C. concisus* from saliva, feces and mucosal biopsies in patients with IBD and healthy subjects, in order to characterize differences in isolates recovered from the same person.

Methods: We aim to include 100 patients with IBD and 50 healthy subjects. Saliva and stool samples are collected as well as 3-7 biopsies extracted during colonoscopy. *C. concisus* is isolated by cultivation and identified by q-PCR. *C. concisus* isolates are stored at -80°C pending DNA-purification for whole-genome sequencing.

Results: At present, 35 IBD patients and 14 healthy subjects have been included. Rates of *C. concisus* cultivation from different locations were as follows for IBD and healthy persons, respectively: Saliva: 65%/61%, mucosal biopsies: 51%/67%, feces: 20%/14%. For IBD patients 77 % were positive at any location while 34% were positive in more than one location. For healthy subjects, this was 93% and 39%, respectively.

Conclusions: Isolation rates of *C. concisus* are high for both IBD patients and healthy subjects, for approximately 35% it is possible from more than one location. Genetic investigations on these isolates will provide us with more knowledge about the species heterogeneity in health and disease.

O009

Whole genome sequences of oral and intestinal *Campylobacter concisus* strains and molecular typing using the ribosomal RNA operon (rrn)

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Background: *Campylobacter concisus* is a heterogeneous species of phenotypically indistinguishable strains belonging to different genomospecies.

Objective: As data on whole genome sequences for oral *C. concisus* strains is lacking, we sequenced whole genomes of oral strains for bioinformatics analysis.

Methods: The genomes of three strains, isolated in this study, were sequenced using in-house next generation sequencers. RMIT-JF1 and O17 are oral isolates from a Crohn's disease and a healthy child respectively; RCH 26 is a faecal strain from a child with gastroenteritis. Two 1.9 Mb (JF1 & RCH 26) and 1.8 Mb (O17) genomes were assembled. A comparative analysis of the genomes was performed in comparison with available sequences in the database.

Results: Bidirectional comparison of *C. concisus* 13826 with JF1, RCH26 and O17 showed 1741, 1758 and 1742 genes shared, respectively. More than 300 genes were unique in the reference strain while 323, 283 and 220 genes were respectively unique in our strains. RCH 26 has two plasmids (22kb and 3.3kb). The 3.3kb plasmid is unique. Phylogenetic trees were generated based on *rrn* from 11 strains. The regions from each *rrn* operon produced different phylogenetic trees demonstrating sequence differences between strains for the 5S rRNA, 23S rRNA, 16S rRNA and other intergenic regions. Furthermore, genomospecies A strains were characterized by the presence of an Intervening Sequence within the 23S rRNA gene.

Conclusions: *C. concisus* can be classified into two genomospecies (A & B) based on the arrangement of the three *rrn* operon copies and by the arrangement of the 5S rRNA, 23S rRNA and 16S rRNA genes within each operon.

O010

Comparative genomic analysis of 17 *Campylobacter concisus* genomes suggests at least two species

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Campylobacter concisus (CC) has been isolated from both healthy and diarrhoeic stool samples, leading to debate as to the role of this species in human gastroenteritis. The species has also been shown to be genetically heterogeneous suggesting there may be variation in the pathogenic potential of different CC strains. Genomes from 17 human strains (the type strain, eight publicly available strains, and eight strains from our collection) from a range of sites and disease states were analysed using ribosomal Multi-Locus Sequence Typing and four genome-wide comparison methods. All the methods clearly separated the genomes into two groups coinciding with AFLP-based genomospecies and homology with published 23S rRNA primers. Large-scale BLAST score ratio was used to predict genes present in all nine genomospecies 2 (GS2) strains and absent, or highly divergent, in all eight genomospecies 1 (GS1) strains. Seven of these predicted genes had significant homology to genes of known function. A profile-based homology scoring method was used to evaluate orthologous genes in all 17 CC genomes and identified 25 genes where the bitscore of the amino acid sequence was significantly higher in GS1 genomes compared to GS2 genomes. Some of these genomospecies-specific gene variants look like good candidates for molecular detection. Although the genomospecies predicted by these analyses do not separate the available strains based on their association with human gastroenteritis, the potential to provide taxonomically meaningful separation of this heterogeneous group may lead, in time, to a better understanding of the role these genomospecies play in human illness.

1110-1230 Room C

O011

The *Helicobacter pylori* cag secretion system suppresses inflammation via translocation of DNA into host cells

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Background: The *H. pylori cag* type IV secretion system (T4SS) translocates the pro-inflammatory effector CagA into host cells. However, the *cag* T4SS also shares structural homology with the archetypal *A. tumefaciens* T4SS, which utilizes DNA as a substrate. Toll like receptor 9 (TLR9) is an immune receptor that recognizes hypo-methylated CpG DNA motifs and, when activated, can induce either pro- or anti-inflammatory responses. Objective: To determine whether *H. pylori* translocates DNA into host cells via the *cag* T4SS and define the immune ramifications of TLR9 activation *in vitro* and *in vivo*.

Methods: TLR9 activation assays, super-resolution confocal microscopy, and flow cytometry were used to assess *H. pylori*-mediated DNA translocation *in vitro*. Wild type or *tlr9-/-* C57Bl/6 mice and human gastric biopsies were used for *in vivo* assays.

Results: Multiple *H. pylori* strains readily translocated DNA into host cells via the *cag* T4SS, leading to robust activation of TLR9. In mice, TLR9 activation significantly suppressed the intensity of the immune response to *H. pylori*. *H. pylori* isolated from patients residing in a high gastric cancer risk region induced significantly higher levels of TLR9 activation and infected high risk patients expressed higher gastric epithelial levels of TLR9 when compared to isolates or patients from the low risk region.

Conclusion: *H. pylori* uses a previously ascribed virulence constituent to reciprocally suppress the host immune response. This provides fresh insights into understanding how *H. pylori* can utilize a single component as an inflammatory rheostat, which may facilitate long-term persistence within human hosts.

O012

The preferred refuge of *Helicobacter pylori* in macrophages: phagosomes, LC3-associated phagosomes, autophagosomes or cytoplasm?

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Background: *Helicobacter pylori* has been suggested to induce and utilize autophagic processes and thereby contribute to persistent infection. Recently, an autophagy-like process, LC3 (microtubule associated protein 1 light chain 3)-associated phagocytosis (LAP), has been reported to be associated with the intracellular survival of several bacteria. However, whether *H. pylori* infection induces canonical autophagy and/or LAP is yet to be clarified.

Objectives: We sought to determine the extent of induction of canonical autophagy and/or LAP in *H. pylori*-infected macrophages. We also investigate the influence of the *H. pylori cag* pathogenicity island (*cagPAI*) on these processes.

Methods: Immunofluorescence confocal microscopy was used to detect the GFP-LC3 puncta and their co-localization with *H. pylori*. Transmission electron microscopy was used to analyse the membrane nature of *H. pylori*-containing intracellular vesicles.

Results: Only a very small percentage (0.5-6%) of intracellular *H. pylori* was targeted to autophagosomes. The majority (85-95%) was found in LC3-negative phagosomes and a small proportion (4-14%) appeared "free" in the cytosol. No statistically significant difference was observed in the relative distribution of *H. pylori* in various intracellular locations between wild-type and *cagPAI* mutant bacteria.

Conclusions: *H. pylori* infection does not induce LAP, but can induce, to small extent, canonical autophagy in macrophages. The appearance of free *H. pylori* in the cytosol suggests that these bacteria might have escaped from phagosomes into cytosol and then be sequestered by autophagosomes. The *cagPAI* of *H. pylori* has little or no effect on the occurrence of these processes.

O013

The molecular evolution of Nod1-dependent immune suppression induced by *Helicobacter pylori* within the stomach

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Background: *Helicobacter pylori* (*Hp*) is a chronic pathogen that has evolved multiple strategies to evade the host immune response. Recently, we reported that pre-activation of NOD1 reduces *Hp*-induced injury in Mongolian gerbils, while inhibition of NOD1 prior to *Hp* infection *in vitro* leads to increased activation of NF-κB.

Aim: To define the role of Nod1 and downstream immune effectors in mediating *Hp*-induced inflammation using a genetic model of *Nod1* deficiency.

Methods: Wild-type (WT) or *Nod1*$^{-/-}$ mice challenged with the *Hp cag*$^{+}$ strain PMSS1 were euthanized 2, 20, or 90 days post-infection to assess colonization, histopathology, and cytokine expression using a multiplex bead array.

Results: No differences in colonization were observed between *Hp*-infected *Nod1*$^{-/-}$ or WT mice. Infected *Nod1*$^{-/-}$ mice developed significantly increased levels of inflammation compared to WT mice at 20 days of infection ($p=0.04$). Two days post-challenge, *Hp*-infected *Nod1*$^{-/-}$ mice harbored significantly ($p<0.05$) higher gastric mucosal levels of acute inflammatory mediators (IL-1β, TNFα, IL-6 and KC), chemokines (including MIP-1α, MIP-1β, and MIP-2), and cytokines that mediate humoral and cellular immune responses (including IFNγ, IL-12 and IL-17). However, 20 days post-infection, this pattern changed as *Hp*-infected *Nod1*$^{-/-}$ animals maintained higher levels of only Th1/Th17 cytokines compared to *Hp*-infected WT mice. By 90 days, *Hp*-infected WT mice expressed significantly higher concentrations of regulatory and Th2 cytokines compared to infected *Nod1*$^{-/-}$ mice.

Conclusion: NOD1 activation affects multiple components of the immune response directed against *Hp*. Manipulation of Nod1, therefore, may represent a novel strategy to prevent disease outcomes induced by *Hp* infection.

O014

Structure-function analysis of *Helicobacter pylori* cell shape proteins

Csd4, Csd5 and Csd6

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Helical cell shape of *Helicobacter pylori* has been shown to be important for efficient colonization in a murine colonization model but the molecular factors that govern shape determination are poorly understood. A cell's shape is determined by its peptidoglycan (PG) cell wall, a single macromolecular structure that encases the cell. Prior genetic screens identified cell shape determinant (csd) genes including two PG hydrolases (Csd4 and Csd6) and a protein without predicted enzymatic domains (Csd5) that share a straight null-mutant phenotype. When examining a panel of human isolates collected from one individual at different sites within the stomach, we observed abundant morphologic variation. Of the isolates with straight morphology, all had mutations in *csd4*, *csd5* or *csd6*. While the *csd4* and *csd6* mutations all lead to protein truncation, the *csd5* mutant causes a non-synonymous change within a putative SH3 domain. Cell shape and protein expression analyses of single point mutants constructed in additional conserved residues in the RT-Src loop of the SH3 domain suggest this loop is important for normal function and stability of Csd5 protein. Additional homology modeling and phylogenetic alignments uncovered a repeat motif adjacent to the SH3 domain in Csd5. Genetic complementation experiments with *csd5* alleles with differing repeat lengths yield strains with altered shape parameters. We currently are investigating possible SH3-domain mediated protein-protein interactions among these Csd proteins and the selective forces that may drive the accumulation of shape variation during human infection.

O015

One genome to rule them all: sequencing a partially clonal population of *Helicobacter pylori* SS1

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Although it is well known that many bacterial genomes are highly variable, it is nonetheless traditional to refer to, analyse, and publish "the genome" of a bacterial strain. Variability is usually minimised ("only sequence from a single colony"), ignored ("just publish the consensus"), or placed in the 'too hard basket' ("analysis of raw read data is more robust"). *Helicobacter pylori* is well known for having a highly variable genome with a high mutation and recombination rate. Nonetheless, little is known about the degree of variation to expect in typical working stocks, and researchers rely heavily on single fixed reference genomes for their strains. In this talk, I will discuss the variability seen in a typical laboratory culture of *H. pylori* strain SS1, as revealed by a combination of next-generation sequencing and traditional laboratory techniques. This includes nearly 50 SNPs at over 5% prevalence, variation in transposon IS607 locations, and copy-number variation of the *cagA* gene. This work reveals that reliance on a single-colony genome or consensus assembly may be misleading, even at the level of a typical laboratory working stock.

O016

Molecular epidemiology of *C. jejuni* and *C. coli* circulating in a model ecosystem with intense agricultural activity and high rates of campylobacteriosis.

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Southwestern Alberta (SWA) possesses campylobacteriosis rates that are among the highest in North America. The region possesses ~150K people (~50:50 urban-rural split), contains a single predominant watershed, and high densities of livestock including ~1 million cattle. We have examined the molecular epidemiology of *C. jejuni* (CJ) and *C. coli* (CC) of isolates circulating in this model ecosystem in an effort to examine factors underlying the high incidence of campylobacteriosis in SWA. CJ-CC isolates (n=8,770) from various sources including livestock (n=4,414), environmental waters (n=1,549), and human stools (n=2,742) submitted to the diagnostic facility within the region (2007-2014) were subtyped using Comparative Genomic Fingerprinting (CGF40). Subtypes were compared to the national CGF40 database, which is comprised of >18,000 isolates from various sources across Canada. A subset of isolates was also subjected to whole genome sequencing. CGF40 subtypes observed among CJ-CC human clinical cases in SWA were widely distributed temporally and geographically, and comprised isolates from multiple animal and environmental sources. A significant proportion (32%) of human clinical isolates belonged to subtypes in which cattle was the dominant source; clinical isolates from SWA also showed higher association with subtypes derived from environmental sources including water. In contrast to what has been observed in other regions of Canada, where chicken is a primary source of clinically-associated subtypes, our results indicate that in agroecosystems such as in SWA, beef cattle are a significant contributing factor to the epidemiology of campylobacteriosis and that water may play an important role in the transmission of CJ-CC.

O017

Comparative genomic fingerprinting for outbreak and sporadic Isolates of *Campylobacter jejuni* in British Columbia, Canada

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Campylobacter is the most common cause of bacterial gastroenteritis in Canada, with *Campylobacter jejuni* as the most commonly isolated species in human illness. Campylobacteriosis is a nationally notifiable disease in Canada, yet surveillance in the country is limited. The high volume of cases impedes epidemiologic investigation, and current molecular subtyping tools are under-utilized due to case volumes, cost and labour constraints and long turn-around-times. Comparative Genomic Fingerprinting (CGF) was developed for high resolution and cost-effective subtyping of *Campylobacter jejuni*. CGF involves multiplex polymerase chain reaction (MPCR) amplification of 40 targets (CGF40) and comparison of subtypes. The British Columbia Public Health Microbiology and Reference Laboratory (BCPHMRL) evaluated CGF using 93 clinical outbreak and sporadic *C. jejuni* isolates. All outbreak isolates were previously confirmed using alternate subtyping methods (multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE)) and/or epidemiological confirmation. Results were compared and analyzed with a Canadian *Campylobacter* CGF database containing over 16,600 isolates. CGF40 accurately clustered outbreak isolates and a subset of 25 targets (CGF25) was equally discriminatory for outbreak isolates as CGF40. Yet CGF40 provided further resolution of sporadic CGF25 subtypes, which could enhance surveillance. Additionally, CGF40 was performed on 472 isolates of *C. jejuni* over a 1-year period from 2013-2014 in BC. Amongst the 472 isolates, CGF40 produced 203 different subtypes and CGF25 produced 174 different subtypes, demonstrating enhanced resolution of CGF40 over CGF25. CGF, in combination with the Canadian CGF database, is therefore an informative tool for surveillance and outbreak investigations of *C. jejuni* in a public health laboratory.

O018

Outbreak investigation using MLPA-based binary typing (MBiT)

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In a previous study (Epidemiology & Infection. 141:1253-1266) we demonstrated that genotyping isolates of campylobacter from human cases was crucial to identifying sources of infection. However the cost and time taken to complete genotyping (whether by pulsed field gel electrophoresis (PFGE), multi locus sequence typing (MLST) or whole genome sequencing), limits the role of genotyping to a confirmation of source, rather than the possibility of using genotyping to guide epidemiological investigations. A new genotyping method MBiT (Multiplex ligation-dependent probe amplification (MLPA) based Binary Typing) allows rapid analysis of isolates (less than 24 hours), in high throughput format (100 isolates a day by single technician), at a consumable cost of less than US$15. In this paper we evaluated over a 1000 campylobacter isolates using MBiT to compare; (a) The discriminatory ability of MBiT relative to PFGE and MLST; and (b) Whether the same clusters of cases identified in the 2012 paper, would have been identified using MBiT. We also describe the use of MBiT to characterise outbreaks of campylobacteriosis related to contaminated water, and a foodborne outbreak.

O019

Campylobacter infections identified through culture-independent methods and their impact on the estimated incidence of Campylobacter infections — Foodborne Diseases Active Surveillance Network, USA, 2010–2014

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Background: Campylobacter is estimated to be the most common US bacterial enteric infection. Incidence rates (IRs) include only culture-confirmed cases; however, culture-independent diagnostic tests (CIDT) are increasingly being used instead of culture.

Objective: Describe impact of CIDT use on incidence of campylobacteriosis

Methods: The Foodborne Diseases Active Surveillance Network (FoodNet) conducts population-based surveillance in ten states for Campylobacter infections diagnosed by culture or by CIDT. We compared these cases and calculated IRs using US census data.

Results: Among 37,252 Campylobacter infections reported during 2010–2014, 33,081 (89%) were diagnosed by culture and 4,171 (11%) only by CIDT. Of 3,028 CIDT reports with test information, 84% were antigen-based commercial tests, 15% locally-developed PCR tests, and 1% DNA-based syndrome panels. CIDT reports increased from 7.7% in 2010 to 14.2% in 2014. If CIDTs were included in IR calculations, IRs would have been 8.4% higher in 2010 (14.7 vs 13.5 cases per 100,000) and 16.5% higher in 2014 (15.7 vs. 13.4). The increase was greatest among persons ≥70 years old. Persons diagnosed by CIDT had a higher median age, were more likely to be female, and less likely to have diarrhea or fever than those diagnosed by culture.

Conclusion: The percentage of Campylobacter infections diagnosed by CIDTs is increasing. Most CIDT reports are based on antigen tests. Differences among persons diagnosed by CIDT versus culture may reflect differences in test performance or in testing practices. Continued collection of CIDT information is important to quantify the impact on measured trends.

O020

Relation between broiler and human Campylobacter strains isolated in Belgium during the period 2011-2013

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Background: *Campylobacter* infections in humans are the predominant zoonosis in Europe but they are mostly sporadic. The characterization of broiler meat monitoring isolates and their comparison with human clinical isolates can contribute to a better understanding of *Campylobacter* epidemiology.

Objective: To compare the *Campylobacter* isolates obtained from the Belgium broiler meat monitoring plan and from human cases during 2011, 2012 and 2013, using different typing methods.

Methods: *Campylobacter* isolates were characterized by multi-locus sequence typing (MLST), RFLP-*flaA* typing, antibiotic microbiological resistance (AMR), presence/absence of 5 putative virulence genes, and exclusively for *C. jejuni*, determination of A to E lipooligosaccharide (LOS) class.

Results: The predominant *C. jejuni* MLST clonal complex (CC) for both human and broiler strains was CC-21, with 29% and 20% of the isolates, respectively. Besides the sequence types (STs) that comprise CC-21, the most frequent STs were ST-48 (human isolates) and ST-464 (broiler isolates), with 10% and 6% of the *C. jejuni* isolates, respectively. For *C. coli* broiler isolates, CC-828 (84%), including those within ST-854 (13%), was predominant. The most frequent AMR profile for *C. jejuni* and *C. coli*, and for both analyzed populations (broiler and human isolates), was the combined resistance of ciprofloxacin, nalidixic acid and tetracycline. The presence of all 5 putative virulence genes was the most frequent virulence gene profile for both tested *Campylobacter* species and all but the LOS A class were equally found.

Conclusions: The most frequent CCs for both human and broiler isolates were CC-21 for *C. jejuni* and CC-828 for *C. coli* in Belgium.

1415-1530 Room B

O021

Specific patterns of phase-variable gene expression are associated with persistence of *Campylobacter jejuni* strain NCTC11168 in chickens

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Surface structures of Campylobacters are subject to a high degree of variability due to phase variation mediated by mutations in hypermutable repetitive sequences. These mutations cause frequent, reversible switches in expression of outer membrane proteins and addition of glycans and other moieties to the capsule, flagella and lipooligosaccharide. Phase variation of these surface proteins and epitopes is predicted to facilitate host adaptation through evasion of adaptive immune responses. In order to determine whether phase variation contributes to host adaptation, we inoculated four groups of twelve two week-old chickens with hypermotile (11168H) or chicken-adapted (11168ca) variants of *C. jejuni* strain NCTC11168. Changes in the polyG/polyC tract lengths of the 28 phase-variable loci of this strain were monitored using a multiplex GeneScan assay for ~3,000 colonies collected at 0, 14, 28 and 52 days post inoculation. Overall expression states for each gene were relatively stable for the first two weeks of colonisation before shifts occurred in multiple genes with most of the variation occurring in the phase-variable epitopes of the flagella. We are now investigating whether these changes are associated with combinatorial expression states (phasotypes) and adaptation to immune responses. The effect of pre-existing immune responses on phase-variable gene expression was investigated by immunising birds with whole cell lysates of the homologous strain prior to challenge. Colonisation levels were similar to non-immunised birds. Fluctuations in the in vivo phase-variable expression states will be presented in the context of on-going research into in vitro growth effects of phasotypes and experimental/theoretical studies of mutation rates, bottlenecks and selection coefficients.

O022

Pathogenicity of *Campylobacter jejuni, C. upsaliensis* and *C. helveticus* in the invertebrate disease model *Galleria mellonella*

Bojanic, K[1]; Midwinter, AC[1]; Biggs, PJ[1]; Marshall, JC[1]; Acke, E[1];

Massey University[1]

Campylobacter jejuni (CJ) is a well-established human pathogen but many other species in the genus have discordant or a lack of evidence of pathogenic potential. *C. upsaliensis* (CU) is also a recognized human pathogen and *C. helveticus* (CH), despite a high level of genetic similarity to CU, is not. The invertebrates have a high degree of functional and structural homology with the mammalian innate immune system and larvae of the Greater Wax Moth, *Galleria mellonella*, has been described as an animal model for CJ. This study aimed to compare the pathogenicity of CJ, CU and CH in this model. Thirty-one isolates of CJ, 19 of CU and 11 of CH were used for inoculation into the haemocoel via the pro-leg of 4,596 larvae. Controls consisted of 313 phosphate-buffered saline inoculated and 306 un-inoculated larvae. Experiments were performed with (i) live bacteria in varying doses; (ii) heat and freeze-thaw inactivated whole cells, insoluble and soluble cellular components; (iii) room and 37°C incubating temperatures and, (iv) aerobic and H_2-enriched microaerobic atmospheres. Technical and biological replicates were used and survival was monitored up to 8 days post-injection. Survival of larvae was dependent on the species of inoculum, bacterial dose, bacterial components/products and environmental conditions. Generally, species differences were more pronounced with live cell assays where CJ caused a higher and faster larval death rate than CU and CH. The observations of differences in the pathogenicity of *Campylobacter* species in this disease model correlate with observations from comparative epidemiological and genomic studies.

O023

TLp11 - galactose chemosensory receptor of *Campylobacter jejuni.*

Day, C.J.[1]; King, R. M.[1]; Shewell, L. K.[1], Tram, G.[1]; Hartley-Tassell, L. E.[1]; Fleetwood, A.[2]; Zhulin, I. B.[2]; Korolik, V.[1]
Griffith University[1]University of Tennessee[2]

A rare *C. jejuni* chemotaxis receptor, initially named Tlp11, was first identified in a highly virulent clinical isolate *C. jejuni* 520 and *C. jejuni* strain 84-25, isolated from a meningitis case. Tlp11 was subsequently found in clinical isolates that resulted in hospitalisation of infected individuals. Chemotaxis ligand and small molecule array screening determined galactose to be the ligand specific for this sensory receptor. Chemotaxis assays using wild type and isogenic inactivation, allelic addition and complemented mutant strains, confirmed the chemotactic response of *C. jejuni* to galactose was Tlp11 dependant. Motility and autoagglutination were significantly altered by Tlp11 expression with decrease in motility and increased autoagglutination in the tlp11 isogenic mutant, as opposed to wild type strain, similar to that seen for *C. jejuni* multiligand receptor CcmL. This novel *C. jejuni* receptor was consequently named CcrG, campylobacter chemoreceptor for galactose. *C. jejuni* 520 ccrG mutant was shown to have significantly reduced ability to adhere to Int-407 and Caco2 intestinal cells lines and significantly reduced ability to colonise chickens. Comparative genomic analysis of the CcrG sequence revealed that the sequence encoding this receptor is unique and likely to have arisen via fusion of periplasmic sensory domain of CcaA, the aspartate receptor, and signalling cytoplasmic domain of CcmL, the multiligand receptor. The findings in this study provide further insight into chemotaxis receptor protein-ligand interactions with implications not just for *C. jejuni* chemotaxis but for all bacterial chemotaxis.

O024

Understanding the genetics of asymptomatic carriage of Campylobacter by malnourished children in the Peruvian Amazon

Pascoe, B[1]; Murray, S[1]; Meric, G[1]; Wilkinson, TS[1]; Kosek, M[2]; Sheppard, SK[1]; Swansea University[1]John Hopkins Bloomberg School of Public Health, Maryland[2]

Background: *Campylobacter* infection in developed countries peaks during infancy and again during early adulthood, with most infections the result of consumption or handling of contaminated poultry products. In developing countries, the epidemiology of disease is quite different - *Campylobacter* infections are endemic among young children, especially those below 2 years old. Asymptomatic infection is common in both children and adults, whereas, in developed countries, asymptomatic *Campylobacter* infection is unusual.

Objectives: We investigate the genetic basis of asymptomatic carriage of *Campylobacter* isolates collected from malnourished children in the Peruvian amazon (442 children aged 0–72 months).

Methods: Tissue culture experiments were used to test host response to our collection of 101 *Campylobacter* isolates. These data were compared to detailed patient records of the symptoms each child suffered. Isolates were also sequenced and genome-wide association studies (GWAS) used to identify genetic elements associated with asymptomatic carriage and specific host immune responses.

Results: GWAS highlighted the potential involvement of glycosylation genes with asymptomatic carriage of *Campylobacter*. This association is currently being tested with phenotype assays in the lab.

Conclusions: Characterisation of the genetic traits associated with asymptomatic carriage of *Campylobacter* will inform intervention strategies against Campylobacteriosis, including potential vaccine development.

O025

Immunoglobulin G response in patients with *Campylobacter concisus* diarrhea

Nielsen, HL[1]; Kaakoush, NO[2]; Mitchell, HM[2]; Nielsen, H[3];

Department of Clinical Microbiology, Aalborg University Hospital, Aalborg, Denmark[1]School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, Australia[2]Department of Infectious Diseases, Aalborg University Hospital, Aalborg, Denmark[3]

Background: The emerging enteric pathogen *Campylobacter concisus* has been associated with gastroenteritis. Although specific immunoreactive proteins have been reported, limited information is available for the assessment of the systemic immunoglobulin response in patients infected with *C. concisus*.

Objectives: To detect and characterize (by ELISA) anti-*C. concisus* antibodies in serum of individuals with *C. concisus* gastroenteritis.

Methods: Two serum samples were collected from 88 adult patients at the time of diagnosis and after four weeks. Pooled donor serum was used as control. IgG antibodies specific for *C. concisus* were measured in serum using an in-house ELISA.

Results: The mean optical density at diagnosis was 0.772 ± 0.067 for the 88 adult patients and 0.455 ± 0.045 in pooled control serum. When using an optical density value equal to the mean plus three intervals of standard error for the control serum, 44/88 (50%) of patients had increased IgG levels to *C. concisus*. Overall, at four weeks follow-up, the IgG levels were not significantly different from initial value. Patients with increased IgG levels were more often females and had more often reported headache and vomiting. In contrast, IgG levels were unrelated to age, duration of diarrhea, number of stools per day, consistency of stools and weight loss.

Conclusions: While *C. concisus*-positive patients had detectable and increased IgG antibodies in half of adult cases with gastroenteritis, a humoral immune response was not associated with clinical features such as duration of diarrhea or severity of diarrheal disease.

Guillain Barré Syndrome
Monday 2nd November

1415-1530 Room C

O026

Guillain-Barre Syndrome incidence declines following successful countrywide control of campylobacteriosis

Baker, MG[1]; Kvalsvig , A[1]; Zhang, J[1]; Lake, R[2]; Sears, A[1]; Wilson, N[1];
University of Otago, Wellington[1]ESR Christchurch[2]

Background: Campylobacter infection is a known precipitating event for Guillain-Barre syndrome (GBS). **Objectives:** To assess the relationship between hospitalisation for campylobacteriosis and subsequent risk of GBS and test the hypothesis that GBS incidence had responded to a marked rise and then decline in campylobacteriosis rates in New Zealand (NZ).

Methods: This study used hospitalisations records for GBS, campylobacteriosis, and selected other conditions; mortality data for GBS; and campylobacteriosis notification data for the years 1988-2010. Hospitalisation data were filtered to remove readmissions and examined for evidence of an association between the incidence of campylobacteriosis and GBS.

Results: We identified 2056 first admissions for GBS, an average rate of 2.32/100000 population/year. Over the 1989-2008 period there were 56 deaths recorded for GBS, a case fatality risk of 3.0%. Annual GBS hospitalisations were closely correlated with campylobacteriosis notifications (p=0.002). Those hospitalized for campylobacteriosis had a significantly increased risk of GBS hospitalization during the following month (rate ratio 319.0, 95%CI 201.5-506.4). Successful interventions to lower Campylobacter contamination of fresh poultry meat were followed by a 52% decline in campylobacteriosis notifications, with GBS hospitalisations declining by 13% (RR 0.87, 95%CI 0.81-0.93) in the 3-year post-intervention period. The sub-population of GBS cases associated with campylobacteriosis had a similar age (median 54 years) to the GBS population in general (median 52.5 years).

Conclusions: This study adds further evidence that campylobacteriosis is an important cause of GBS. Furthermore, these data suggest that regulatory measures to prevent food-borne campylobacteriosis have the health and economic co-benefit of preventing GBS.

O027

Lipooligosaccharide allele genotyping enables the prediction of ganglioside mimicking structures on *Campylobacter jejuni*

Heikema, AP[1]; Horst-Kreft, D[1]; Louwen, R[1]; Huizinga, R[2]; Gilbert, M[3]; Li, J[3]; Parker, CT[4]; Endtz, HP[1];

Department of Medical Microbiology and Infectious Diseases, Erasmus MC, University Medical Centre Rotterdam, The Netherlands[1]Department of Immunology, Erasmus MC, University Medical Centre Rotterdam, The Netherlands[2]Human Health Therapeutics, National Research Council Canada, Ottawa, Canada[3]Produce Safety and Microbiology Research Unit, Agricultural Research Service, United States Department of Agriculture, Albany California, USA[4]

Background: The *Campylobacter jejuni* lipooligosaccharide (LOS) locus genotypes A and B are associated with the development of Guillain-Barré syndrome (GBS). However, high prevalence of these genotypes in strains isolated from patients with uncomplicated enteritis suggests that additional bacterial factors could contribute to the onset of GBS.

Objective: To assess whether allele variations within the *C. jejuni* LOS locus of A and B genotypes can differentiate GBS- from uncomplicated enteritis-associated strains, or determine the structure of the ganglioside mimic produced.

Methods: PCR and sequencing were performed to assess the prevalence of LOS alleles A1/2 and B1/2 in a large collection of GBS- and enteritis-associated strains. Mass spectrometry was used to determine the LOS structures produced by the strains.

Results: The A1 and B2 alleles were most prevalent (each ~80%) among LOS class A and B strains isolated from GBS as well as enteritis patients. Sialylation of the inner galactose of the outer core LOS was only observed for strains with an A1 or B1 allele. *C. jejuni* with the A1 allele predominantly (88%) synthesized GM1a and GD1a ganglioside mimics. In strains with the A2 and B2 allele, GM1b and/or GD1c-mimics were frequently (86%) observed. Point mutations within LOS biosynthesis genes explained alternate LOS structures in specific strains.

Conclusions: Allele variations within the LOS locus A and B genotypes do not distinguish GBS- from uncomplicated enteritis-associated strains. However, LOS allele genotyping is a powerful tool that can be used to predict which ganglioside mimicking structures are synthesized by *C. jejuni* strains.

O028

Comparative genomic analysis of human bacteraemic *Campylobacter jejuni* isolates from New Zealand

Biggs, PJ[1]; Blackmore, T[2]; Reynolds, AD[3]; Midwinter, AC[1]; French, NP[1];

Massey University, Palmerston North, NZ[1]Capital and Coast DHB, Wellington, NZ[2]AgResearch, Palmerston North, NZ[3]

Campylobacter jejuni is one of the most common causes of global gastroenteritis, and rarely causes more serious sequelae such as bacteraemia. We analysed by performing comparative pathogenomics, a series of 10 invasive *C. jejuni* human infections occurring in the greater Wellington region between 2010 and 2012 (one prosthetic joint and nine blood isolates). They were compared to a set of published faecal strains from the UK and two from New Zealand. A variety of genomic analysis methods were performed on the 10 samples, with synthetic STs typical for human infection being detected by MLST. A core genome of 1340 genes was found for these 10 strains. Twenty random gastroenteritis isolates matched by ST were also included for further analysis. The genomes grouped together by ST, rather than by disease type (faecal vs. invasive). Analysis using four methods at various biological information levels for the 30 isolates – nucleotide (ribosomal MLST and core SNPs), amino acid (core genome) and gene function (based on absence/presence matrices from COG classifications) – was performed, with subsequent visualisation using NeighborNets. The two NZ ST50 isolates – from the same part of Wellington – were noticeably different to their ST-matched gastrointestinal isolates, indicating an underlying difference in these two ST50 isolates. Multiple methods of comparative pathogenomic analyses on *C. jejuni* indicate that similarities and differences are detectable in the genome overall, but the exact causes lies within the accessory genomes of these isolates. This analysis indicates that with the possible exception of ST50, invasive strains of *C. jejuni* do not appear different to those causing diarrhoea.

O029

Comprehensive analysis of lipo-oligosaccharides in *Campylobacter jejuni* isolated from Guillain–Barré syndrome and enteritis patients, and healthy controls

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Background: *Campylobacter jejuni* is one of the most frequent causes of acute gastroenteritis and is associated with post-infectious sequelae such as Guillain–Barré Syndrome (GBS). The lipooligosaccharide (LOS) of *C. jejuni* has been reported as an important determinant to develop GBS.

Objective: This study aimed to investigate the association of *C. jejuni* LOS classes with GBS and enteritis patients and healthy controls in Bangladesh.

Method: We studied 30 *C. jejuni* strains from GBS patients, 152 from enteritis patients and 215 from healthy control in Bangladesh. All strains were analyzed by PCR for 14 LOS classes (A-S).

Results: A total of 314/397 (79%) *C. jejuni* strains assigned to different LOS classes. LOS class A was significantly associated with GBS-associated strains compared to enteritis patients (47% vs 3%, $p < 0.01$) and healthy controls (47% vs 7%, $p < 0.01$). LOS Class B was frequent in GBS (50%), enteritis patients (40%) and healthy controls (53%). LOS class E was identified more frequently in enteritis patients compared to healthy controls (16% and 8% respectively). A total of 28 (7%) *C. jejuni* either from enteritis or healthy controls belonged to one of the following classes D, I, Q, K, N, F, J, or S. Two strains from enteritis patients had LOS class N and two from healthy control had LOS class S, J or F.

Conclusion: Our study identified LOS class A being responsible for the induction of GBS; however, further effort is warranted for proper classification of the strains which could not be typed by the existing LOS classification scheme.

1600-1740 RoomA

O030

Wild bird associated *Campylobacter jejuni* isolates are a consistent source of human disease, in Oxfordshire, United Kingdom.

Cody, A.J.[1]; McCarthy, N.D.[2]; Wimalarathna, H.M.L[1]; Jansen van Rensburg, M.[1]; Bray, J.E.[1]; Colles, F.M.[1]; Dingle, K.E.[4]; Waldenström, J.[5]; Maiden, M.C.J.[13];

Dept of Zoology, University of Oxford, Oxford, UK.[1]Warwick Medical School, University of Warwick, Coventry, UK.[2]NIHR Health Protections Research Unit in Gastrointestinal Infections, University of Oxford, Oxford, UK.[3]Nuffield Department of Clinical Medicine, Oxford University, John Radcliffe Hospital, Oxford, UK.[4]Centre for Ecology and Evolution in Microbial Model Systems, Linnaeus University, Kalmar, Sweden.[5]

Background: Host-association of phylogenetically distinct *Campylobacter jejuni* genotypes is strong among wild birds, exceeding that of geographic origin. The availability of ten years of continuous *Campylobacter* genotype surveillance data from Oxfordshire, United Kingdom (UK) facilitated a robust estimation of the contribution of this infection source to the burden of clinical infection.

Objectives: To determine the contribution of wild-bird attributed *C. jejuni* to human disease in Oxfordshire, UK over a ten-year period.

Methods: The probable origin of clinical isolates, as described by multi-locus sequence typing (MLST), was determined by comparison to reference populations of isolates from farm animals and five wild bird families, using the STRUCTURE algorithm.

Results: Wild bird-attributed isolates accounted for between 476 (2.1%) and 543 (3.5%) cases annually. This proportion did not vary significantly by study year (P=0.934) but varied seasonally, with wild bird attributed genotypes comprising a greater proportion of isolates during summer compared to winter months (P=0.003). The highest proportion of wild bird-attributed illness occurred in August (P<0.001), with a significantly lower proportion in November (P=0.018). Among genotypes attributed to specific groups of wild birds, seasonality was most apparent for *Turdidae*-attributed isolates, which were absent during winter months.

Conclusions: These results are consistent with some wild bird species representing a persistent source of human campylobacteriosis, and contributing a distinctive seasonal pattern to disease burden. If Oxfordshire is representative of the UK as a whole in this respect, these data suggest that the national burden of wild bird-attributed isolates could be in the order of 10,000 annually.

O031

A model for assessing the concordance between genetic and epidemiological similarity of Campylobacter isolates: towards improved application of genomic epidemiology in public health.

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Department of Biological Sciences, University of Lethbridge, Lethbridge AB, Canada.[1]Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Lethbridge AB, Canada.[2]

Campylobacter jejuni represents a leading bacterial foodborne pathogen in Canada, with yearly per capita incidence as high as 1% of the population, representing a cost of over $350 million to the Canadian economy. Improved methods for attributing sources of exposure are increasingly critical for public health interventions aimed at reducing the burden of illness from *C. jejuni*. With recent advances in whole genome sequencing (WGS), we are targeting WGS of isolates collected through surveillance programs in Canada for use in routine epidemiologic investigations aimed at prevention and control of *C. jejuni*. We aim to develop analytical methods that extract critical information from WGS data to facilitate genomic epidemiology. We have sequenced the genomes of approximately 300 environmental, animal, and clinically derived Canadian isolates of *C. jejuni*, and have developed a framework for assessing the concordance between epidemiological and genetic relationships in the R environment for statistical computing. We have developed a statistical model resulting in a quantitative summary statistic based on traditional epidemiologic parameters allowing for direct assessment of concordance between traditional and molecular epidemiology data. We are currently using this model to optimize the interpretation of *C. jejuni* WGS data in the context of epidemiological investigations. Our model allows for direct comparison between epidemiologic and genomic data, paving the way for improved outbreak detection, source tracking and source attribution. We are developing an online toolkit to be used in the development of interpretation criteria for WGS in epidemiologic investigations of C. jejuni and other high-risk bacterial pathogens.

O032

Multilocus sequence typing of *Campylobacter concisus* from Danish diarrheic patients

Nielsen, HL[1]; Nielsen, H[2]; Torpdahl, M[3];

Department of Clinical Microbiology, Aalborg University Hospital, Aalborg, Denmark[1]Department of Infectious Diseases, Aalborg University Hospital, Aalborg, Denmark[2]Department for Microbiology and Infection control, Statens Serum Institut, Copenhagen, Denmark[3]

Background: The emerging enteric pathogen *Campylobacter concisus* is associated with prolonged diarrhoea and inflammatory bowel disease. Previous studies have shown that *C. concisus* strains are genetically very diverse. Nevertheless, *C. concisus* strains have been divided into two genomospecies, where GS1 strains were isolated predominantly from healthy individuals while the GS2 cluster contained isolates primarily from diarrheic individuals.

Objectives: To determine the diversity of *C. concisus* isolates from Danish diarrheic patients by use of multilocus sequence typing (MLST) and genomospecies.

Methods: MLST, using the loci aspA, atpA, glnA, gltA, glyA, ilvD, and pgm, as well as genomospecies, based on specific differences in the 23S rRNA, were used to characterize 67 isolates (63 faecal and 4 oral), from 56 patients with different clinical presentations (33 with diarrhoea, 9 with bloody diarrhoea, 7 with collagenous colitis and 7 with Crohn's disease).

Results: MLST revealed a high diversity of *C. concisus* with 53 sequence types (STs), of which 52 were identified as"new" STs. Allele sequences showed more than 90% similarity between isolates, with only two outliers. Dendrogram profiles of each allele showed a division into two groups which more or less correlated with GS1 and GS2. However, this subgrouping had no association with the clinical severity of disease.

Conclusions: *Campylobacter concisus* isolates from Denmark were found to be highly genetically diverse. However, dendrogram profiles of each allele, showed a division into two groups, almost similar to GS1 and GS2, but in contrast to previous studies we found no association with clinical presentation.

O033

Epidemiology and Antimicrobial Susceptibility of human Campylobacter strains isolated in Belgium in 2013: Results of the first Belgian multi-center survey.

Martiny, D[1]; Vlaes, L[1]; Retore, P[1]; Attalibi, S[1]; Mahadeb, B[2]; Hallin, M[3]; Vandenberg, O[4];
National Reference Centre for Campylobacter, iris-Lab, Iris-Brussels Public Hospital Network, Brussels, Belgium[1] Department of Microbiology, iris-Lab, iris-Brussels Public Hospitals Network, Brussels, Belgium[2] Department of Molecular Biology, iris-Lab, iris-Brussels Public Hospitals Network, Brussels, Belgium[3] National Reference Centre for Campylobacter, iris-Lab, Iris-Brussels Public Hospital Network, Brussels, Belgium and Environmental health and occupational health Research Centre, Public Health School,[4]

Background: Campylobacter is the leading cause of bacterial gastroenteritis in Belgium. Although most infections are self-limited, antibiotic treatment is required for severe illness. Updating of national empiric treatment guidelines according to recent national antimicrobial resistance trends is needed.

Objectives: With a network of voluntary sentinel clinical laboratories, we conducted a multicentric survey to determine the epidemiology and the antimicrobial susceptibility of human campylobacters in Belgium.

Methods: During a three-month period, the ten first enteric and all invasive campylobacters isolated in each participating laboratory were collected. Identifications were performed using MALDI-TOF MS. According to WHO's recommendations, erythromycin, ciprofloxacin and tetracycline were tested. AST were realized using gradient MIC strips and interpreted following EUCAST breakpoints.

Results: From July 2013 to September 2013, 842 campylobacters from 92 participating laboratories were included in the study. Among these, 90.7% were successfully subcultured at the national reference center (n=764): 85.3% were Campylobacter jejuni (n=655), 13.4% were Campylobacter coli (n=102). Among C. jejuni, 0.5%, 51.0% and 34.7% were resistant to erythromycin, ciprofloxacin and tetracycline respectively. Among C. coli, a higher rate of resistance was recorded for erythromycin (7.8%), ciprofloxacin (67.6%), and tetracycline (67.6%).

Conclusion: This study highlights a high prevalence of Campylobacter resistance to fluoroquinolones and a low level of Campylobacter resistance to macrolides. When clinically indicated, erythromycin remains the treatment of choice for intestinal campylobacteriosis but could lead to treatment failure in case of C. coli infection. These results provide data essential for updating national empiric treatment guidelines and highlight the need for national survey.

O034

Exposures associated with Campylobacter infection in young children-Foodborne Diseases Active Surveillance Network, United States, 2014-2015

Marder, E[1]; Geissler, A[1]; Cieslak, P[2]; Dunn, J[3]; Hurd, S[4]; Lathrop, S[5]; McGuire, S[6]; Mahon, B[1]; Henao, O[1];
Centers for Disease Control and Prevention[1]Oregon Health Authority[2]Tennessee Department of Health[3]Yale Emerging Infections Program[4]University of New Mexico[5]New York State Department of Health[6]

Background: The incidence rate of campylobacteriosis is highest among males and children aged 1–4 years. The Foodborne Diseases Active Surveillance Network (FoodNet) conducts active population-based surveillance for culture-confirmed campylobacteriosis in sentinel US sites.

Objectives: To identify possible sources of campylobacteriosis among children aged 1–4 years, by sex.

Methods: We compared food, water, animal, and environmental exposures among children 1–4 years of age with campylobacteriosis to children with salmonellosis reported to FoodNet during January 2014 to February 2015. We calculated site-adjusted odds ratios (aOR), excluding outbreak- and international travel-associated infections.

Results: Among 236 *Campylobacter* and 274 *Salmonella* infections, 158 (67%) and 188 (69%) patients, respectively, had exposure data. Girls with *Campylobacter* infection were more likely than girls with *Salmonella* infection to have had contact with a sick pet (23% vs. 2%; aOR=10.58; p<0.01). Boys with *Campylobacter* infection were more likely than boys with *Salmonella* infection to have had contact with a sick pet (18% vs. 1%; aOR=10.17; p=0.04), a cat (30% vs. 13%; aOR=4.07; p=0.01), or to have visited a farm (30% vs. 9%; aOR=5.13; p<0.01).

Conclusions: Several animal exposures, especially contact with a sick pet, were more strongly associated with *Campylobacter* than *Salmonella* infections in young children. The sex differences in animal and environmental exposures could reflect differences in exposures for either or both pathogens. Although case-case comparisons are influenced by similarities and differences in causal pathways, our results identify plausible differential exposures for *Campylobacter* infection and suggest targets for prevention.

O035

Little evidence that *Campylobacter jejuni* strains are exchanged between livestock animals and passerine birds inhabiting the same Salinas Valley agricultural lands

Parker, C[1]; Chapman, M[1]; Huynh, S[1]; Cooper, K[2];

USDA-ARS[1]California State University- Northridge[2]

Background: In a large study of *Campylobacter jejuni* isolates in the UK, strains from wild birds were shown to be genetically distinct from strains from farm animals. Here, we compared the genotypes of *C. jejuni* isolates from livestock (21), water (8) and wild passerine birds (31) located in the agriculturally important Salinas Valley.

Objective: Further explore the potential exchange of *C. jejuni* isolates between livestock and wild passerine birds located in the same region.

Methods: Whole-genome sequencing was performed using Illumina MiSeq, reads were assembled using Newbler, and genomes were annotated using Geneious'-BLAST feature. Seven-locus MLST allelic profiles were determined via PubMLST for each isolate and the lipooligosaccharide locus (LOS) was compared to published LOS classes.

Results: The MLST profile (ST) and clonal complex (CC) were defined for most isolates from livestock (18/21). These isolates mostly possessed previously defined LOS classes (19/21) including A (8) and C (8). The MLST profiles for the passerine isolates were not all defined (22/31 ST and 6/31 CC). ST-1224 was associated with crows (10/20) and CC-177 was associated with starlings (4/6). Five newly described LOS classes were identified and were predominant among the passerine isolates. We observed that one cow isolate was ST-1224 and nearly identical to a crow isolate.

Conclusions: The *C. jejuni* genotypes associated with livestock animals and passerine birds were mostly distinct from each other with only one isolate sharing ST between the two animal groups. Furthermore, distinct LOS classes predominated among the passerine isolates and may represent host specific genes.

Campylobacter Pathogenesis
Monday 2nd November

1600-1740 Room B

O036

Characterisation of the host response to *Campylobacter concisus* pathotypes

Kaakoush, NO[1]; Deshpande, NP[1]; Wilkins, MR[1]; Mitchell, HM[1];

The University of New South Wales[1]

Background: The emergent pathogen *Campylobacter concisus* has been associated with several diseases including gastroenteritis, Barrett's oesophagus, and inflammatory bowel diseases. Recently, we hypothesised that based on specific pathogenic properties within different isolates, *C. concisus* strains may be divided into pathotypes including adherent and invasive *C. concisus* (AICC) and adherent and toxigenic *C. concisus* (AToCC), in addition to commensal strains.

Objectives: To characterise the epithelial and macrophage response to infection by different *C. concisus* pathotypes.

Methods: Comprehensive transcriptome and methylation analyses were performed on intestinal epithelial Caco-2 cells and THP-1 derived macrophages infected with AICC and AToCC strains using RNA-seq and HM450 methylation arrays.

Results: A significant difference was found in the epithelial response to *C. concisus* pathotypes; however, similarities in specific responses suggested common antibacterial responses against all *C. concisus* strains. Atypical epithelial recognition of AToCC through pattern recognition receptors not commonly associated with *Campylobacter* infection was observed. Further, a potential mechanism by which AToCC strains could cause acute gastroenteritis was identified. A comprehensive global profile of innate immune responses to AICC infection was determined in THP-1 derived macrophages. Differential expression of pattern recognition receptors and robust upregulation of DNA- and RNA-sensing molecules was observed, in particular, IFI16 inflammasome assembly. Thirteen microRNAs and 333 noncoding RNAs were significantly regulated upon infection, including MIR221, which has been associated with colorectal carcinogenesis.

Conclusions: A substantial difference in the host response to *C. concisus* pathotypes exists, and this difference reflects the potential association of specific pathotypes with either an acute or chronic infection.

O037

Virulence characteristics of hcp+ *Campylobacter jejuni* and *Campylobacter coli* isolates from retail chicken

Corcionioschi, N[1]; Gundogdu, O[2]; Moran, L[1]; Kelly, C[1]; Scates, P[1]; Stef, L[3]; Cean, A[3]; Wren, B[2]; Dorrell, N[2]; Madden, RH[1];
Agri-Food & Biosciences Institute[1]London School of Hygiene and Tropical Medicine[2]Banat University of Animal Sciences and Veterinary Medicine[3]

Most commercially raised poultry carry campylobacters, with 87% of broiler carcasses in the UK found to be contaminated. Recently the type six secretion system (T6SS), which can play a significant role in bacterial survival and pathogenesis, was reported in *Campylobacter* spp. and a key component of the T6SS is the hcp gene. Because campylobacteriosis is associated with the consumption of infected poultry meat, this study aimed to investigate *C. jejuni* (n=59) and *C. coli* (n=57) isolated from retail raw chicken for the presence of the hcp gene, and for other virulence characteristics, in order to investigate the association of T6SS with virulence. Multiplex PCR found a significantly higher prevalence of hcp in *C. coli* isolates (56.1%) than in *C. jejuni* (28.8%) and AFLP analysis showed a high degree of genetic similarity between isolates carrying the hcp gene. Genome sequencing data showed that 84.3% of the *C. coli* and 93.7% of the *C. jejuni* isolates had all 13 T6SS open reading frames. Moreover, the virulence characteristics of hcp+ isolates, including motility and the ability to invade human intestinal epithelial cells in vitro, were significantly greater than in the control strain *C. jejuni* 12502; a human isolate which is hcp positive. Overall, it was discovered that hcp+ *C. coli* and *C. jejuni* isolated from retail chicken isolates posses genetic and phenotypic properties associated with enhanced virulence. However, since human infections with *C. coli* are significantly less frequent than those of C. jejuni, the relationship between virulence factors and pathogenesis requires further study.

O038

CRISPR-Cas in *Campylobacter jejuni* controls membrane integrity and pathogenicity

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The bacterial cell envelope is the most important structure to counteract the early stages of (a)biotic stress. In *Campylobacter jejuni*, a zoonotic human intestinal pathogen, modification of the bacterial cell envelope and *C*lusters of *R*egularly *I*nterspaced *S*hort *P*alindromic *R*epeats and *as*sociated genes (CRISPR-Cas) have both been linked to defense and virulence features. Current evidence suggests that a dual function exists between the CRISPR-Cas system in *C. jejuni* and the regulation of cell envelope exposed outer surface structures. The objective of this study is to elucidate how the CRISPR-Cas system in *C. jejuni* modulates cell envelope exposed outer surface structures during (a)biotic stressors. Here, we show that loss of cell envelope structures or the CRISPR-Cas marker gene *cas9* significantly alters membrane permeability, antibiotic resistance, swarming behavior and immune recognition. Bioinformatics and RNA analysis reveal a potential mechanism showing that Cas9 and CRISPR RNAs control the expression of cell envelope structures, as evidenced in other microorganisms. This study indicates that the CRISPR-Cas system in *C. jejuni* is actively involved in the regulation of cell envelope exposed outer surface structures affecting antibiotic resistance, virulence features and immune recognition. *C. jejuni* could utilize its CRISPR-Cas system to regulate its cell envelope structures in order to survive encountered (a)biotic stress in the environment or inside a host during infection. Currently, experiments are ongoing to unravel the exact regulatory mechanism.

O039

The outer surface capsule modulates the binding of *Campylobacter jejuni* to sialoadhesin-expressing cells

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Background: Sialylated lipooligosaccharides (LOS), present in the outer membrane of *Campylobacter jejuni*, enhance the activation of the innate immune system and are critical for the induction of Guillain-Barré syndrome (GBS). Binding of sialylated LOS to the immune-receptor sialoadhesin leads to bacterial uptake and the release of proinflammatory cytokines by primary human macrophages. However, recent research has indicated that LOS are not always exposed to the external environment. In this study, we determined whether the outer surface capsule shields off the LOS and may prevent the binding of *C. jejuni* to sialoadhesin.

Methods: Capsule and sialic acid transferase knock-out mutants were generated. Cholera toxin was used to assess the exposure of the LOS to the environment. For sialoadhesin binding experiments, bacteria were FITC-labelled and incubated with sialoadhesin-expressing cells. Cholera toxin and sialoadhesin binding was assessed using flow cytometry.

Results: Cholera toxin binding experiments revealed that the capsule of *C. jejuni* almost completely shields off the LOS from the environment. This effect was observed for at least three capsular serotypes, HS:1, HS:4 and HS:19. In mutant strains without a capsule, the binding to sialoadhesin was largely enhanced when compared to the encapsulated parental strains. In fact, wild type strains and sialic acid transferase mutants showed comparable low binding to sialoadhesin-expressing cells.

Conclusion: Our results demonstrate that the capsule of *C. jejuni* can prevent the recognition of *C. jejuni* by the immune system. Bypassing the capsule to induce a cross-reactive immune response to LOS is a critical step in the pathogenesis of GBS.

O040

Exploration of *Campylobacter jejuni* survival mechanisms in house flies

Jensen, A.N.[1], de Vries, S.P.W.[2], Gupta, S.[2], Baig, A.[2], Pedersen, H.S.[3], Wolanska, D.P.[2], Maskell, D.J.[2], Hald, B.[1], Grant, A.J.[2]

[1]National Food Institute, Technical University of Denmark, Denmark [2]Department of Veterinary Medicine, University of Cambridge, United Kingdom, [3]Department of Agroecology - Entomology and Plant Pathology, Aarhus University

Background: Houseflies have been shown to play an important role in the transmission of *Campylobacter jejuni* to poultry in poultry houses. An experimental fly model has been established to investigate the survival dynamics of *C. jejuni* in the housefly.

Objectives: Identification of *C. jejuni* genes required for persistence/survival in the housefly.

Methods: A mariner transposon mutant library of ~10,000 unique transposon insertion mutants was constructed in *C. jejuni* M1. Five groups of 10 flies were individually inoculated with 10^6 CFU of the mutant library in a 1µl volume via their proboscis. After 4 h incubation at 20°C mutants were recovered from flies on mCCDA-chloramphenicol plates. To identify the mutants that were unable or less able to survive in flies, the relative abundance of each mutant (inoculum versus recovered) was determined by massively parallel sequencing of transposon insertion sites (Tn-seq). Directed gene deletion mutants were constructed for validation using an overlap PCR method.

Results: After correction for genes required for in vitro growth of *C. jejuni* M1, a total of 48 genes were identified for which mutants showed >2-fold attenuated (Padjusted < 0.05) survival in flies. A number of gene deletion mutants have been generated for validation of the Tn-seq data and for further characterization.

Conclusions: Using a combination of high-density transposon mutagenesis and genome-wide targeted sequencing of transposon insertion sites, we have identified a set of genes which may be required for survival/persistence in the house fly, and which could potentially serve as novel targets for intervention strategies.

O041

The interaction between *C. concisus* and enteric epithelial cells in vitro

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Background: *Campylobacter concisus* is an extremely fastidious bacterium which is known to be genetically diverse. It is considered as a human oral cavity coloniser which has been linked to gastroenteritis and other infections. However, the potential pathogenic role of *C. concisus* in human infections is still not ratified with only few virulence factors been thoroughly investigated.

Methods: Adhesion and invasion assays were performed on oral and intestinal *C. concisus* strains using the human intestinal cell line INT407. In addition, semi-quantitative SYBR green assays were developed to evaluate the expression of *C. concisus* putative virulence genes (*cjaC, cjaA* and *dnaJ*) in bacterial cultures sustained in different synthetic growth media (basic and enriched) and in a cell culture environment with the enteric host cells INT407.

Results: *C. concisus* strains were more likely to adhere than invade unlike the control strains *C. jejuni* 81116 and *C. coli* NCTC 11366. Enteric invasive *C. concisus* strains were detected for the first time in the oral cavity of healthy individuals. The expression of *cjaC* and *cjaA* was slightly supressed when the bacterial cells were grown in an enriched medium. However, *dnaJ* expression was significantly higher in bacterial cells introduced into INT407 than in bacterial cells sustained in the tissue culture medium only.

Conclusions: Enteric invasive strains can be isolated from healthy individuals. *dnaJ* may be involved in cellular invasion and the virulence of this bacterium. Further studies are required to investigate the expression of *dnaJ* and other virulence genes *in vivo*.

Genetic and Proteomic Responses for Adaptation - *Campylobacter*
Monday 2nd November

1600-1740 Room C

O042

Understanding the dynamics of oxygen-regulated gene and protein expression in *Campylobacter jejuni* using continuous chemostat cultures

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Background and Objectives: The relationship between *Campylobacter jejuni* and oxygen is one of the major defining features of the biology of this microaerophilic food-borne pathogen, but the molecular responses to oxygen are poorly understood. To study this, the ability to fix the growth rate of the cells while varying oxygen availability in the absence of variation of other environmental parameters is a key requirement, which cannot be achieved in batch cultures. We therefore studied oxygen-dependent changes in gene and protein expression in strain NCTC 11168, using serine-limited continuous cultures in which the growth rate was fixed.

Methods and Results: We show that in steady-states established over a wide-range of oxygen availabilities (from 1.25% v/v to 17% v/v in the gas-flow), the cellular acetate excretion flux can be used as a measure of the degree of aerobiosis perceived by the cells. At 100% aerobiosis the acetate flux is zero and metabolism is fully aerobic. Proteomic analyses by 2D-PAGE and mass-spectrometry at each steady-state provided an exceptionally detailed picture of the effect of oxygen on protein abundance. In a temporal transition experiment between steady-states at 150% and 38% aerobiosis, microarray analysis showed transient up-regulation of many genes associated with alternative pathways of electron transport, while some catabolic genes like proline dehydrogenase (putA) were strongly down-regulated. Some well-studied genes that were previously not known to be regulated by oxygen, were identified as such from this study.

Conclusions: Chemostat culture allows the response to oxygen to be studied in detail and has enabled the identification of hitherto unrecognised oxygen-regulated genes.

O043

Campylobacter bacteriophages encode a flagellar glycan-binding effector protein that reduces cell motility and growth

Sacher, J. C.[1]; Javed, M. A.[1]; Patry, R. T.[1]; Szymanski, C. M. [1];

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The extensive glycosylation of surface structures in *Campylobacter jejuni* provides many essential functions. Greater than 10% of *C. jejuni* flagellar mass is contributed by O-linked sialic acid-like sugars such as pseudaminic acid (Pse) and legionaminic acid. O-glycosylation mutants in *C. jejuni* are non-motile and display impaired gut colonization. We have identified a protein, Gp047, derived from the lytic Campylobacter bacteriophage NCTC 12673 that binds specifically to acetamidino-modified Pse on *C. jejuni* flagella. Because *gp047* is encoded by all *Campylobacter* phages sequenced to date, we sought to elucidate its role. Gp047 was not identified during NCTC 12673 proteome analysis, anti-Gp047 antibodies do not label this phage, and the phage does not bind flagella, suggesting that Gp047 is an effector rather than a structural protein. Overexpression of Gp047 and addition to *C. jejuni* cultures resulted in bacterial agglutination and motility reduction, and overexpression of Gp047 in *C. jejuni* cells resulted in reduced motility. Interestingly, Gp047 also reduces *C. jejuni* growth when spotted onto agar plates. We hypothesize that as virions escape from infected cells, Gp047 is released, agglutinating/slowing neighboring cells and promoting subsequent rounds of phage infection. Gp047 may alternatively induce down-regulation of flagellar gene expression and/or cell growth, potentially altering phage burst size or *Campylobacter* colonization/dissemination. These results implicate a mechanism by which *Campylobacter* phages may use glycan-binding proteins to modulate the lifecycle of their host.

O044

Campy has a memory? Environmental origins of two closely related *Campylobacter jejuni* strains correlated after long-term storage at low temperature

On, SLW[1]; Shi, Z[2]; Dawson, C[2]; Clerans, S[3]; Hussain, MA[2];
ESR[1]Lincoln University[2]AgResearch[3]

Using a high-resolution, gel-free approach, we examined and compared the proteomes of two isolates of *C. jejuni* strains that represented the same clone: SVS 5141 was recovered from the contaminated drinking water that caused an outbreak in 1995-1996, of which SVS 5001 represents a human isolate. These strains have been stored at -80°C ever since their initial recovery. To examine in detail their responses to cold shock, strains were cultured directly from frozen to blood agar in microaerobic conditions for 24 h before culturing at 42°C in BHI broth microarobically, after which time aliquots were subjected to refrigeration (4°C) in microaerobic conditions. Viable cell counts were examined after 6 h, 1 day, 2 days, 6 days and 8 days and whole-cell protein extracts prepared at T0 and at these intervals. Proteins were resolved and identified using iTraq labelling coupled to UPLC-MS/MS. The viability profile of SVS 5141 indicated its average cell death rate to be lower than that of SVS 5001. Furthermore, in SVS 5141, the number of proteins that were up-regulated, down-regulated, or associated with the cessation of protein synthesis or cold induction were 138, 11, 28 and 9 after six days of cold storage. In contrast, the corresponding results for SVS 5001 were 86, 1, 54 and 10. Within these categories, there were also qualitative differences in the individual protein expressions. These data suggest that the adaptive responses of *C. jejun*i to new environments may persist as proteomic "memories" and can be preserved for long periods of time.

O045

Varying fast or slow: do differences in phase variation rates matter?

Aidley, J[1]; Sørensen, M C H[2]; Brøndsted, L[2]; Bayliss, C D[1];

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Phase variation is a common feature of many pathogenic bacteria including *Campylobacter jejuni* which has many genes exhibiting high frequency, stochastic, reversible switching between ON and OFF expression states. Genetically, this variation results from instability in homonucleotide (poly-G/C) repeat tracts in the coding regions of multiple genes producing downstream frame shift mutations. Repeat tract length influences rates of variation, with shorter tract lengths resulting in lower phase variation rates. To investigate the biological significance of differences in switching rate, we are developing a cyclic selection assay for ON and OFF states of a single phase-variable gene. Our model gene is Cj1421c. We constructed a Cj1422c deletion mutant in strain NCTC11168 and showed that switching OFF of Cj1421c mediates resistance to phage F336 and, conversely, we showed that the ON state of Cj1421c provides resistance to human sera. Our data demonstrates that >95% of surviving colonies are in the desired OFF state after selection by phage and >90% in the ON state following selection with serum. We report on the results of initial cyclical experiments. We have also developed an *in silico* model of cyclical selection incorporating empirical data for switching rates and selection coefficients. Results from this model indicate that the favoured tract length varies with the strength of selection and frequency of change in selection direction (e.g. for ON or for OFF). Introducing experimental data from the *in vitro* cyclical selection assay allows for predictions of the conditions that will favour evolution of long and short repeat tract lengths.

O046

The first structure and functional analysis of a bacterial MORN protein: *Campylobacter jejuni* MceL is involved in lipid-binding and cell-envelope maintenance

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Background: Bacterial lipoproteins play a key role in membrane stability, protein targeting and virulence. There are in excess of forty predicted lipoproteins in *C. jejuni* strain 11168, many of which remain largely uncharacterised. One such lipoprotein, the Maintenance of Cell Envelope (MceL) contains Membrane Occupation and Recognition Nexus (MORN) repeats which are found in eukaryotic and prokaryotic proteins but are of unclear function. *mceL* is part of a three gene operon which is under the control of a sensor-repressor system (see White *et al*. - poster).

Objectives: Using structural and protein-lipid interaction studies, we aimed to functionally characterise the MceL lipoprotein.

Methods and Results: Cellular fractionation showed that MceL is an outer membrane anchored lipoprotein that faces the periplasm. We have determined the 3D crystal structure of MceL, which consists of a highly unusual flattened beta-ladder. Lipid overlay assays demonstrated that heterologously produced MceL was able to bind an array of polar and negatively-charged phospholipids, indicating a role in lipid-binding. We have reconstituted this protein into liposomes, which also encapsulated a fluorescent probe. Probe leakage caused by cationic antimicrobial peptide (CAMP)-induced membrane disruption, was significantly reduced in the presence of intra-liposome MceL, suggesting that it can protect the bilayer from CAMP attack.

Conclusions: This study suggests that MceL binds phosholipids in the outer membrane and may assist with membrane stability and protection. To understand the wider role of MceL, a number of putative *mceL* gene homologues within other Epsilon and Gamma Proteobacteria have been identified and are under investigation.

O047

Mutations in an outer membrane protein enhances the virulence of *Campylobacter jejuni*

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Iowa State University[1]National University of Singapore[2]

Bacterial pathogens constantly evolve in adaption to changes in environmental or host conditions. *Campylobacter jejuni* as an enteric pathogen predominately resides in the intestinal tract of animal hosts. Recently, a hypervirulent clone of *C. jejuni* (clone SA) has emerged as the predominant cause of sheep abortion, which has also been associated with foodborne outbreaks of gastroenteritis, in the United States. The hallmark of clone SA is its ability to translocate across gut epithelium and induce systemic infection. Although several previous functional genomics analyses have identified a number of candidate loci that might be related to virulence, the causative genetic basis for the hyper-virulence of clone SA has remained elusive. In this study, we took advantage of natural competence of *C. jejuni*, positive selection in animal model, and next generation sequencing to directly identify the genetic determinant causing abortion. Transformation of *C. jejuni* NCTC 11168, a non-abortifacient strain, using genomic DNA of clone SA made it fully virulent in abortion induction. Deep sequencing and comparative genomics analyses of the isolates from the aborted animals indicated that transfer of several point mutations in a gene encoding an outer membrane protein was associated with gain of virulence. Directed genetic experiments including allelic exchanges proved that sequence polymorphisms in the outer membrane protein are both necessary and sufficient for inducing abortion in pregnant animals. These findings identify a key virulence determinant responsible for abortion induction by Campylobacter and illustrate the power and necessity of combining experimental evolution with genomic approaches for understanding bacterial pathogenesis.

Comparative Genomics and Genomic Epidemiology (sponsored by Applied Maths)
Tuesday 3rd November

0815-1040 Room A

O048

Genome-wide association study identifies genes associated with the survival of *C. jejuni* from farm to human disease

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Bacteria display extraordinary variation in phenotypes such as virulence, tissue specificity and host range, and understanding the genetic basis of this diversity is a pervasive aim in microbiology. Advances in whole genome sequencing are providing new opportunities for improved understanding of bacterial epidemiology and evolution. However, while many laboratories are now sequencing 1000s of bacterial genomes, developing methods for analyzing this data remains a significant challenge. A promising approach, pioneered in human genetics, is genome-wide association mapping where DNA sequence variation across the genome is related to particular phenotypes. This approach has been challenging to apply to bacteria because of their strong population structure resulting from clonal reproduction, but here we present new method and apply it to investigate genetic variation in the zoonotic food poisoning pathogen Campylobacter as it passes through the food chain. Contaminated poultry meat is a major source of human infection and the strains infecting humans are a subset of those found in chickens on the farm. To better understand the genes and alleles associated with survival at different stages, we sequenced more than 500 genomes from chickens on the farm, abattoirs, retail poultry meat and human disease. The genomes were divided into overlapping 30bp words, allowing simultaneous analysis of homologous and non-homologous sequence variation, and words that were significantly overrepresented in particular stages, compared to expectation based upon the population structure, were highlighted. Words that showed significant association across different clonal complexes were identified, and mapped onto an annotated reference genome to investigate their functionality. This provided a list of candidate genes, that could be important in the passage of Campylobacter from farm to fork, including many with predicted functions in oxidative stress response. In in vitro fitness experiments, clinical isolates showed a higher growth rate than farm isolates in the presence of increasing atmospheric oxygen concentrations. Using knock-out mutants, we investigated additional phenotypes involved in metabolism and survival. This work suggests that Campylobacter lineages have distinct genomic and ecological signatures of survival and transmission from farm to humans, and this may be associated with resistance to oxidative stress.

O049

Multilocus sequence types of *Campylobacter jejuni* isolates from different sources in Eastern China

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Campylobacter jejuni is a major food-borne pathogen that causes human gastroenteritis in many developed countries. In our study, we applied multilocus sequence typing (MLST) technology to 167 *C. jejuni* isolates from diverse sources in Eastern China to examine their genetic diversity. MLST defined 94 sequence types (STs) belonging to 18 clonal complexes (CCs). Forty-five STs from 60 isolates (36%) and 22 alleles have not been previously documented in an international database. One hundred and two isolates, accounting for 61.1% of all isolates, belonged to eight clonal complexes. The eight major clonal complexes were also the most common complexes from different sources. The most common ST type of isolates from human and food was ST-353. The dominant ST type in chicken and foods was ST-354. Among 21 STs that contained two or more different sources isolates, 15 STs contained human isolates and isolates from other sources, suggesting that potentially pathogenic strains are not restricted to specific lineages.

O050

Towards the development of a robust Core Genome MLST (cgMLST) scheme for *Campylobacter jejuni:* optimization of analytical approaches for mitigating the effects of sequence data quality on strain typeability.

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Core genome multilocus sequence typing (cgMLST) is a sequence typing method in which the core genome is used in a conceptual extension of classical multilocus sequence typing (MLST). By expanding from 7 loci in MLST to hundreds of loci, cgMLST promises a dramatic increase in discriminatory power, an essential feature for future epidemiology and public health applications. Problems relating to data loss hinder attempts to develop robust cgMLST schemes. In particular, the frequency of contig truncations in draft genome assemblies poses problems to strain typeability, an issue that is exacerbated as the number of genomes analyzed increases. Our aim was to examine approaches for optimizing the identification of cgMLST target loci. 732 *Campylobacter jejuni* core genes were queried against 2,585 *C. jejuni* draft genome assemblies. Contig truncations were located and the information content of alleles was determined using Shannon Entropy. Although most genomes (n=1,872; 72.41%) did not present contig truncations, a significant number of genomes (n=107; 4.13%) had 1% or greater incomplete loci. Similarly, although most loci possessed low probability of contig truncation, some loci (n=19; 2.6%) had incomplete data in 1% or more of the genomes analyzed. Criteria for inclusion of loci in cgMLST should include avoidance of elevated probability of truncation and preservation of high information content. We propose the use of optimal subregions of genes to improve data completeness whilst maximizing discriminatory power. A scheme designed using these objective criteria will provide public health agencies and epidemiologists a reliable and robust approach to cgMLST.

O051

Transforming public health microbiology for Campylobacter with whole genome sequencing: PulseNet and beyond

Pruckler, J[1]; Wagner, D[1]; Williams, G[1]; Carleton, H[1]; Bennett, C[1]; Joseph, L [1]; Trees, E[1]; Huang, A[1]; Katz, LS[1]; Gladney, L[1]; Maiden, MCJ[2]; Miller, WG[3]; Chen, Y[3]; Zhao, S[3]; McDermott, P[3]; Whichard, J[3]; Pouseele, H[3]; Ribot, EM[3]; Fitzgerald, C[3]; Gerner-Smidt, P[3];

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2. Department[23]

Background: Conventional phenotypic and genotypic methods employed for identification and subtyping of Campylobacter are labor intensive, expensive, and imprecise. We have begun development of Enteric Reference Identification and Campylobacter subtype characterization whole genome sequence (WGS) databases. The PulseNet infrastructure (BioNumerics) will be used in conjunction with the existing BIGSdb platform to build the databases, with the goal of characterizing Campylobacter in a single workflow using WGS.

Methods: Reference genomes (n=103) provided by FDA and USDA and strains (n=100) sequenced at CDC were used to develop the database. Assemblies and annotations were performed using the Computational Genomics Pipeline v0.4. These genomes cover the known members of the species and genera within *Campylobacteraceae* and the known genetic diversity of *C. jejuni*. The data will be used to set criteria for the current PubMLST. org/campylobacter locus definitions. Multiple subschema are being set up within the databases to perform identification and scalable, hierarchical subtyping that will include seven locus, ribosomal, core genome and whole genome (MLST, rMLST, cgMLST and wgMLST).

Results: To date 203 reference genomes have been sequenced, annotated and used for development of the BioNumerics databases, including an additional 600 isolates being used to validate the prototype databases.

Conclusions: These WGS BioNumerics databases will provide a single, unified, cost-effective approach for accurate species identification and subtyping to aid the surveillance of sporadic and outbreak related Campylobacter infections. Through continued collaboration with domestic and international partners, we will test and refine

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O052

A blast from the past: insights from *Campylobacter* genomes from a South African archive

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Background: The epidemiology of human campylobacteriosis is thought to differ in developed and developing countries; however, epidemiological data from the latter are limited. South Africa is an exception, as researchers from the University of Cape Town (UCT) carried out long-term *Campylobacter* surveillance at a paediatric referral hospital between 1982 and 2000, establishing a valuable isolate collection.

Objectives: To characterise isolates from the UCT archive using whole-genome sequencing (WGS), thereby providing insights into the epidemiology and evolution of *Campylobacter* in South Africa.

Methods: WGS data were obtained from 110 freeze-dried *Campylobacter* collected from Red Cross War Memorial Children's Hospital in 1991. Using whole-genome multilocus sequence typing, isolates were compared to each other and to *Campylobacter* collected in Cape Town and the United Kingdom (UK) between 2011 and 2014.

Results: Isolates corresponded to *C. upsaliensis* (42%), *C. jejuni* subsp. *doylei* (24%), *C. jejuni* subsp. *jejuni* (22%), *C. coli* (5%), *C. fetus* (3%), *C. concisus* (1%), and unknown *Campylobacter* spp. (3%). *C. upsaliensis* and *C. jejuni* subsp. *doylei* isolates were highly diverse, forming numerous small clusters. *C. jejuni* subsp. *jejuni* and *C. coli* isolates predominantly belonged to global livestock-associated lineages. These isolates and contemporary *C. jejuni* subsp. *jejuni* and *C. coli* from Cape Town and the UK did not segregate according to geographical origin.

Conclusions: Sources of emerging *Campylobacter* species in Cape Town remain unclear, but *C. upsaliensis* and *C. jejuni* subsp. *doylei* diversity suggests that these reservoirs are entrenched. The presence of livestock-associated *C. jejuni* subsp. *jejuni* and *C. coli* lineages indicate that the food chain is an established source of campylobacteriosis in Cape Town.

0815-1040 Room B

O053

Physiological alterations in *Campylobacter jejuni* post-infectious irritable bowel syndrome

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Background: *Campylobacter jejuni*, a leading cause of food-borne illness, is implicated in development of post-infectious irritable bowel syndrome (PI-IBS) with risk ranging from 9% to 13%. The mechanisms of PI-IBS following infection with *C. jejuni* are incompletely understood.

Aim: To determine changes in GI transit, *in vivo* permeability and mucosal barrier function among a cohort of post-*C. jejuni* IBS patients and matched set of healthy volunteers.

Methods: We identified 45 patients with IBS (Rome III criteria) following culture-positive *C. jejuni* enteritis. To date, we have recruited 7 PI-IBS patients (4 IBS-M, 3 IBS-D) and 7 age- and gender-matched healthy volunteers. Participants completed questionnaires, underwent scintigraphic GI transit and *in vivo* permeability. Colonic transepithelial resistance (TER) and FITC Dextran (4 kDa) flux were measured. Tissue responses (Change in short circuit current, Isc) to serosal acetylcholine (Ach) were determined. Unpaired two-sided t-tests were used for comparisons.

Results: One of 7 patients with PI-IBS had colonic transit (GC24h) >4.4, the 95th percentile for 319 normal controls in our laboratory. The main group differences were in mucosal barrier function (TER, FITC flux and *in vivo* permeability) and secretory functions *in vitro* with a 37% decrease in TER in PI-IBS, a 4-fold increase in FITC flux and increased *in vivo* colonic permeability. Serosal Ach caused nearly 2x increase in Isc in PI-IBS biopsies than controls.

Conclusions: Patients with post-*C. jejuni* IBS have an impaired *in vivo* colonic permeability and colonic mucosal barrier function and increased secretory response to ACh. These changes in mucosal function may play a role in symptom generation in *C. jejuni* PI-IBS.

O054

Comparative genomics of invasive *Campylobacter jejuni*

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Little is known about the *C. jejuni* mechanisms involved in establishing invasive infections. Earlier, we have used MLST to study clinically well-characterized blood isolates from Finland and found that MLST lineage ST677CC represented almost half of these isolates, yet this lineage only represents ~10% of Finnish *C. jejuni* faecal isolates. We performed whole genome Illumina sequencing and comparative genomic analyses of ST677CC isolates derived from blood (n=31) and faeces (n=24) in order to find genetic features potentially involved in the invasive phenotype associated with bacteremia. We combined the genome analyses with phenotypical evidence on serum resistance and this was correlated with degradation of an O-methyl phosphoramidate transferase and phase variation of *wcbK*; both capsular biosynthesis features. We found novel epigenetic regulatory mechanisms, specifically restriction-modification systems and a new integrated element, CJIE5, encoding two additional DNases. On the contrary, features previously considered to be related to pathogenesis were either absent, such as GGT and fucose permease, or disrupted, such as the *cdt* operon, among our strains. This shows that 'virulence genes', described for a limited number of strains, may actually not be widely distributed among the species. Our results refine the role of two capsular features and suggest that epigenetic processes play an important role in the potential of *C. jejuni* to establish invasive infections. Our study highlights the importance of comprehensive genomic and phenotypical characterization of a representative number of strains associated with particular clinical outcomes.

O055

The prevalence of *Campylobacter concisus* (genomospecies B) in adults with inflammatory bowel diseases

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Background: *Campylobacter concisus* is an oral bacterium that has been isolated from faecal specimens of gastroenteritis patients. Recent studies suggested its association with inflammatory bowel diseases (IBD) due to a higher DNA detection rate in colonic biopsies from patients than from healthy individuals. *C. concisus* is known to be subtyped to either genomospecies A or B based on the 23S rDNA sequence, however, it is not known if a particular genomospecies is associated with diseases.

Methods: The presence of *C. concisus* in adults with IBD and in healthy individuals was investigated by culture and molecular techniques in 350 intestinal biopsies from 51 participants. New nested PCR techniques were developed to detect *zot*, a virulence gene encoding tight junction toxin, and to type *C. concisus* to the genomospecies level directly from clinical samples.

Results: The prevalence of *C. concisus* in intestinal biopsies of IBD patients was significantly higher (59.5%) than in healthy individuals (21.4%). Remarkably, the prevalence of genomospecies B was significantly higher in IBD patients (35.1%) than in healthy individuals (7.1%). Furthermore, for the first time, *C. concisus* was isolated from duodenal and ascending colonic biopsies. Yet, no significant difference in the detection rate of zot was found in IBD patients compared to healthy individuals.

Conclusions: Our data indicate a potential association of genomospecies B with IBD. Yet z*ot* does not seem to be essential for *C. concisus* colonisation in the gastrointestinal tract. Further studies are required to investigate the association of other *C. concisus* virulence genes with IBD.

O056

Campylobacter concisus zonula occludens toxin causes disruption of epithelial integrity and production of pro-inflammatory cytokines

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Zonula occludens toxin (Zot) is an enterotoxin secreted by *Vibrio cholerae* that increases intestinal permeability. Zot has been detected in *Campylobacter concisus*, an oral bacterium that is associated with inflammatory bowel disease (IBD). The current study investigates the effect of *C. concisus* Zot on intestinal epithelial integrity and induction of proinflammatory cytokines. *zot* gene was amplified in *C. concisus* strains isolated from a patient with active IBD and a healthy control. They were cloned into pETblue-2 protein expression system. Zot proteins were purified and added to Caco-2 and HT-29 cells. Transepithelial electrical resistance (TEER) and pro-inflammatory cytokine secretion levels were measured. Zot proteins from patient and healthy control caused a rapid decrease of TEER in Caco-2 cells within the first two hours of exposure. This was significant as compared to the vector control (without recombinant Zot) ($p<0.05$). The decrease in the TEER level caused by Zot from patient with active IBD was significant as compared to that from the healthy control. After removal of toxins, the TEER in Caco-2 cells treated with Zot toxins continued to decrease for 24 hours. Zot treated HT-29 cells produced significantly higher levels of IL-8 and TNF-α as compared to the vector control ($p<0.05$). In conclusion, these data show that *C. concisus* Zot may play a role in initiating IBD by damaging intestinal epithelial barrier and inducing production of pro-inflammatory cytokines.

Government and Regulatory Aspects
Tuesday 3rd November

0815-1040 Room C

O057

Raw drinking milk: Using science to make good policy

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Ministry for Primary Industries[1]

The Ministry for Primary Industries (MPI) is responsible for setting and managing import, export and domestic requirements; and market assurances for animal welfare, biosecurity and food safety. The requirements and options for risk management should be informed by science and knowledge, and be risk based as far as possible within the decision making process. The current law allows milk producers to sell up to five litres of raw milk at any one time from the farm direct to a consumer. However, the consumer demand for raw milk appears to be growing in New Zealand. The number of foodborne illness outbreaks where raw milk consumption was recorded as a risk factor has increased since 2009. Most of the campylobacteriosis outbreaks recorded by Notifiable Diseases Surveillance Database where dairy products were implicated were associated with raw milk consumption. Recently MPI has reviewed the requirements for the sale of raw milk to consumers. As part of the review, MPI commissioned a microbiological survey to provide data on the presence of *Campylobacter* spp. in raw milk, and conducted a risk assessment that looked at the effect of different interventions on the incidence of Campylobacter along the raw milk supply chain. This presentation will explain the results from the microbiological survey and risk assessment activities; and how the information obtained from these is helping to inform the risk management decision making process for the provision of raw drinking milk to consumers including the development and implementation of science based control measures.

O058

25 Years of the Swedish Campylobacter monitoring program

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National Veterinary Institute, Sweden[1]Swedish Poultry Meat Association[2]

In 2015 the Swedish Campylobacter program celebrates 25 years anniversary. *Campylobacter* spp. have been monitored since 1991. The objective is to reduce the occurrence of Campylobacter in the food chain through preventive measures, starting with primary production. Basically, the program involves sampling of all conventional broiler flocks at slaughter, together with occasional additional samplings. A revised Campylobacter surveillance program for broilers commenced in 2001. This program replaced the earlier monitoring program of Campylobacter conducted by the industry. At least ten cloacal or caecal samples have been taken for analysis from each flock. In summary, the main findings are:

- The annual incidence of Campylobacter positive flocks decreased from 23% in 2001 to 9% in 2013
- A regional variation in Campylobacter incidence was seen
- A seasonal summer peak in incidence occurred
- Approximately half of the producers seldom delivered positive flocks, (<10% positive flocks/year)
- Risk factors associated with Campylobacter positive flocks were identified, i.e. other livestock nearby, split slaughter, and low biosecurity
- Contamination during transport to slaughter due to dirty crates could cause a problem
- Flocks tested positive at farm had a higher concentration of Campylobacter on carcasses compared to flocks in which Campylobacter was found only at slaughter (slaughter contamination) Knowledge gained from the program is used for interventions such as:
- Swedish producers are continuously informed and educated about the importance of strict bio security and hygienic barriers
- Regular feedback to producers on results of monitoring and other studies
- The producers get a premium for slaughter batches that are free from Campylobacter

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O059

International Standard ISO 10272 for detection and enumeration of Campylobacter in the food chain

Jacobs-Reitsma, WF[1];

On behalf of the CEN/TAG 19 working group[1]

Draft International Standard ISO/DIS 10272 for detection and enumeration of *Campylobacter* in the food chain was under international technical voting from February – May 2015. Elaboration of comments will be followed by a final editorial FDIS voting of 2 months and then the official publication of this revised ISO is anticipated early 2016. Both parts now also include the performance characteristics as established in the inter laboratory studies carried out under the CEN Mandate M381. Part 1 on detection: Part 1A: Bolton broth (4-6 h at 37°C then 40-48 h at 41.5°C), isolation on mCCDA and a second selective medium, with a principle different from mCCDA. E.g. cooked or frozen products (except frozen raw poultry meat). Part 1B: Preston broth (22-26 h at 41.5°C) plus isolation on mCCDA. High background flora containing products like raw meats, raw milk, frozen raw poultry meat. Part 1C: Direct isolation from sample material or a primary dilution onto mCCDA. E.g. faeces, poultry caecal contents or raw poultry meat. Like for agar plates, all enrichment broths are to be incubated in microaerobic atmosphere. Part 2 on enumeration: Plating on mCCDA in single (ISO 7218:2007). Confirmation in both parts include microscopic examination, detection of oxidase and absence of aerobic growth at 25°C. Optionally, *Campylobacter* species are identified by specific biochemical tests (catalase, hippurate hydrolysis, indoxyl acetate). New work was recently started to prepare guidance on the use of molecular methods for *Campylobacter* species identification, which may be added to ISO 10272 at a later stage.

O060

Notification Based Risk Assessment

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New Zealand Ministry for Primary Industries[1]

The Ministry for Primary Industries is responsible for establishing import requirements for food imported into New Zealand. A risk assessment is required to evaluate the likely impact of fresh chicken meat imports on the current rate of human foodborne campylobacteriosis and salmonellosis. A conventional farm to consumption risk assessment approach is unlikely to be successful due to the paucity and non-comparability of data on hazard levels in chicken meat from exporting countries. A human illness notification-rate based risk assessment (NBRA) model was developed that uses country-reported human campylobacteriosis notification rates, adjusted for underestimates using published correction factors, to estimate the likely disease burden. Source attribution studies were then used to determine the proportion of human campylobacteriosis cases attributable to the consumption of chicken meat, expressed as the number of cases per kg chicken meat consumed. Currently, the output of the model is independent of pathogen control measures (e.g. cooking) carried out prior to consumption and qualitatively assumes (in the absence of evidence to the contrary), that domestic and imported chicken meat will be similarly handled in New Zealand. In the future, market research on commercial and consumer handling of imported chicken meat will further inform development of the model. This presentation will demonstrate how a NBRA model can be used to simulate the likely human health impacts of imported chicken meat in relation to current impacts resulting from consumption of New Zealand-produced chicken meat. A model such as this can be effectively used to inform risk management decisions.

O061

Survey of National Reference Laboratory (NRL) capacity for campylobacteriosis surveillance in low and middle incomes countries.

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Background: The World Health Organization (WHO) plays an important part in international surveillance of Campylobacteriosis through WHO Global Foodborne Infections Network (GFN). This network aims to enhance the capacity of countries to conduct integrated surveillance of foodborne and other enteric infections. Main activities of GFN are international training courses, organizing external quality assurance system (EQAS), performing focused regional and national projects, providing reference services, and an electronic discussion group.

Objectives: In 2014, an e-mail survey was conducted to assess the capacity National Reference-level Laboratories (NRL) of Low and Middle Incomes Countries (LMIC) to conduct integrated surveillance of campylobacteriosis.
Methods: The survey was sent to 199 contact points identified in the GFN Member States on 11 June 2014. The deadline for returning the questionnaire to ECDC was 11 July 2014. In addition to the laboratory methods assessment, we also included questions on training and further needs of laboratories.

Results: 33 laboratories members of the GFN network located in 27 LMIC participated to the study. All laboratories used culture-based methods for isolation and identification of Campylobacter. Antimicrobial susceptibility testing was performed by 60% of responders whereas 80% participated to EQAS. Laboratories generally underlined the need for further training and harmonization of methods.

Conclusions: This study highlights the capacity of NRL of LMIC to assess the health burden of campylobacteriosis. However, future initiatives are needed to harmonize NRL services across the world in terms of methods and to ensure the minimum operational capacity required to contribute to a wide network for network for campylobacter's protection.

Comparative 'Omics: *Campylobacter*
Tuesday 3rd November

1110-1230 Room A

O062

A novel method for efficient inference of recombination hot regions in bacterial genomes and its application to 200 *C. jejuni* genomes

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In eukaryotes, detailed surveys of recombination rates have shown variation at multiple genomic scales and the presence of "hotspots" of highly elevated recombination. In bacteria, studies of recombination rate variation are less developed, in part because there are few analysis methods that take into account the clonal context within which bacterial evolution occurs. Here we focus in particular on identifying "hot regions" of the genome where DNA is transferred frequently between isolates. We present a computationally efficient algorithm based on the recently developed "chromosome painting" algorithm, which characterizes patterns of haplotype sharing across a genome. We compare the average genome wide painting, which principally reflects clonal descent, with the painting for each site which additionally reflects the specific deviations at the site due to recombination. Using simulated data, we show that hot regions have consistently higher deviations from the genome wide average than normal regions. We applied our approach to previously analysed *Escherichia coli* genomes, and revealed that the new method is highly correlated with the number of recombination events affecting each site inferred by ClonalOrigin, a method that is only applicable to small numbers of genomes. Furthermore, we analysed recombination hot regions in *Campylobacter jejuni* by using 200 genomes. We identified three recombination hot regions which are enriched for genes related to membrane proteins. Our approach and its implementation, which is downloadable from https://github.com/bioprojects/orderedPainting, will help to develop a new phase of population genomic studies of recombination in prokaryotes (Yahara 2014, *Molecular Biology and Evolution*).

O063

Recombination and the limits of the species concept in Campylobacter

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Hybridization between distantly related organisms can facilitate rapid adaptation but is constrained by epistatic fitness interactions. The zoonotic pathogens *Campylobacter coli* and *C. jejuni* differ from each other at an average of nearly 40 amino acids per gene, comparable to the nucleotide divergence between *E. coli* and *Salmonella*. Nevertheless, they have started to exchange substantial amounts of DNA. By analysing whole genome data from 500 *Campylobacter* isolates our results describe how a *C. coli* diversified into three clades that could be considered different species. The clade 1 *C. coli* lineage has successfully colonized the agricultural niche. Descendants fall into two groups, the ST-828 and ST-1150 clonal complexes both of which have been progressively accumulating *C. jejuni* DNA. The 1150 complex is less common among genotyped isolates but has undergone a substantially greater amount of introgression, leading to replacement of up to 23% of the *C. coli* core genome as well as import of novel DNA. By contrast, 828 complex strains have 10-11% introgressed DNA and *C. jejuni* and non-agricultural *C. coli* strains each have less than 2%. Using ChromoPainter analysis software we find `haplotypes' in sequence data, each of which has been "painted" as a combination of all other sequences quantifying the number of recombination events at all sites, and the donor genomes. These findings show the remarkable interchangeability of basic cellular machinery even after a prolonged period of independent evolution and highlight the limits of the species concept in bacteria.

O064

Inferring recombination graphs and population dynamics of Campylobacter and other bacterial species

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Understanding the evolution and population dynamics of *Campylobacter spp.* and other bacterial pathogens is important for inferring transmission and source attribution. Bayesian phylogenetic methods are sometimes used to infer evolutionary and demographic model parameters from genetic sequence data. In this context, the phylogeny itself is merely the glue which ties the sequence data to the population genetics/dynamics models that describe the larger-scale behaviour of the population. This style of inference has been used to great effect in the study of viral evolution, where it has been used to infer both epidemiological parameters and pathogen prevalence dynamics. However, its application to the study of bacterial evolution is hampered by the confounding effects of frequent recombination. We present here a new method for performing joint Bayesian inference of bacterial ancestral recombination graphs (ARGs) and the demographic model parameters governing the populations that generate them. Our method uses a novel MCMC algorithm to perform inference under the ClonalOrigin model (Didelot et al., Genetics, 2010) and is implemented as an extension to the popular phylogenetic inference tool BEAST 2 (Bouckaert et al., PLoS Comp Biol, 2014). After discussing the technical details of the inference scheme and its implementation, we will prove the validity of the method by applying it to simulated genetic data. We will then go on to demonstrate the genuine practical utility of the method by applying it to the joint inference of ARGs and population size dynamics from several real datasets collected from several organisms including *Campylobacter* and *E. coli*.

O065

Campylobacter jejuni flagellar biogenesis: the role of FlhF

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Amphitrichous *Campylobacter jejuni* lack the flagella biosynthesis master regulator (*flhDC*) found in well-studied model organisms such as *E. coli* and *Salmonella*. Like most polar flagellate bacteria, *C. jejuni* carry tandem flagellar genes Cj0064c:*flhF* and Cj0063c:*flhG* (a GTPase SRP-pathway subfamily protein, and a MinD homologue, respectively) shown to be involved in the regulation of flagellar biogenesis. Deletion of *flhF* by insertional mutagenesis caused an aflagellate and non-motile phenotype in the NCTC11168 strain. Pseudo-revertant isolates, obtained through repeated subculturing and selection of motility of the *ΔflhF* isolates, are motile and predominantly unipolar. Genome sequencing of 9 independently-derived pseudo-revertants revealed point mutations in flagella-associated genes. Although recent research has yielded new insights into the functions of FlhF and FlhG, their precise role is still uncertain. The aim of this study is to further our understanding of the role of FlhF through the use of comparative phenotypic, transcriptomic and structural analysis of the wild-type, *ΔflhF* and pseudo-revertant isolates. Eight of the nine pseudo-revertants had at least one non-synonymous point mutation in *fliF* and one had a similar mutation in *fliG*. These proteins encode the MS and C rings of the flagellar basal body. FliF is the first protein inserted into the inner membrane during flagellar synthesis, and requires FliG for stability. Global gene expression analysis and *in silico* protein structure prediction along with phenotypic data suggest that structural/conformational properties of the MS and C ring-proteins play a key role in the completion of *C. jejuni* flagella through the induction of the σ54 regulon.

1110-1230 Room B

O066

***Arcobacter butzleri* is overestimated when using enrichment culturing**

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Background: Studies from food products show *Arcobacter butzleri* as the most prevalent species followed by *Arcobacter cryaerophilus*. On the basis of that the former species is included in the list of microbes considered a serious hazard to human health by the International Commission on Microbiological Specifications for Foods.

Objective: To determine if the importance attributed to *A. butzleri* is influenced by the employed culture approach.

Methods: Presence of *Arcobacter* from 29 wastewater samples was determined by m-PCR, direct culturing and after an enrichment step (CAT-broth). Isolates were genotyped with ERIC-PCR and identified with the 16S rRNA-RFLP.

Results: More positive samples were found after enrichment either by m-PCR (26/29, 89.6%) or by culture (25/29, 86.2%) when compare with direct m-PCR detection (16/29, 55.2%) and direct plating (13/29, 44.8%). After enrichment *A. butzleri* was detected by m-PCR and recovered by culture in the 100% of the positive samples while *A. cryaerophilus* was only detected by m-PCR in 19.2% (5/26) and recovered by culture in 4% (1/25). However, *A. cryaerophilus* was detected in 13 of 16 (81.25%) positive samples by direct m-PCR and was recovered in 11 of the 13 (84.6%) positive samples by direct plating. The 449 studied isolates belonged to 287 ERIC genotypes (strains), 97.2% (172/177) were obtained after enrichment and corresponded to *A. butzleri* while by direct plating 68.2% (75/110) corresponded to *A. cryaerophilus*.

Conclusions: The enrichment step provides more positive samples but will always give the wrong idea that *A. butzleri* is the prevailing species, when in reality, it is not. Acknowledgments: Projects AGL2011-30461-C02-02 and FP7/2007-2013 (no. 311846).

O067

A viable quantitative approach (v-qPCR) for detecting *Arcobacter* species.

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Background: Methods are necessary for the detection of only viable cells of potentially pathogenic microbes like *Arcobacter* spp, which have been involved in food and water borne outbreaks. DNA- binding dyes like propidium monoazide (PMA) to the DNA enable to detect only viable cells. The recently developed PMA-PCR method detect *Arcobacter butzleri* and *Arcobacter cryaerophilus* in biofilms. However, this approach does not allow quantification.

Objective: To develop a viable quantitative PCR (v-qPCR) for detection and quantification of Arcobacter spp.

Methods: The specificity of primers targeting the 23S was assessed. Living and dead *Arcobacter* cells (killed by heat treatment) with and without PMA were evaluated. The PMA and cell concentration, photo activation and incubation time were optimized. Once the conditions were established, the v-qPCR was assessed using SYBRgreen.

Results: Primers were specific for the 20 species that comprises the genus *Arcobacter*. Optimal PMA conditions were established at a concentration of 20µM PMA, 5 minutes incubation at room temperature, and photo activation time of 15 minutes for a concentration of cells up to 10^7 CFU/ml. Similar Ct values were obtained for living cells (with and without PMA) and dead cells not treated with PMA. No amplification signal was seen from the dead cells treated with PMA and the V-qPCR had an efficiency of 98.3%.

Conclusion: This is the first study that uses a v-qPCR for detection and quantification of *Arcobacter* spp. Further studies are needed to implement this methodology in food safety. Acknowledgments: Projects AGL2011-30461-C02-02 and FP7/2007-2013 (no. 311846). Fellowship URV-IRTA-BS.

O068

Molecular epidemiology of *Arcobacter butzleri* in a model ecosystem

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Background: *Arcobacter butzleri* is an emerging enteric pathogen of human beings, but its pathogenicity and epidemiology remain poorly understood.

Objective. Southwestern Alberta (SWA) was used as a model ecosystem to ascertain the importance of *A. butzleri* as an incitant of enteritis, and isolates/samples obtained over a 1-year period from diarrheic and non-diarrheic humans, livestock, wildlife, surface waters, and wastewater were examined.

Methods: Isolates/samples were analyzed with novel molecular tools developed using comparative whole genome sequence analysis. These included PCR primers for direct detection/quantification of *A. butzleri*, and a comparative genomic fingerprinting assay for high resolution subtyping of the bacterium.

Results: Direct detection PCR indicated that a significantly higher proportion of stools from diarrheic (62.5%; n=1183) than non-diarrheic (45.5%; n=88) people were positive for *A. butzleri* DNA. The frequency of detection peaked in June and October for diarrheic individuals, which also corresponded to the peak of *A. butzleri* DNA detected in wastewater. Preliminary high resolution subtype analysis of isolates (n=992), including those from human stools (n=58) showed a high population diversity (464 clusters). Among the subtypes recovered from human diarrheic stools (n=10), only three subtypes were observed in multiple individuals.

Conclusions. Evidence indicated that *A. butzleri* is a resident of the intestines of human beings and is ubiquitous in wastewater and surface waters in SWA. However, the frequency and peaks of detection of *A. butzleri* in diarrheic people coupled with evidence that a limited number of subtypes occur in diarrheic individuals indicates that pathogenic genotypes of the bacterium exist.

O069

Fluoroquinolone and macrolide susceptibility of *Arcobacter butzleri* and *Arcobacter cryaerophilus* human clinical isolates

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Background: *Arcobacter* are human pathogens causing diarrheal disease in all age groups. Infants and immunocompromised patients may need treatment. Macrolides became preferred therapy for *Campylobacter* infections due to rising fluoroquinolone resistance. Data on *Arcobacter* antibiotic susceptibility remain scarce.

Objectives: To obtain susceptibility data and minimum inhibitory concentration (MIC) distribution ranges of erythromycin (ERY), azithromycin (AZI) and ciprofloxacin (CIP) for human clinical *Arcobacter* isolates.

Methods: 106 *Arcobacter* strains were tested to ERY and CIP by gradient strip diffusion of a 1 McF inoculum on Mueller-Hinton-F with 5% horse blood and incubation for 24 hours in microaerobic conditions at 35 +-2°C. CLSI breakpoints for *Campylobacter* were used for interpretation. A selection of 41 strains was additionally tested for AZI susceptibility by the same method.

Results: *Arcobacter butzleri* showed high susceptibility to CIP (87%, n=55) while half of *Arcobacter cryaerophilus* strains (46%, n=20) showed high level resistance (MIC >32 mg/L). ERY retained 78% (n=83) susceptibility for *Arcobacter* spp. MICs for AZI ranged between 0.5 and >256 mg/L, showing a protracted monomodal distribution for both species.

Conclusions: A pronounced level of resistance to CIP for *A. cryaerophilus* was observed. Mutations in the quinolone resistance-determining region (QRDR) of the gyrA gene have to be considered. Resistance to ERY for *Arcobacter* is high when compared to *Campylobacter*. Notwithstanding categoral agreement, MICs for AZI are at least equal or higher than those for ERY. AZI specific clinical breakpoints could clarify interpretation of susceptibility results. A multicenter data set of MIC results can validate the obtained distribution ranges and permit the development of an epidemiological cut-off values (ECOFF) set.

1110-1230 Room C

O070

Helicobacter pylori infection is modulated by a molecular rheostat that tunes the cellular immune response and promotes persistent infection

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Background: CagY is an essential component of the H. pylori Type IV secretion system (T4SS) that can undergo recombination in vivo and alter T4SS function.

Results: To determine which arm of adaptive immunity is necessary for cagY-mediated modulation of T4SS function, B and T cell knockout mice were challenged with H. pylori. Similar to RAG-/- mice, T cell knockout mice failed to select for loss of T4SS function or change in cagY, whereas strains from B cell knockout mice looked similar to those from BL6 mice. Adoptive transfer experiments confirmed that CD4+ T cells are necessary for cagY selection. We next challenged IFNgR-/- and IL-10-/- mice to provide a reduced and an enhanced inflammatory environment, respectively. Strains recovered from IL-10-/- mice lost the ability to induce IL-8 and change their cagY much faster than strains from BL6 mice. In addition, strains from IL-10-/- mice with changed cagY and loss of IL-8 induction had a higher bacterial burden. Strains recovered from IFNgR-/- mice were similar to those from RAG-/- and T cell knockout mice, indicating that selection for cagY variants is IFNg mediated. Co-infection of mice with H. pylori and Salmonella recapitulated many of the findings with IL10-/- mice, suggesting that CagY-mediated down modulation of T4SS function is a bacterial strategy to adapt to a robust host inflammatory response.

Conclusions: IFNg-dependent immune pressure selects for variation in CagY, yielding a pool of strains with varying inflammatory potential, which permits *H. pylori* to adapt to changing inflammatory conditions and maximize persistent infection.

O071

Association of *H. pylori* cagA gene and vacA allele type with histopathologic diagnoses in Arctic Canada

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Background: Patterns of disease due to infection with *H. pylori* vary among different ethnicities and geographic regions. Virulence genes *cagA* and *vacA s1i1m1* allele type may be more frequent in populations where severe *H. pylori* disease is common. The prevalence of *H. pylori* infection in Arctic Canada Aboriginal communities is 2-3 times higher than in non-Aboriginal populations. Estimates of association of virulence genes with disease manifestations in these Aboriginal communities are of interest.

Objective: To describe the distribution of virulence genes (*cagA, vacA* allele types) in *H. pylori* isolated from residents of 4 Aboriginal communities in Arctic Canada, and to estimate their associations with histopathologic diagnoses.

Methods: Genomic DNA extracted from 206 *H. pylori* isolated from members of 4 communities in Arctic Canada. *cagA* and *vacA* allele types were identified by PCR and DNA sequence analysis. Crude odds ratios were calculated to estimate the associations of *cagA* and *vacA* allele types with histopathologic diagnoses determined by the Sydney classification system.

Results: The histopathologic diagnoses included 16% severe acute gastritis and 46% severe chronic gastritis, 42% atrophy, and 18% intestinal metaplasia. *cagA* positivity was associated with severe gastritis. Individuals infected with *vacA s1* positive *H. pylori* had greater odds of severe gastritis, gastric atrophy and intestinal metaplasia than *H. pylori*-infected individuals without *vacA s1*. The other vacA alleles or combination allele types were less strongly associated with disease manifestations.

Conclusion: Although study numbers were small, *cagA* gene positivity and *vacA* allele type influenced severe disease outcomes in *H. pylori*-infected residents of Aboriginal communities in Arctic Canada.

O072

Secular trends for peptic ulcer disease and gastric cancer in Arizona, 1993-2013

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Gastroduodenal disorders affect various regions of the stomach and are significant causes of morbidity and mortality globally. Infection with *Helicobacter pylori* is associated with an increased risk of peptic ulcer disease and gastric cancer. In the United States, secular trends for peptic ulcer disease and gastric cancer hospitalization rates have been declining. These declines are attributed to decreases in *Helicobacter pylori* infections due to improved antibiotic eradication. We aimed to evaluate secular trends of peptic ulcer disease and gastric cancer in Arizona, from 1993-2013. Retrospective cross-sectional study using Health Care Utilization Project State Inpatient Database. Joinpoint regression was used to analyze temporal trends, from 1993-2013, to identify statistically significant rate changes, over the entire time period, as well as from year to year. The mean age of patients diagnosed with peptic ulcer was 64 ±18 years. More females, 53%, than males, 47%, were diagnosed with peptic ulcer disease. The reverse was the case for gastric cancer, 44% females versus 56% males. Peptic ulcer hospitalization increased by 69.27% ($p< 0.001$) from 1993 to 1995, and by 26.43% ($p= 0.01$) from 2006 to 2009. Gastric cancer hospitalization rates increased by 11.94% ($p= 0.03$) from 2006 to 2009. Secular trends indicate that peptic ulcer and gastric cancer hospitalization rates in Arizona increased significantly over multiple years, within the 1993-2013 time period. These findings are in contrast to recent national trends showing a downward trajectory for peptic ulcer and gastric cancer. Further research is needed to understand increasing trends in these diseases in Arizona.

O073

Casting a long shadow: the role of household crowding in *Helicobactor pylori* infection and excess stomach cancer incidence among Maori and Pacific people

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University of Otago, Wellington[1]

Background: *Helicobacter pylori* has been linked with household crowding and is considered a necessary causal factor for non-cardia stomach cancer. Māori and Pacific peoples in NZ experience greater household crowding, *H. pylori* infection and stomach cancer incidence.

Objectives: To estimate the contribution of household crowding to *H. pylori* seroprevalence by ethnicity and estimate the excess stomach cancer incidence in Māori and Pacific attributable to *H. pylori*.

Methods: A systematic review and meta-analysis was conducted to summarise the association between household crowding and *H. pylori*. The meta-analysis odds ratio was used to estimate the contribution of crowding to *H. pylori* seroprevalence by ethnicity and birth cohort. Rate ratios (RRs) for excess age-standardised Māori and Pacific stomach cancer incidence compared to European/Other were adjusted for *H. pylori*, smoking and non-cardia subsite. Observed RRs were compared with adjusted RRs to give an 'excess RR proportion'.

Results: Persons experiencing greatest vs. the least household crowding had 1.73 (95% CI 1.48-2.03, n=28, I2=87%) greater odds of *H. pylori* infection. Household crowding amongst children born 1971-85 contributed to 44% (95% CI: 32-54%) of Pacific, 36% (95% CI: 25-47%) of Māori, and 14% (95% CI: 9-20%) of European seroprevalence. Pooled average *H. pylori* seroprevalence was greatest among Pacific (62%), followed by Māori (35%) and European (18%). *H. pylori* and smoking (to a lesser degree) contributed to 53-65% of the excess non-cardia stomach cancer among Māori and74-90% of the excess non-cardia stomach cancer among Pacific.

Conclusions: Household crowding is a major contributor to Māori and Pacific *H. pylori* seroprevalence and the primary driver of excess non-cardia stomach cancer incidence among Māori and Pacific.

Attribution Workshop
Tuesday 3rd November

1415-1530 Room A

O074

Source attribution and case-control analyses to identify exposures associated with campylobacteriosis in the United States

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Background: Foodborne *Campylobacter* causes an estimated 850,000 domestically-acquired illnesses in the United States annually. Understanding the sources and risk factors for infection can help target prevention efforts.

Objectives: To identify exposures associated with sporadic infection with different *Campylobacter* strains.

Methods: We analyzed 789 *Campylobacter* isolates from sporadic human infections ascertained by the Foodborne Disease Active Surveillance Network (FoodNet) in 1998 and 2008 using multilocus sequence typing (MLST). We used isolate source data from published studies in an asymmetric island model to attribute cases to animal and environmental sources. Using exposure data from cases and controls collected during a 1998 FoodNet study, and data from cases in 2008, we conducted weighted multivariable logistic regression to identify exposures significantly associated with MLST profiles attributed to each source.

Results: Consumption of chicken, turkey, pork, and seafood were associated with isolates attributed to poultry (n=177). Among isolates attributed to ruminant sources (n=117), consumption of beef, fresh fish, eggs, contact with live poultry and sick pets were significant exposures. Isolates attributed to the environment (n=120) were associated with consumption of turkey or other poultry, pork, and seafood, and contact with live poultry. Visiting or living on a farm was a significant exposure for all isolates.

Conclusions: We identified exposures associated with specific *Campylobacter* strains using exposure data collected from cases and controls and MLST profiles attributed to specific sources. Our results suggest that exposure to isolates from different reservoirs occurs via multiple transmission routes.

O075

Dynamic source attribution modelling for campylobacteriosis surveillance

Marshall, JC[1]; French, NP[1];

Massey University[1]

Until recently, New Zealand had the highest per-capita incidence rate of campylobacteriosis in the world and, despite recent efforts, notification rates are still significantly higher than in comparable countries. This is not only a significant public health issue, but also an important economic issue, as it is estimated that the 7,000 cases currently notified represent direct and indirect costs to New Zealand of approximately $32 million dollars per annum. For the majority of these cases there is little epidemiological information, making it difficult to determine the likely source or reservoir of infection. Genotyping techniques, such as Multi-locus sequence typing, however, allow each case to be categorised, allowing assignment of human cases to the most likely source based on the distribution of genotypes on those sources. Campylobacteriosis notifications are highly seasonal, with some evidence that the incidence of specific genotypes is also seasonal. Further, an intervention targetted at the poultry industry in 2007 correlated with a decline in human cases, particularly in urban areas. Thus, it is expected that the proportion of cases attributed to each source changes through time, and may differ between urban and rural areas. A Bayesian model for the attribution of campylobacteriosis cases through time will be presented, with application to cases from the Manawatu region of New Zealand.

O076

Monitoring the impact of poultry industry interventions on the burden of human disease with MLST and whole genome sequence data.

Cody, A.J.[1]; McCarthy, N.D.[2]; Bray, J.E.[1]; Wimalarathna, H.M.L.[1]; Jansen van Rensburg, M.[1]; Colles, F.M.[1]; Dingle, K.E.[3]; Maiden, M.C.J.[4];

Department of Zoology, University of Oxford, Oxford, UK.[1]Warwick Medical School, University of Warwick, Coventry, UK. NIHR Health Protections Research Unit in Gastrointestinal Infections, University of Oxford, Oxford, UK.[2]Nuffield Department of Clinical Medicine, Oxford University, John Radcliffe Hospital, Oxford, UK.[3]Department of Zoology, University of Oxford, Oxford, UK. NIHR Health Protections Research Unit in Gastrointestinal Infections, University of Oxford, Oxford, UK.[4]

Background: Human campylobacteriosis in the United Kingdom (UK) costs in excess of £0.5bn annually, with 71,365 reported cases in 2012. Nucleotide sequence-based attribution analyses using multi-locus sequence typing (MLST) have identified poultry meat as the single most important source of human infection.

Objectives: To detect changes in the incidence and source of human *Campylobacter* isolates, from Oxfordshire, UK, between June 2011 and June 2014, which relate to food chain interventions.

Methods: Isolates were characterised by hierarchical gene-by-gene analysis of whole genome sequence data. Their attribution to probable origin was determined by comparison of MLST data to that of reference populations using STRUCTURE. Ciprofloxacin resistance was assessed from specific *gyrA* allele sequence mutations.

Results: The incidence of human campylobacteriosis remained unchanged in Oxfordshire over this period, reflecting national trends. Complete MLST data was obtained for 99.7% isolates, describing 31 *C. jejuni* (n=2269, 89.0%) and 2 *C. coli* (n=280, 11.0%) clonal complexes. Only minor changes were observed in the prevalence of isolates belonging to the twenty most common complexes, which contributed 1583 (62.1%) isolates. Farm animals, notably poultry, were identified as being the most probable source of human disease, with the majority of these assigned to chicken associated genotypes. The proportion of ciprofloxacin resistant isolates increased from 38.1% to 43.1%, with a significant [χ^2 ($P < 0.001$)] number of these attributable to chicken.

Conclusions: There was no evidence of any substantial change in either the incidence or source of human disease over the three year period during which intervention strategies were implemented.

O077

Source attribution of *Campylobacter jejuni* infections in Ontario, Canada sentinel site using integrated surveillance, comparative genomic fingerprinting and comparative exposure assessment

Pollari, F[1]; Taboada, E[2]; Mutschall, S[2]; Nadon, C[3]; Maki, A[4]; Hurst, M[1]; Pintar, K[1]; Marshall, B[1]; Nesbitt, A[1]; David, J[5]; Ravel, A[6];

Centre for Food-borne, Environmental, and Zoonotic Infectious Diseases, Public Health Agency of Canada, Guelph, Ontario, Canada[1]Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Lethbridge, Alberta, Canada[2]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada[3]Public Health Ontario Laboratory, Public Health Ontario, Toronto, Ontario, Canada[4]Swine epid[56]

Background: *Campylobacter jejuni* is the most commonly isolated species from bacterial gastroenteritis cases in Canada. Source attribution modeling has been limited due to limited epidemiologic investigation of sporadic cases and molecular subtyping. FoodNet Canada is an integrated enteric pathogen surveillance system that utilizes the sentinel site approach to focus resources to gather enhanced exposure information and molecular subtyping information from human cases, retail foods farms and surface water.

Objectives: To derive source attribution estimates for human campylobacteriosis in Canada.

Methods: From 2006 – 2012, 1768 *Campylobacter* isolates (250 clinical, 985 farm, 481 retail and 52 water) were obtained from the FNC Ontario site. Isolates were subtyped using Comparative Genomic Fingerprinting (CGF), an efficient multiplex PCR method (targeting 40 genes) and assigned a subtype using a national database housed at the Laboratory for Foodborne Zoonoses (LFZ-Lethbridge). Estimates of the average daily exposure to *Campylobacter* from food, animal and water sources, obtained from a *Campylobacter* Comparative Exposure Assessment analysis, were then incorporated into the Dutch attribution model using CGF subtyping results from cases, food, farm and water *C. jejuni* isolates.

Results: Overall, 1768 isolates analyzed. The 250 human isolates were distributed among 104 CGF subtypes, 56 of these subtypes (197 isolates) being found in at least one of the other sources. Preliminary Dutch model results, suggest that chicken meat is the largest contributor, followed by cattle manure.

Conclusions: The results of the source attribution model, combined with the exposure assessment and integrated surveillance data, confirm the importance of chicken meat as well as environmental sources for human campylobacteriosis.

1415-1530 Room B

O078

Evaluation of MALDI-TOF MS for identification of Campylobacter related organisms

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Enteric Diseases Program, National Microbiology Laboratory, Winnipeg, Canada[1] Proteomics & Mass Spectrometry Core Facility, National Microbiology Laboratory, Winnipeg, Canada[2]

Background: Conventional identification of Campylobacter related organisms can be problematic and an alternate method would be advantageous.

Objectives: MALDI-TOF mass spectrometry was evaluated as an identification tool for Campylobacter related organisms as compared to conventional means.

Methods: 433 clinical and reference isolates representing 53 taxa were subjected to conventional short or full biochemical plus molecular testing (PCR-RFLP, species-specific PCR, 16S sequencing) all in parallel with MALDI-TOF analysis. In a second validation stage, MALDI-TOF was the initial screen followed by selective confirmatory conventional testing.

Results: MALDI-TOF results were in agreement with conventional results at the species level: 100% (330/330) for *Campylobacter* sp., 100% (22/22) for *Arcobacter* sp., 88% (70/80) for *Helicobacter* sp., and 100% (1/1) for *Wolinella* sp. MALDI-TOF correctly identified the genus of each isolate with the exception of 1 H. pullorum and 3 *H. pylori* isolates which generated no reliable information. Biochemical testing determined biotypes and phenotypic variants (hippurate-negative *C. jejuni*, urea-positive *C. lari*, aberrant antimicrobial susceptibility profiles) while molecular testing identified atypical PCR-RFLP patterns. Although MALDI-TOF did not detect biochemical or genetic variability, it did identify C. fetus isolates belonging to a recently reported third subspecies. Conventional identification was completed in 5 to 21 days (short and full biochemical testing, respectively) whereas MALDI-TOF was completed in 2 to 3 days.

Conclusions: MALDI-TOF is an efficient and effective means of identification offering a substantial reduction in time and resources. Although unable to determine biotypes, biochemical variants, or molecular anomalies, due to its speed and accuracy identification by MALDI-TOF is recommended to either supplement or substitute conventional species-level identification methods for Campylobacter related organisms.

O079

Subspecies typing of environmental and human health-related *Campylobacter jejuni* strains using Maldi-Tof mass spectrometry

Penny, C.[1]; Zhang, L.[2]; Grothendick, B.[2]; Fagerquist, C.K.[3]; Zaragoza, W.J.[3]; Miller, W.G.[3]; Ragimbeau, C.[4]; Mossong, J.[4]; Cornelius, A.J.[5]; Gilpin, B.J.[5]; On, S.L.W.[5]; Cauchie, H.M.[1]; Sandrin, T.R.[2];

Luxembourg Institute of Science and Technology, Luxembourg[1]Arizona State University, USA[2]US Department of Agriculture, USA[3]National Health Laboratory, Luxembourg[4]Environmental Science & Research, New Zealand[5]

Background: Maldi-Tof mass spectrometry has been widely adopted for the routine identification of bacteria at the species level in clinical, veterinary, agri-food and environmental microbiology labs. Though, its utility as a robust and complementary tool for characterizations of *Campylobacter* at the subspecies level has only been scarcely investigated.

Objectives: A world-collection of *C. jejuni* was subjected to profile-based diversity assessment and clustering through Maldi-Tof-MS analysis. Classification at the subspecies level was linked to multi-locus sequence typing (MLST) fingerprints, the geographical origin of the strains and their human, animal and water-related origins.
Methods: 172 *C. jejuni* isolates from the USA, Luxembourg, South Africa and New Zealand were selected for screening by Maldi-Tof-MS. A reliable workflow for protein extraction mass spectrometry on a Bruker MicroflexLT was validated. Bioinformatics analyses (spectrum trimming, similarity calculations) were performed using the BioNumerics software.

Results: Clustering of the *C. jejuni* collection was largely consistent with the MLST genotype profiles. Genetic diversity information on *C. jejuni* isolates could be trustfully transcribed through Maldi-Tof-MS fingerprints. Fine-tuning of the methodology is nevertheless required through the homogenization of large datasets (spectrum normalization). Currently, the identification of characteristic biomarkers and signatures specific for selected groups of strains through top-down proteomics, and the comparative typing with state-of-the-art methods such as whole-genome-sequencing (MLST+) and multiplex binary typing (MBiT) are ongoing.

Conclusions: Straightforward and cost-efficient diversity (pre-)screening of *Campylobacter* collections is made possible by Maldi-Tof-MS. This approach goes beyond simple species identification and allows barcoding of *Campylobacter* for epidemiology, risk assessment and source tracking purposes.

O080

Discrimination of Campylobacter species by a degenerate PCR-RFLP method based on gyrB gene sequence

Fukuda, H[1]; Kojima, F[2]; Fujimoto, S[2];

Kyushu University graduate school of medical science[1]Kyushu University department of medical science[2]

Background: Conventional molecular biological methods for discriminating *Campylobacter* species usually target for common species like *C. jejuni* and/or *C. coli*, with one or some specific primer pairs. However, these methods cannot identify other untargeted species and need additional methods for further investigation.

Objectives: The aim of this study is to establish a PCR-RFLP method using degenerate primers, which can distinguish at least 14 *Campylobacter* species including *C. jejuni* and *C. coli*.

Methods: The degenerate PCR primers were designed based on *gyr*B gene sequences of 190 strains (14 *Campylobacter* species) from the Genbank database. Acquired PCR products were treated with *Dde*I or *Mbo*I and the RFLP patterns were used for species identification by comparing with reference patterns (putative RFLP patterns based on the sequence data from the 190 strains).

Results: We examined 86 strains isolated from patients with *Campylobacter* gastroenteritis using this method and we could get the PCR products in all strains. RFLP analysis showed that among the 86 strains, 76 strains were *C. jejuni* and 7 strains were *C. coli*. The remaining 3 strains could not be identified because the RFLP patterns of the strains were identical to none of the reference patterns.

Conclusions: Molecular diagnosis for *Campylobacter* infection is important in clinical microbiology. This study indicate that the method using the degenerate primers is useful to discriminate between *C. jejuni* and *C. coli*. This method could be able to discriminate the 14 *Campylobacter* species theoretically and might be one of the tools for discriminating *Campylobacter* species.

O081

EU Reference Laboratory- Campylobacter Proficiency Tests for National Reference Laboratories. Progress of performance in five years.

Hansson, I[1]; Nyman, A[1]; Lahti, E [1]; Harbom, B[1]; Pudas, N[1]; Svensson, L[1]; Olsson Engvall, E[1]; EURL Campylobacter[1]

In the European Union, a network of reference laboratories, EU Reference Laboratories (EURLs), and National Reference Laboratories (NRLs), has been established to ensure the quality of analytical methods applied for samples taken in the official control. The major tasks for EURL-Campylobacter are to provide scientific and technical assistance to the Commission (DG Sante) and the NRLs-Campylobacter. The EURL for Campylobacter, situated in Uppsala Sweden, organizes annual proficiency tests for the NRLs. In 2010 to 2014, the PTs have included detection and enumeration of *Campylobacter* spp. in different food matrices using the standardized protocols of ISO 10272. The number of participating NRLs have been between 34 and 36. The proportion of NRLs that have reported correct results of detection in all samples has ranged from 56% to 74% with the highest in 2014. Enumeration has been performed by colony count technique, and the proportion of NRLs that have reported correct results has ranged from 65% to 91%. Most incorrect results have been reported on samples with a mixed culture of *Campylobacter* and ESBL *E. coli*. To be considered to have an excellent or good performance in detecting *Campylobacter* spp. or in enumeration the NRLs need to correctly detect *Campylobacter* spp. or have an enumeration within 2 SD in 85.7 % of the *Campylobacter* spp. positive samples, respectively. During the years, the majority of the NRLs have had excellent or good performance results. The EURL-Campylobacter assists the NRLs that need to improve their performance by organizing training courses and on-site visits.

1415-1530 Room C

O082

Comparative distribution of the gastric microbiome in a pediatric population colonized or not with *Helicobacter pylori*

Perez Perez, GI[1]; Llorca Otero, L[2]; Urruzuno, P[3]; Martinez, MJ[4]; Alarcon, T[2];

New York University Langone Medical Center[1]Hospital Universitario de la Princesa[2]Hospital Universitario 12 de Octubre[3]Hospital del Nino Jesus[4]

Background: *H. pylori* colonizes the human stomach of 50% of the world's population, and increases risk of gastric diseases, including gastric cancer. Aims. To compare the taxonomical distribution of the gastric microbiota in pediatric patients with and without *H. pylori* colonization.

MethodS: We studied 51 children [32 males and 19 females (mean age 11.1 years, range 1-17)] that underwent gastric endoscopy due to dyspeptic symptoms in Madrid, Spain. Gastric biopsies were obtained from each child for RUT, culture only for the positive RUT, and DNA extraction. From purified DNA of 51 gastric biopsies, total bacteria and *H. pylori* were enumerated by qPCR and V4 16S rRNA high-throughput sequencing (Illumina) was performed.

Results: A total of 16 children were *H. pylori* positive and 35 were negative. We found a good correlation between RUT and qPCR results to determine *H. pylori* status. *H. pylori* positive and negative specimens were similar in alpha and beta diversity, regardless of subject clinical condition, gender and age. Taxonomic analysis of the gastric microbiota at species level confirmed that *H. pylori* positive subjects had a higher relative abundance of *H. pylori* sequences (mean 50%) than *H. pylori* negative subjects (mean 0.1%). By LEfSe analysis, the samples from *H. pylori* positive were dominated by Proteobacteria phyla. In contrast *H. pylori* negative were dominated by Firmicutes and Bacteroidetes. Proteobacteria was present in *H. pylori* positive and negative biopsies but with different families represented. For *H. pylori* negative gamma- and beta-Proteobacteria were the most abundant. For *H. pylori* positive as expected was epsilon-proteobacteria. Four phyla (Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria) accounted for >97% of all reads.

Conclusions: In the gastric microbiota, *H. pylori* is an important component of the community. However, the rest of the gastric microbiota is nearly identical in children independently of the *H. pylori* status.

O083

Identifying genes responsible for genomic variation and virulence in *Helicobacter pylori*

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Swansea University[1]University of Tokyo[2]

An estimated 50% of all people carry the stomach bacterium *Helicobacter pylori*. This organism is responsible for various gastric problems like gastritis and gastric ulcers and is one of the major causes of gastric cancer worldwide. Large numbers of people carry this organism asymptomatically and many questions remain about why serious symptoms develop in a subset of infected humans. We hypothesise that these extremely recombinant bacteria may take different evolutionary trajectories in different people and that some genomic changes may be associated with gastric cancer. A pan-genome, including all genes present in at least one of the 557 sequenced genomes in the Swansea *H. pylori* BIGS database, was created and used to study core and accessory genome variation. These analyses were run with large diverse isolates collections and lead to the identification of loci with interesting genomic variations, in relation to the geographic clustering and phenotypic characteristics of the strains. Phenotypic characterization included motility and DNA damage assays, patient background information which were compared to genomic variations to identify genomic elements in *H. pylori* associated with gastric cancer.

O084

Synthetic small molecules disrupt *Helicobacter pylori* cag type IV secretion system activity

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Vanderbilt University Department of Pathology, Microbiology, and Immunology, Nashville, TN[1]Umeå University Department of Chemistry, Umeå Centre for Microbial Research, Umeå, Sweden[2]University of Kentucky Department of Pharmaceutical Sciences, Lexington, KY[3]Umeå University Department of Chemistry, Umeå, Sweden[4]Vanderbilt University Department of Medicine, Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN[5]Vanderbilt University Department of Medicine, Nashville, TN[6]

Type IV secretion systems (T4SS) are complex bacterial nanomachines that translocate diverse effector proteins or DNA into target cells. However, the mechanism by which translocation machineries deliver cargo across the bacterial envelope remains poorly understood. *Helicobacter pylori* exploits the *cag* T4SS to inject CagA and peptidoglycan into gastric epithelial cells. Here, we describe two synthetic small molecules (C10 and KSK85) that disrupt *cag* T4SS effector translocation. We show that KSK85 impedes biogenesis of the *cag* T4SS-associated pilus at the bacteria host-cell interface, while C10 inhibits *cag* T4SS function without disrupting pilus assembly. We provide evidence that these small molecules target CagA in a manner that is independent of their effects on T4SS activity. Using super-resolution microscopy, we demonstrate that a fluorescent inhibitor analog segregates to a helical distribution along the periphery of bacterial cells in a *cag* T4SS-independent manner, and co-localizes with a determinant of cell elongation that coordinates peptidoglycan synthesis. Our data indicated that these compounds target a component of the bacterial elongasome that impacts T4SS function. Consistent with this hypothesis, we demonstrate that these compounds disrupt inter-bacterial conjugative DNA transfer in *Escherichia coli*, and inhibit T-DNA delivery *in planta* by the prototypical *Agrobacterium tumefaciens vir* T4SS. Thus, our data support a model in which these compounds interfere with conserved components of the cell wall synthetic machinery to perturb dynamics of type IV secretion in divergent proteobacteria. Finally, we present a molecular scaffold that can be manipulated to develop versatile chemical tools to dissect and disarm these important machineries.

O085

Functional genomics of virulence potential and antimicrobial resistance markers by in silico genomotyping of *Helicobacter pylori*

van Vliet, Arnoud[1]; Kusters, Johannes[2];

Institute of Food Research, Norwich, UK[1]University of Utrecht, The Netherlands[2]

Background: The development of genome sequencing technologies has made large collections of genome sequences available for pathogenic bacteria such as *Helicobacter pylori*. The H. pylori genome displays very high levels of diversity generated through mutation, recombination and horizontal gene transfer, which complicates comparative genomics and molecular epidemiology.

Objective: To use alignment-free phylogenomic and functional genomics approaches to study population structure, genome evolution and antimicrobial resistance in *H. pylori*.

Methods: Alignment-free phylogenetic analyses were coupled to in silico genotyping and comparative genomics approaches, using ~400 publicly available genomes of *H. pylori* isolates and other gastric *Helicobacter* species.

Results: Alignment-free phylogenetic trees of whole genomes of seven gastric *Helicobacter* species matched those obtained by analysis of 16S rDNA and ribosomal proteins, and comparison of alignment-free methods of 63 *H. pylori* genomes with core genome SNP-based methods showed again comparable phylogenetic clustering, consistent with the genomotypes identified using multi-locus sequence typing (MLST). Analysis of the distribution of virulence markers and antimicrobial resistance markers in 379 *H. pylori* whole genomes and proteomes showed a good conservation of genomotypes and linkage with phylogeographic and phenotypic characteristics, but no association with virulence markers, except for those previously described to be linked such as the cag pathogenicity island and vacA s1.

Conclusions: Alignment-free phylogenetics can be used to rapidly analyse large collections of *H. pylori* genome sequences, which are too divergent for SNP-based analyses. This is a powerful tool for analysis of linkages between genomotypes, phylogeographic information and pathogenicity and antimicrobial resistance potential.

O086

Dominantly persistent infection in gastric mucosa of Mongolian gerbils with *H. pylori* strain isolated from 3 children compared to those from their parents.

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Department of Infectous Diseases, Kyorin University School of Medicine[1]

It has been ecologically shown that *H. pylori* infection usually almost occurs under 2 years old in human life. Although transmission route of *H. pylori* has not been fully understood, its transmission from family member (particularly mother) to child is most frequently detected in Japan. In the present study, several strains isolated from family members were used for animal experiment to compare the infectivity of the isolates to host and adhesion activity to gastric epithelial cells. *H. pylori* K21 or K22 strain was isolated from father or mother, respectively, and K23-25 strains were isolated from three children of the family. The sequence types (ST) determined by MLST (multilocus sequencing type) were ST2760, ST2761 and ST2762 for K21, K22 and K23-25, respectively. The ST(2762) of child strains was exactly the same. Mongolian gerbils were inoculated with K21 or K22 strain at 1st inoculation and also inoculated with K25 at 2nd inoculation 10 days after the 1st inoculation (Groups K21/K25 and K22/K25). An opposite inoculation order experiment was also done (Groups K25/K21 and K25/K22). Five weeks after the 2nd inoculation, gastric *H. pylori* were cultured from stomach of Mongolian gerbils. Bacterial DNA was extracted from each colony and analyzed by RAPD fingerprinting and direct-sequencing of trpC. The ability of adherence to gastric cell line (AGS) and growth of three strains were compared. Molecular type of K25 was highly frequently detected in the colonies of *H. pylori* in the gerbils of 4 groups (77.8 %; 84/108 colonies isolated). The remaining 24 colonies were shown not to be opposite K21 or K22 molecular type. It was also shown that the adhesion ability of K25 to AGS cells was significantly higher than that of other strains, suggesting that K25 child strain with enhanced adhesion activity dominantly persists rather than parental strains in gastric mucosa of Mongolian gerbil.

1600-1740 Room B

O087

Development of rapid diagnostic tests for the identification of pathogenic *Campylobacter jejuni* isolates

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The prevention and control of campylobacteriosis is complicated by the broad ecological distribution and high prevalence of *Campylobacter jejuni* in wild and domesticated animals, including in most companion and food production animals, and environmental sources such as water, and soil. Evidence suggests that a small number of "pathogenic" lineages are responsible for a large proportion of campylobacteriosis cases. Our aim was to use a comparative genomics approach to identify genetic markers associated with such lineages. The Canadian National Comparative Genomic Fingerprinting database, which contains subtyping data for >18,000 *Campylobacter* isolates was used to select a set of 166 *C. jejuni* isolates representative of prominent "pathogenic" and "non-pathogenic" lineages in Canada for whole genome sequencing. Comparative genomic analysis of gene content was used to identify genes with significant differences in carriage between the lineages. A total of 33 high quality markers were identified and assessed in silico against an expanded set of over 2,000 publicly-available *C. jejuni* genomes. Three markers, when used in combination were present in 97% versus 46% of strains from "pathogenic" and "non-pathogenic" lineages, respectively. These markers were targeted for the development of a PCR assay to identify potentially pathogenic strains of *C. jejuni*. Effective mitigation strategies will require identification of reservoirs with the highest prevalence of strains posing an increased risk to human health. Employing a comparative genomic approach on "pathogenic" and "non-pathogenic" lineages, we have developed a PCR-based risk assessment tool for public health authorities to rapidly identify *C. jejuni* strains that are potentially pathogenic to people.

O088

Evaluation of a hybrid, extraction-free MPN-PCR method for detection of Campylobacter in estuarine waters

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Background: The complex dynamics of estuarine systems has limited the use of culture-based and molecular assays for Campylobacter enumeration. Hybrid culture-PCR methods represent alternative, rapid quantification tools for use in these environments. However, evaluation is still required.

Objectives: This paper evaluates and compares a hybrid culture-PCR (MPN-PCR) assay to that of the Australian Standard (AS/NZS 4276.19:2001) for the rapid enumeration of pathogenic *Campylobacter* spp. in estuarine systems.

Methods: 147 samples were collected over a two year period (2012-213) from the Yarra River estuary, Australia. Concurrently, climatic, hydrological and biological data for 14 parameters were collected to evaluate pathogen-environment relationships. Samples underwent quantification by AS/NZS 4276.19:2001 and MPN-PCR; MPN-PCR was conducted on enriched sample cultures after 48hrs incubation. A 200bp 16S rRNA amplicon of *C. jejuni, C. coli, C. lari* and *C. upsaliensis* was PCR amplified. Isolates were collected and retained for speciation and subtyping by MLST.

Results: The intra-laboratory performance of the MPN-PCR exceeded that of AS/NZS 4276.19:2001 ($\rho = 0.7912$, $P<0.001$; $\kappa = 0.701$, $P<0.001$) with an overall diagnostic accuracy of ~94%. Genetic data demonstrated the presence of both generalist and niche ST-complexes with *C. coli* the most abundant species (85% of isolates). Comparative studies identified the potential introduction of bias during environmental parameter relationship analysis.

Conclusion: The MPN-PCR assay was superior to AS/NZS 4276.19:2001 for the enumeration of Campylobacter in estuarine waters. The potential for method introduced bias, which affects the assessment of pathogen-environment relationships, (information essential for microbial risk assessments) requires further evaluation.

O089

Enhancing performance of ISO 10272:2006 Standard for Enumeration and Detection of Campylobacter in chicken meat by the use of chromogenic-like media

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Background: Better quantification and detection of *Campylobacter* in fresh poultry meat is under consideration, by national and international authorities, as an important step for setting microbiological criteria for such important foodborne zoonoses. The commonly applied method for detection and enumeration of *Campylobacter* is the ISO standard 10272. The current standard suffers certain limitations, and there are on-going research efforts to restore its selectivity, sensitivity and diagnostic convenience.

Objectives: We evaluate the recommended ISO 10272:2006 versus alternative procedures for *Campylobacter* enumeration and enrichment in naturally contaminated chicken meat samples.

Methods: In the first experiment, using 49 samples of naturally contaminated chicken meat samples, two enrichment media were evaluated (Bolton broth (BB) and Preston broth(PB)) in combination with three selective plating agars; the ISO recommended mCCDA, and the chromogenic-like media CampyFood agar (CDA; bioMérieux) and Brilliance CampyCount agar (BCC; Oxoid). In the second experiment, using 59 samples of naturally contaminated chicken meat samples, we compared the ISO recommended mCCDA to the chromogenic-like media RAPID Campylobacter (RC; Bio-Rad); in this experiment we also compared the standard formulation of Bolton broth supplement to three supplement alterations (using Irgasan, Clavulanic acid and Polymyxin B).

Results: In the first experiment, direct plating on CFA provided the highest number of *Campylobacter* positive samples (17/49); but was not statistically different from numbers of positive samples recovered by direct plating on mCCDA (15/49) or BCC agars (14/49). Enrichment of chicken meat samples in BB for 48 h and subsequent plating on CFA provided significantly higher ($P < 0.05$) *Campylobacter* detection compared to PB. Enrichment in PB for 24 h followed by plating on mCCDA gave a higher number of positive samples (20/49) than 48 h enrichment in BB and plating on mCCDA (15/49). In the second experiment, enrichment in BB for 48 h followed by plating on RC recovered significantly higher *Campylobacter* positive samples (25/59) as compared to the current ISO method (17/59). Alteration of BB supplementation in the second experiment by using irgasan and clavulanic provided equivalently higher *Campylobacter* positive samples (22/59) as compared to the standard BB formulation (17/59) after plating on mCCDA. Interestingly, enrichment in BB with either of the previous supplement alterations followed by plating on RC provided the most positive *Campylobacter* samples (28/59) throughout all of the compared enrichment and direct plating combinations.

Conclusions: Compared to the current ISO method, some alternative combinations of enrichment and agar media could provide significantly better detection and enumeration of *Campylobacter* in chicken meat. Including new chromogenic-like media enhances the convenience and overall diagnostic sensitivity of the current ISO method.

O090

Identification of *Campylobacter fetus* subspecies *venerealis:* culture or PCR?

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Bovine genital campylobacteriosis (BGC) is difficult to diagnose due to the fastidious growth of *Campylobacter fetus* subspecies *venerealis*, and the lack of subspecies-specific PCR assays. This study isolated *C. fetus* and *Campylobacter* like organisms from fresh bull samples to compare identification methods using boiled lysates (qPCR), culture/biochemical phenotyping, and PCR of gDNA. The cultured isolates (n=54) were identified according to OIE standards and the gDNAs were analysed using: qPCRs (*parA*, IS*Cfe*1, and *nahE*), and conventional PCRs (*cstA/parA* duplex and IS*Cfe*1). Biochemical methods identified 30/54 *C. fetus* subsp. *venerealis* isolates with 16 of these were confirmed as *C. fetus* in the *nahE* qPCR and 15/54 positive in *parA* and IS*Cfe*1 *C. fetus* subsp. *venerealis* qPCRs if low cycle thresholds are adopted for cut-offs for positives. An *Arcobacter*-like isolate was positive in all molecular assays suggesting incorrect phenotyping and one isolate was negative in the *parA* (qPCR) and *cstA/parA* (duplex PCR) assays. Although the *parA* qPCR methods can detect as few as 4 cells prepared from pure cultures, non-specific primer dimers and non-*C. fetus* species are also detected at Cq scores above 22 cycles. In addition, screening bull prepuce lysates using the *parA* qPCR showed that Cq scores do not correlate with the subsequent isolation of *C. fetus* subsp. *venerealis*. Specificity could only be demonstrated in molecular screening of gDNA from pure cultures. Subspecies can be identified using a combination of *C. fetus* subsp. *venerealis* culture phenotyping, and PCR of pure cultures in *nahE* qPCR and two *C. fetus* subsp. *venerealis* PCRs.

O091

LOD_{50} is dependent on choice of *Campylobacter* strain and food matrix

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Due to abundant growth of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* during enrichment it is hard to isolate and recognize *Campylobacter* colonies on mCCDA. Therefore, in the current revision of the ISO protocol (ISO 10272-1), next to Bolton Broth (BB), Preston broth (PB) is suggested as enrichment broth to inhibit competitive flora in samples where high levels of background flora such as ESBLs are suspected. To validate this revised ISO 10272-1, an Inter Laboratory Study (ILS) was performed where different matrices were used in the enrichment procedures: frozen spinach, minced meat, raw milk and chicken skin. Each matrix was inoculated with a different strain of *C. jejuni* or *C. coli* and the results were expressed as LOD_{50} (Level of Detection) which is the lowest contamination level that can be detected with a probability of 50%. Since different strains were used for each matrix, results of the ILS are possibly influenced by the strains' characteristics. Therefore, in this study we tested the enrichment procedures for spinach, minced meat, milk and chicken skin with each of the strains used in the ILS. The LOD_{50} of all strains tested in spinach was in the range of 0.7 cfu/sample which complies with the ILS-results and which is also the theoretical value. Preliminary results for the other product types, however, show a large variation of LOD_{50} between strains which indicates that the choice of strain will influence the LOD50. In conclusion care should be taken to extrapolate the ILS results to other strains.

Environmental Survival
Tuesday 3rd November

1600-1740 Room C

O092

Enhanced biofilm formation and multi-host transmission evolve from divergent genetic backgrounds in *Campylobacter jejuni*

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Background: Multicellular biofilms are an ancient bacterial adaptation that offers a protective environment for survival in hostile habitats. In microaerophilic organisms like *Campylobacter*, biofilms play a key role in transmission to humans as the bacteria are exposed to atmospheric oxygen concentrations when leaving the reservoir host gut.

Objectives: Genetic determinants of biofilm formation differ between species, but little is known about how strains of the same species achieve the biofilm phenotype with different genetic backgrounds.

Methods: Our approach combines genome-wide association studies with traditional microbiology techniques to investigate the genetic basis of biofilm formation in 102 *Campylobacter jejuni* isolates. We quantified biofilm formation among the isolates and identified hotspots of genetic variation in homologous sequence that correspond to variation in biofilm phenotypes.

Results: Forty-six genes show a statistically robust association including those involved in adhesion, motility, nitrosative and oxidative stress. The genes associated with biofilm formation were different in the host generalist ST-21 and ST-45 clonal complexes, which are frequently isolated from multiple host species and clinical samples, but both had enhanced biofilm formation compared to host specialists.

Conclusions: This suggests the evolution of enhanced biofilm from different genetic backgrounds and a possible role in colonisation of multiple hosts and transmission to humans.

O093

Survival of *Campylobacter jejuni* in soil from the dairy farm environment is dependent upon strain, temperature and presence of other micro-organisms

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University of Liverpool[1]Chulalongkorn University[2]

Background: Currently, little is known about the persistence of *Campylobacter* in soil and the factors that influence its survival.

Objectives: To investigate the ability of two different *C. jejuni* strains to survive in soil microcosms under different experimental conditions.

Methods: Sterilised and non-sterilised soil aliquots, collected from the same location on a dairy farm, were seeded with ~10^4 CFU of *C. jejuni* M1 (ST-137/CC45) or *C. jejuni* 13126 (ST-21/CC21) and incubated at 20°C and 4°C in dark conditions. Enumeration was performed at 0, 1, 2, 3 and 7 days and then at weekly intervals using the Miles and Misra method.

Results: In the sterilised soil, no *C. jejuni* M1 was detected 48 hours post inoculation. However, *C. jejuni* 13126 was still present 3 days after inoculation. In the non-sterilised soil, *Campylobacter* stored at 5°C showed better survival compared with storage at 20°C. No difference was observed between the two strains when stored at 5°C in non-sterilised soil, where both were still present for up 2 weeks. However, at 20°C strain 13126 survived longer than M1.

Conclusions: This study suggests that *Campylobacter* is capable of surviving in soil and this may be important in the maintenance of *Campylobacter* in the environment. This study has also shown that the presence of other micro-organisms in the soil is particularly important for the survival of *Campylobacter* in soil and that *Campylobacter* strains differ in their ability to survive in the same soil environment.

O094

Characterization of biofilm formation in *Campylobacter concisus* and the role of the luxS gene

Huq, M[1]; Elshagmani, E[1]; Allemailem, K[1]; Smooker, P[1]; Istivan, T[1];

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Background: *Campylobacter concisus* is a fastidious, hydrogen-requiring bacterium of the human oral cavity which has been considered as an emerging pathogen. *C. concisus* is known to produce biofilms on glass, stainless steel, and polystyrene plastic.

Objectives: Regulation of biofilm formation has been linked to *luxS* in many oral pathogens. However, no thorough investigation on biofilms has been performed in this species.

Methods: Biofilm formation by 15 clinical and 19 oral *C. concisus* strains was investigated by crystal violet assay. The biofilms were phenotypically characterized by phase contrast and confocal microscopy. PCR amplification of *luxS* was performed and further molecular intervention was applied to knock out this gene in a selected strain.

Results: All tested *C. concisus* strains were capable of producing biofilms in different levels, with the oral strains being the highest. Different morphological state stages were observed by phase contrast microscopy. Completely developed biofilms were detected on day 5 with a mixture of dead and live bacteria. A *luxS* PCR product (309 bp) was amplified from all clinical and oral strains. In addition, a *luxS* mutant was successfully created by inserting a kanamycin cassette within the gene of the highest biofilm producing strain. Molecular studies are currently undertaken to characterise the *luxS* mutant in comparison with the parental strain.

Conclusions: *C. concisus* oral strains are higher biofilm producers than clinical strains. The role of *luxS* in biofilm formation and its expression level in different strains are currently under investigation.

O095

Architecture and matrix characterization of biofilms developed by *Campylobacter jejuni* 11168 and 81-176 under microaerobic and oxygen-enriched conditions

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As the leading cause of alimentary infections in developed countries, *Campylobacter jejuni* has to survive in the environment without permanent loss of viability and virulence, despite its fastidious growth requirements. The mechanisms responsible for the survival of *Campylobacter* remain unknown, but one of the survival strategies might be linked to its ability to adhere to abiotic surfaces and form biofilms. We have compared the biofilm development of two *Campylobacter jejuni* strains cultivated under microaerobic and oxygen-enriched conditions using confocal laser scanning microscopy (CLSM). We observed that the biofilm architecture differed between the two strains from finger-like structure with voids and channels to compact multilayer-like structure. Despite their structuration, the biofilms of both strains contained motile cells. Fluorescent lectin-binding analysis (FLBA) using 73 different lectins revealed strain-specific patterns with only 6 lectins interacting with biofilm matrix of both strains. Moreover, we observed the presence of amyloids in the matrix. Exposure of cells to oxygen-enriched conditions prior to and during biofilm formation enhanced biofilm development, especially in its early stages. We also observed that the regulator CosR plays a role in biofilm maturation as its overexpression in the poorer biofilm-forming strain resulted in enhanced biofilm formation with a high level of structuration. To conclude, biofilm formation of *C. jejuni* is strain-dependent with CosR playing an important role in the maturation of the biofilm structure. The process of biofilm formation is enhanced under oxygen-enriched conditions suggesting that biofilms could be a protective niche facilitating survival of *C. jejuni* in the environment.

O096

C. jejuni M1 survival in nutrient poor water: gene expression profiles during the viable but non-culturable (VBNC) state

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Background: Although *Campylobacter* is able to survive in the natural environment, creating a potential reservoir for livestock and human infection, little is understood about the mechanisms involved.

Objectives: Here we compare different *C. jejuni* strains for their ability to retain culturability in water. Focusing on one strain that rapidly loses culturability but retains viability (*C. jejuni* M1), we analyse bacterial gene expression in order to better understand the mechanisms involved in the survival process.

Methods: Water survival experiments were carried out to compare different *C. jejuni* strains. Exponentially growing cells were inoculated into 100ml sterile distilled water (SDW) for 0, 24 (25°C) or 72hrs (4°C), and Mueller Hinton Broth at 37°C (control). Samples were prepared in triplicate and strand-specific RNA-Seq was performed.
Results: Strain M1 lost culturability in SDW more rapidly compared to other strains. However live-dead staining and RNA-Seq confirmed that the cells were viable. Clear differences in gene expression profiles were observed between control cultures and bacterial populations surviving in water, with further variations between the three SDW samples. RNA-Seq showed that most of the significant upregulation in gene expression (34%) occurred at 0hrs. For 85 genes statistically significant upregulation was detected in all three water samples. Notably, the *luxS* gene was significantly up-regulated in all water samples.

Conclusions: *C. jejuni* strains differ significantly in the levels of culturability they maintain and thereby their survival strategy when coping with adverse conditions. *luxS* gene expression (and therefore quorum sensing) is implicated in the survival of strain M1 in water.

Control strategies for *Campylobacter* (sponsored by the UK Society for Applied Microbiology) Wednesday 4th November

1000-1040

O097

Estimation of on-farm interventions to control *Campylobacter*

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Before making risk management decisions to control Campylobacter prevalence in broiler flocks, it is useful to identify effective interventions. A given risk factor may seem to have a large effect, but in practice interventions related to this risk factor may have only limited effect due to a relative small proportion of the farms that can actually be intervened for the given risk factors. We present a novel tool for risk assessors to obtain such estimates of the effect of interventions before it is implemented at the farms. A statistical method was developed in order to estimate the flock prevalence if an intervention was to be implemented in a given population of broiler farms. The method is anchored in the ideas behind standardized population estimations. In order to obtain a country wise population estimate the predicted prevalence values are multiplied with elements from a reference population. In the present study risk factor estimates from a European study was used and the reference population consisted of data from the risk factor study plus extra data from a large questionnaire survey to improve the representativeness of the reference population. The results showed that some individual interventions gave only a limited reduction in prevalence if the biosecurity was not accounted for. Furthermore, the effect of the interventions differed between countries, depending on current farm management practices and Campylobacter prevalence. The most effective interventions were "building new houses with strict biosecurity for all houses older than 15 years" and "apply drinkers with nipples without cups".

O098

The efficacy of broiler farm boot-dip disinfectants against *Campylobacter jejuni*

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Animal and Plant Health Agency[1]

Campylobacteriosis is a common cause of bacterial enteritis and scientific opinion suggests that 50% to 80% of cases are attributed to the chicken reservoir. Reducing *Campylobacter* colonisation in chicken flocks could help lower the burden of disease to society. Disinfectant boot-dips at access points to broiler flocks are associated with a reduced risk of flock colonisation. The type of disinfectant and how it is used varies and it is known that, after dilution, disinfectants may lose efficacy over time, and may also become inactivated by organic matter. This study aimed to assess the suitability of disinfectants for use in boot-dips as on-farm *Campylobacter* controls. Twelve products that covered the main disinfectant classes used on farms were selected for testing. A disinfectant boot-dip model was created to simulate use through daily loading with poultry litter and to assess the effect of contact time. A test method for disinfectant efficacy against *Campylobacter* (based on BS6734:1986) was developed for this study. All products were effective (>4 log10 *Campylobacter* reduction) after a 30 minute contact time in clean boot-dips; however a shortened contact time or increasing contamination of boot-dips with poultry litter resulted in some products becoming ineffective. After seven days of simulated boot-dip usage (with litter loading), only chlorocresol products remained effective at the one minute contact time. In conclusion, different disinfectant groups vary in the time needed to inactivate *Campylobacter* and resistance to interference by organic matter. These properties should be considered when selecting and managing boot-dips for optimal *Campylobacter* control.

Non-poultry sources of Campylobacter spp.
Wednesday 4th November

1000-1040

O099

Wild birds are second to domestic poultry as a source of environmental water contamination with campylobacters

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Background: Campylobacters are widespread in the environment where they indicate recent faecal contamination, including agricultural run-off and sewage effluent. Environmental water is generally considered as a 'sink' collecting campylobacters from different amplifying (animal) hosts, whose relative contributions are largely unknown, though wildlife is believed to play a major role. The devastating avian influenza outbreak hitting the Netherlands in 2003 showed that the massive poultry culling was associated with a 44-50% decrease in human campylobacteriosis cases in the culling areas (Emerg. Infect. Dis. 2012;18:466-8), suggesting a major role of environment-mediated spread of poultry-borne campylobacters.

Objectives: To quantify the contribution of domestic poultry relative to wild birds, ruminants, and pigs to the environmental water contamination with campylobacters.

Methods: Multilocus sequence typing (MLST) was performed on 450 *Campylobacter jejuni/coli* isolates from environmental water, including rivers, ponds, recreational water, and wastewater treatment plants in Luxembourg and the Netherlands. MLST-typed isolates from poultry (n=694), ruminants (n=273), wild birds (n=142), and pigs (n=77) were obtained from different research/surveillance activities on farms, slaughterhouses, and retail. Data were collected during 2000-2012. The asymmetric island model for source attribution was used to attribute the environmental water isolates to the animal sources.

Results: Most environmental water *Campylobacter* isolates were attributed to poultry (48.5%, 95%CI 28.9-70.6%), followed by wild birds (30.9%, 16.7-53.7%), ruminants (11.5%, 6.1-22.6%), and pigs (9.1%, 0.6-26.4%).

Conclusions: Wild birds are the second most important source of environmental water contamination by campylobacters, after poultry. Human campylobacteriosis acquired from environmental water is therefore more likely to originate from poultry than wild birds.

O100

Do raccoons play a significant role in the transmission dynamics of *Campylobacter jejuni* at the interface of wild animal populations and livestock?

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Wildlife is increasingly recognized as a potential reservoir of *Campylobacter*. The role of wild animals such as raccoons, which live at the interface of rural, urban, and wild environments, in *Campylobacter* transmission or to the epidemiology of campylobacteriosis is presently unknown. We explored the prevalence and genotypic diversity of *Campylobacter* in raccoons to determine if: they are a significant reservoir of *Campylobacter*; they harbor host-restricted subtypes or share subtypes common with livestock; and if subtypes have been previously associated with human clinical cases. From 2011 - 2013, fecal swabs (n=1,214) were collected from raccoons trapped near Guelph, Ontario, Canada for *Campylobacter* isolation. Isolates (n=1,630) were subjected to high-resolution subtyping using Comparative Genomic Fingerprinting (CGF40) and compared to a national CGF database containing over 18,000 *Campylobacter* isolates from across Canada. *Campylobacter* prevalence in raccoons was 43.8% and 98.7% of isolates collected were *C. jejuni*. Fifty-seven percent of raccoon isolates had CGF subtypes that have only been observed in raccoons. However, approximately 17% of raccoon isolates are from subtypes that are associated with agricultural environments, and these are also significantly more likely to have human clinical associations. Our results show that raccoons represent a significant reservoir of *C. jejuni* and, although they primarily harbor a number of raccoon-restricted subtypes, they also carry agriculture-associated genotypes that have been observed among human clinical cases. This suggests that raccoons may act as vectors in the transmission of *C. jejuni* subtypes that pose a risk to human health at the interface of rural, urban, and wild environments.

1110-1230

O101

Chlorination in immersion chillers

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MPI[1] Tegel Foods Ltd[2]

To date, biosecurity measures at farm level and hygiene interventions during primary processing are not sufficient to produce poultry meat that is *Campylobacter* free. Consequently, a final intervention at the end of primary processing offers another opportunity to minimise carcass contamination. The trial was designed to separately quantify the decontamination effects of water and chlorine for poultry subject to immersion chilling under commercial conditions. The trial was carried out at a commercial poultry slaughterhouse that had two parallel immersion chillers on the same dressing chain with both being able to be operated at different chlorination levels. Every 20 seconds carcasses from the same flock were diverted to one and then to the other chiller. The chillers were operated with (a) municipal water supply (MWS) without additional chlorination, (b) 50 ppm chlorination (c) 90 ppm at the chiller water inlets. Each day 30 carcass rinsate samples from one flock were taken before immersion chilling and 60 similar samples were taken after immersion chilling (30 for each chiller). In total 15 flocks were sampled. The chillers with either 50 ppm or 90 ppm Cl_2 performed better than the chillers with MWS only. The performance of chillers with 50 ppm Cl_2 was similar to chillers with 90 ppm Cl_2 with mean Campylobacter reductions varying between 1.6 log10 - 2.4 log10 and 1.8 log10 - 2.4 log10 CFU per rinsate respectively when the same flocks were sampled.

O102

Correlation of slaughterhouses´ food safety management system and Campylobacter contamination on produced broiler carcasses

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Background: Broiler meat is identified as the main source of human campylobacteriosis. It can be assumed that slaughterhouses with better performing food safety management system (FSMS) are able to provide less contaminated broiler carcasses.

Objectives: To compare the performance of a FSMS with Campylobacter contamination on carcasses in broiler slaughterhouses.

Methods: The study was conducted in 23 European slaughterhouses. A diagnostic tool was used in an interview with the quality assurance personnel of each slaughterhouse to measure the performance of the implemented FSMSs (consisting out of good hygienic, manufacturing practices and HACCP). Cluster analysis of the individual scores was dividing the slaughterhouses into groups according to the level (basic, generic or advanced) of the implemented control and assurance activities to prevent Campylobacter contamination. Campylobacter enumeration data on post-chill carcasses were collected during other, independent studies.

Results: Two major clusters (I and II) were discriminated. Slaughterhouses from cluster I were operating in less risky context (less changes in their production process, lower workforce flow and stronger power in their supplier relationship). Cluster I had also more advanced control activities (i.e. by better raw material control and effectiveness of washing and chilling). Differences in the assurance activity demonstrated that slaughterhouses from cluster II have less systematic information on how their personnel are following food safety requirements. Despite the more advanced FSMS present in cluster I, no significant differences in mean Campylobacter levels on post-chill carcasses were observed compared to cluster II.

Conclusions: The level of implemented FSMS did not influence the Campylobacter concentrations found on carcasses post-chill.

O103

Efficacy of biosecurity measures in Spanish broiler farms to prevent thermophilic Campylobacter colonization

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Chicken products are considered the main source of campylobacteriosis, the most frequent foodborne bacterial gastroenteritis in the European Union. Hence, a reduction in *Campylobacter* prevalence of broiler flocks is a priority in the UE. Currently, the only effective measure available for *Campylobacter* control in poultry farms is a proper implementation of biosecurity at farm and house level. Thus, a field study in 18 Spanish broiler farms during 12 broiler rearing cycles (August 2013 to November 2015) is currently ongoing to determine the efficacy of increasing farm biosecurity measures. In 12 of these farms, improved biosecurity measures have been implemented and in 5 of those, additionally fly screens were mounted recently, once a sufficient level of biosecurity was reached. The remaining 7 farms are control farms, where no changes have been made. Weekly boot socks samplings are performed in all farms, and *Campylobacter* detection is performed by PCR. Despite to date only 9 flock cycles per farm have been monitored, the results are so far promising. Results show a significant reduction of the number of *Campylobacter*-positive samples in farms with improved biosecurity compared to the control farms. Moreover, the reduction to date is more marked in the group of farms with fly screens, which are expected to provide most influence during the forthcoming summer months, when the insect population is abundant. Updated results will be presented at the congress.

O104

The effect of the cutting process on Campylobacter contamination levels in broiler meat cut

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Background: Quantitative data concerning *Campylobacter* contamination during the broiler slaughter process become increasingly available. However, the occurrence of cross-contamination and the actual *Campylobacter* counts on various types of broiler meat cuts is not well known whereas these broiler meat cuts are increasingly bought by European consumers.

Objectives: Present study aimed to quantitatively assess the effect of cutting process on the *Campylobacter* contamination of broiler meat cuts.

Methods:*:* Two Belgian broiler cutting plants were visited three times each. During every visit, the first four successive batches of the processing day were sampled. From the first batch, six carcasses were collected before the cutting process had started. From each of the following three batches (batch 2, 3 and 4), 9 carcasses, wings, breast caps, thighs and fillets were collected at 1, 15 and 30 min from the start of the process of each batch. *Campylobacter* was quantified by the direct plating method.

Results: *Campylobacter* counts on meat cuts increased in comparison to the whole carcasses (with fillets being an exception). Additionally, the transfer of *Campylobacter* counts on meat cuts from a positive to a negative batch occurred and lasted longer in comparison to the Campylobacter transmission observed on whole carcasses during the prior slaughter process.

Conclusions: The cutting process plays a significant role in the distribution of *Campylobacter* counts on broiler meat cuts and it enhances the transfer of *Campylobacter* contamination between successively processed batches. Presented data describe the numbers of *Campylobacter* that will impact cross-contamination to other foods when preparing broiler meat cuts at consumers' home.

O105

Similar control points for Campylobacter and ESBL/AmpC producing *E. coli* during broiler processing

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We determined steps during broiler processing where the control of both Campylobacter and ESBL/AmpC producing *E. coli* concentrations on carcasses would be most effective. Concentrations in whole carcass rinse samples were investigated in 21 Campylobacter positive batches in two slaughterhouses, after bleeding, scalding, defeathering, evisceration and chilling. Campylobacter was cultured on CampyFood Agar and ESBL/AmpC producing *E. coli* on MacConkey agar with 1 mg/L cefotaxime. The concentrations after bleeding varied between batches in each slaughterhouse of both Campylobacter (from 2 to 6 log10 CFU/ml) and ESBL/AmpC producing *E. coli* (from 2 to 5 log10 CFU/ml), but were comparable between slaughterhouses. The concentrations followed different patterns along the processing line in both slaughterhouses. Scalding, evisceration and chilling performed better in Slaughterhouse 2, and defeathering in Slaughterhouse 1. The overall reduction was higher in Slaughterhouse 2 by 0.6 log10 for Campylobacter and by 0.1 log10 for ESBL/AmpC producing *E. coli*. Processing steps affected Campylobacter and ESBL/AmpC producing *E. coli* concentrations in a similar way in each slaughterhouse, except for defeathering. Campylobacter concentrations increased after defeathering in Slaughterhouse 2 by 0.4 log10 and did not change in Slaughterhouse 1. ESBL/AmpC producing *E. coli* concentrations decreased after defeathering by 0.74 log10 in Slaughterhouse 1 and did not change in Slaughterhouse 2. Interventions at slaughter could include reducing the bacterial levels upon entrance to the processing and improvement of the hygienic performance of scalding, defeathering and evisceration. Differences in the impact of processing steps on concentrations between slaughterhouses suggest that such improvements are attainable and can have a similar effect on multiple hazardous organisms.

O106

Occurrence of *Campylobacter* spp. In shellfish-harvesting areas and their catchments in France

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Background: The microbiological quality of coastal environments can be affected by faecal pollution from urban and agricultural sources.

Objectives: The aims of this 2-year study were to evaluate the presence and diversity of *Campylobacter* spp. in three shellfish-harvesting areas and to compare methods for the isolation of *Campylobacter* spp. from water.

Methods: The sites selected are located on the North-West of France and characterized by intensive livestock farming (cattle, sheep, pigs and/or poultry). Shellfish (n=237), water (n=227) and sediment (n=36) samples were collected monthly from February 2013 to January 2015. The detection frequency of *Campylobacter* spp. was investigated using real-time PCR, the EN.ISO.10272 method and a passive-filtration method (PFM, water samples only). Genotyping of a selection of isolates (n=266) was done by *porA* sequencing.

Results: *Campylobacter* genes were detected in 80.7% water, 47.9% shellfish and 66.6% sediment samples. Among the 1,300 isolates, *C. jejuni* and *C. coli* were most frequently isolated in water whereas *C. lari* was predominant in shellfish. Diversity comparison of *C. jejuni* and *C. coli* from 28 river water samples showed that while equivalent numbers (133 isolates for each technique) of strains and genotypes were retrieved (24 and 26 different *porA* alleles for PFM and ISO, respectively), only 45.8% of the genotypes were shared using both techniques.

Conclusions: Both culture-based detection methods are complementary in detecting an utmost genetic diversity of campylobacters in contaminated surface waters. Overall, the highly frequent presence of *Campylobacter* spp. in shellfish and waters was evidenced, providing important information for microbial risk assessment.

O107

Sources of contamination and dynamic of Campylobacter transmission within a pig farm

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Campylobacter, a major cause of food-borne human infection, is commonly carried in the intestinal tract of livestock animals without clinical signs. Pigs are known to frequently exhibit high counts of *Campylobacter* in their faeces. Targeted control of food-borne pathogens usually requires the identification of contamination sources and major ways of transmission. In terms of risk assessment, the ability to understand the sources of contamination and the dynamic of *Campylobacter* transmission is essential. This work aimed at describing the different sources of contamination in a pig farm and investigating coexistence of different strains in pigs and their environment, in order to understand *Campylobacter* infection dynamics within a conventional pig farm. Quantification and identification of *Campylobacter* in faeces from 30 piglets and their corresponding sows along one production cycle was followed in two farrow-to-finish farms. At each sampling time, environmental and feed samples were also considered. PCR-RFLP method was used to genotype *Campylobacter* isolates to describe the circulation of *Campylobacter* between animals and from environment to animals. *Campylobacter*, mainly *C. coli*, was highly prevalent for sows and their piglets. However, variable counts of *Campylobacter* in different faecal samples for a given animal were observed during their lifetime. Intermittent excretion or succession of elimination/contamination phases could be suggested. The genotyping of the strains isolated in this study helped us to describe the circulation of *Campylobacter* within a conventional pig farm underlining the roles of (i) the sows as a primary source and (ii) the environment for indirect contamination.

O108

Use of MLPA-based binary typing (MBiT) to evaluate source and potential health risk of campylobacter isolated from river water.

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Campylobacters are frequently isolated from freshwater sources in New Zealand with some studies estimating that 5% or more of notified campylobacteriosis cases in New Zealand could be attributable to water contact recreation. The importance of genotyping of campylobacter from rivers has been demonstrated using techniques such as pulsed field gel electrophoresis (PFGE) and multi locus sequence typing (MLST). These studies have revealed that many of the campylobacters found in rivers are of types not found in farm animals or human cases, with wildfowl specific strains often dominating. More routine analysis of campylobacter from water has been limited by the cost of MLST and PFGE, limited throughput, and often lengthy processing time. In this paper we evaluate the application of Multiplex ligation-dependent probe amplification (MLPA) based Binary Typing (MBiT) to analyse campylobacter isolates from a range of rivers in New Zealand. MBiT allows rapid analysis (less than 24 hours), in high throughput format (100 isolates a day by single technician), at a consumable cost of less than US$15. The output is also easily interpretable. Key criteria assessed are typability, diversity of types, comparison with isolates from known sources, and we provide recommendations for implementation of MBiT within a water monitoring context, including the vexing issue of how many isolates to analyse.

O109

Using enteric *Campylobacter* spp. to explore host-microbe dynamics in isolated wildlife populations

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Endangered wildlife populations maintained in reserves present a unique opportunity to investigate host-microbe relationships in a controlled semi-natural environment where diversity, abundance and the movement of species are restricted. The aim of this study was to investigate the prevalence and molecular differentiation of enteric commensal *Campylobacter* carried by fragmented populations of the endangered New Zealand flightless takahe (*Porphyrio hochstetteri*). We explored the effects of geographic isolation, translocation and the presence of reservoir hosts using molecular epidemiological techniques including whole genome sequencing, ribosomal multi-locus sequence typing (rMLST) of *Campylobacter* isolates. Results suggest that intensive conservation management of takahe in different environmental settings has influenced the carriage of *Campylobacter jejuni* and *Campylobacter coli*. A newly discovered rail-associated *Campylobacter* species nova 1 was prevalent in all takahe populations. However, more discriminatory whole genome analysis of isolates detected a significant biogeographic variation in *C.* sp. *nova 1* genotypes. Possible explanations for the observed pattern include the spatial expansion and isolation of hosts resulting in reduced gene flow of *Campylobacter* and allopatric speciation, and the presence of heterogeneous environmental attributes. An assessment of *Campylobacter* carriage in sympatric vertebrate hosts within an island ecosystem indicated cross-species transmission of *Campylobacter* was most likely to occur between closely related host species and could be a factor contributing to the maintenance and geographic distribution of *Campylobacter* in takahe. Results suggest subtle but important differences in host-microbe relationships may occur as a consequence of isolation and conservation management, which has important implications when relocating wildlife populations.

O110

Genomic comparisons and pathogenic potential of Campylobacter isolates found in American crows around Davis, California

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Campylobacter jejuni is the leading cause of gastroenteritis in humans worldwide with recent studies indicating wild birds as zoonotic vectors. American crows, abundant in urban, suburban, and agricultural settings, carry *C. jejuni* as a commensal organism. Studies demonstrating that the crow is a *Campylobacter* zoonotic reservoir are lacking, and no studies have investigated *Campylobacter* genomics for commensal organisms. To examine pathogenic potential, zoonotic exchange and genomic phylogeny, we sequenced 92 *Campylobacter* genomes (79 *C. jejuni*) from crow and non-human primate origin from the Sacramento Valley, CA and compared them to outbreak strains. Many genomes displayed high similarity to isolates implicated in human disease, suggesting these isolates as potential pathogens of public health importance. Tetracycline resistance gene tetO was present in 19.5% of the isolates, and 13 isolates (7 *C. jejuni*, 3 *C. coli*, 3 *C. lari*) contained mutations in gyrA indicative of Fluoroquinolone resistance. Arsenic resistance gene (arsB) was observed in 97% of the isolates, CTD toxin genes were present in 97% of isolates, 100% contained invasion and adherence genes, while 34% of genomes contained a Type IV SS system. Although resistance and virulence genes were broadly distributed throughout these isolates, specific genotypes were associated with individual hosts and a sub-set of isolates were associated with several hosts (i.e. crows, primates, sheep, humans), suggesting that these genotypes may be from zoonotic exchange. In this study we utilized whole genome sequencing (WGS) of *Campylobacter* isolates from multiple hosts in the same geographic area to show antibiotic resistance, virulence and zoonotic potential.

Roundtable discussion on poultry control - international perspective
Wednesday 4th November

1415-1530

O111

The Campylobacter Risk Management Strategy in New Zealand

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A very high rate of foodborne campylobacteriosis was evident in New Zealand in 2006 and attribution studies estimated that more than 50% of human cases were due to the preparation and consumption of poultry meat. This led to the implementation of a national risk management strategy for *Campylobacter* to significantly reduce what was judged to be an unacceptably high human illness burden. The primary focus was on improving the food safety control systems for slaughter and dressing of broiler chickens in order to get a reduction in *Campylobacter* levels on carcasses at the end of primary processing.Improvements in broiler chicken processing, together with improvements in hygienic practice at other steps in the farm-to-plate food chain has resulted in the following reductions in relation to Campylobacter: 49.7% (2007-8) to 27.3% (2014-15) positive carcass rinsates, and 22.4% (2007-8) to 3.9% (2014-15) carcass rinsates greater than 3.78 log10 cfu. Human campylobacteriosis cases in New Zealand have reduced by more than 50% since 2006 (15,873) compared to 2014 (6,776). Source attribution studies from 2005 – 2014 show that the proportion of notified cases of human campylobacteriosis in New Zealand that is attributable to consumption of poultry is changing and a dynamic risk management programme needs to take into account other exposure pathways when developing mitigation measures. These include the increasing proportion of overall notified cases that are attributable to Campylobacter strains carried by ruminants, as well as potential changes in exposure due to the increased consumption of raw milk in New Zealand.

Molecular Epidemiology -2
Wednesday 4th November

1415-1530

O112

Comparative genomics and molecular epidemiology of *Campylobacter coli*

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Background: *Campylobacter coli* causes ~10% of campylobacteriosis cases. Comparative genomics of 25 *C. coli* genome sequences (Sheppard et, Mol Ecol 2013) have shown that the *C. coli* genome is distinct from the *C. jejuni* genome, and that within *C. coli*, distinct agricultural (Clade 1) and riparian lineages (Clades 2, 3) exist.

Objective: To map the genomic diversity in 466 newly sequenced *C. coli* genomes, and to identify markers distinguishing agricultural and environmental isolates.

Methods: *C. coli* isolates were retrieved from strain collections of UK isolates, and sequenced on an Illumina HiSeq 2500 with 100 nt reads. Genomes were assembled with SpaDES, annotated with Prokka and analysed for core and accessory genome.

Results: Phylogenetic clustering of the *C. coli* genomes showed a very distinct pattern, with clearly separated lineages. Comparison with previously classified C. coli genomes showed that four of these represented the agricultural Clade 1 (including ST-1150, n=362), Clade 2 (n=36) and Clade 3 (n=56), and an additional Clade 1 lineage branches off between Clades 2/3 and ST-828 genomes. Genome sizes varied between the lineages, with Clade 3 genomes being smaller (1.59 ± 0.35 Mbp) and Clade 2 (1.79 ± 0.67 Mbp) genomes larger than Clade 1 genomes (1.71 ± 0.68 Mbp). Lineage-specific genes are abundant, and include genes involved in metabolism such as a putative cresol dehydrogenase, metal acquisition systems and putative colonisation factors.

Conclusions: *C. coli* has a very distinct population structure, with clear separation of lineages. The genome sequences determined here will allow future studies into the biological differences between these lineages.

O113

MLST types and antimicrobial resistance of *Campylobacter jejuni* isolates from multiply sources and children in Lithuania

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Background: Campylobacteriosis is a common gastrointestinal infection in humans in the EU. Increase in resistance of Campylobacter to antimicrobials used in clinical practices is observed, indicating a possible effect on public health.

Objectives: The aim of study was to investigate MLST genotypes in association to antimicrobial resistance of *Campylobacter jejuni* strains isolated from various sources including children campylobacteriosis cases.

Methods: In total 379 *C. jejuni* isolates were genotyped with MLST. Antimicrobial resistance was tested with five antimicrobial agents (tetracycline, ciprofloxacin, erythromycin, gentamicin, ceftriaxone) using the agar dilution method.

Results: Altogether 161 *C. jejuni* distinct sequence types (STs) were identified and assigned to 28 clonal complexes (CCs). In total 74 STs were previously unreported at PubMLST database. ST-5 and ST-50 were dominant among children campylobacteriosis cases. ST-21 was dominant among *C. jejuni* isolated from dairy cattle. ST-464 and ST-6410 were dominant in broiler products. All *C. jejuni* from broiler products and the majority of strains from children were resistant to ciprofloxacin. *C. jejuni* isolated from broiler products were sensitive to erythromycin. Meanwhile, all *C. jejuni* from children were sensitive to gentamicin. Preliminary results showed that almost 50% of tested *C. jejuni* strains from broiler products were resistant to tetracycline. Certain bacteria strains of ST-5 and ST-658 isolated from children were confirmed as multiresistant.

Conclusions: Our preliminary data shows some newly described dominant *C. jejuni* MLST genotypes as ST-6410 in broiler products and a high occurrence of a new STs among wild birds isolates. Ongoing examination of antimicrobial resistance of *C. jejuni* strains reveals a very high resistance to some of the 5 tested antibiotics. Acknowledgements This research was funded by a grant (No.MIP-041/2015) from the Research Council of Lithuania.

O114

Exploring genetic diversity of the porA gene of *Campylobacter jejuni* and *Campylobacter coli* isolated from the interior of chicken livers sold at UK retail

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Background *Campylobacter* species are the most commonly reported bacterial food-borne pathogen worldwide. A number of *Campylobacter* outbreaks have been associated with the consumption of undercooked chicken liver pate. However, very little is known about diversity of *Campylobacter* strains found in chicken livers, with most studies focusing on individual outbreaks. Objectives To examine the genetic diversity of the porA gene of *Campylobacter* strains isolated from the interior of chicken livers sold in the UK. Methods Prepacked chicken livers were sourced from two UK retailers with 32 livers sampled from each. Livers were surface sterilised before stomaching followed by enrichment and plating onto mCCDA. Results The majority of livers (58/64) were culture positive for *Campylobacter* and up to 4 colonies were selected from each liver and subjected to porA gene typing. In total, 13 different porA alleles were found amongst the 79 *C. jejuni* (10 alleles) and 38 *C. coli* isolates (3 alleles). The most common alleles among the *C. jejuni* isolates were 1, 6, 73 and 1072 representing 13.9%, 17.7%, 21.5% and 16.5% of the isolates, respectively. The most common allele amongst the *C. coli* isolates was 992 representing 71.0% of the isolates. Conclusions The prevalence of *Campylobacter* and diversity of the porA alleles found within the chicken livers is surprising given the surface sterilisation step. Further work remains to elucidate whether the chicken livers are becoming contaminated with multiple *Campylobacter* strains during slaughter and processing, or whether such strains represent those capable of extra-intestinal spread within the chicken pre-slaughter.

O115

Distribution of *Campylobacter jejuni* sequence types in Polish chicken production

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Background: Multilocus sequence typing (MLST) data have been used to estimate the importance of different *C. jejuni* reservoirs and to analyze transmission routes of human campylobacteriosis. The distribution of *C. jejuni* among broiler production systems may be affected by their structure and intensity. In Poland, they are dominated by small producers located all over the country.

Objectives: To better understand the diversity of *C. jejuni* population in Poland by investigation of sequence type (ST), clonal complex (CC) and antimicrobial resistance (AMR) distribution in broilers.

Methods: During a two-year study period 15 broiler farms located over Poland were sampled for *Campylobacter* at the slaughter level. Altogether, 72 *C. jejuni* from caeca and 61 isolates from the corresponding carcasses were identified for MLST and AMR analyses. BioNumerics®7.5 was used to assemble the sequence, obtain the allele identifiers and sequence types by connection with pubMLST and also for phylogenetic analysis.

Results: A total of 133 isolates were assigned into 40 STs types of which three were novel. 27 out of 40 STs covered 89 (66.9%) strains and were clustered into 13 CCs. The remaining 13 STs were unassigned to any CCs. The most predominate were two STs types: 257 (20 isolates) and 6411 (22 isolates). The CC257 was the most frequent among assigned strains and was joined with resistance to ciprofloxacin and tetracycline.

Conclusions: The Polish *C. jejuni* population in chickens was overall genetically diverse, but certain predominant STs were found which were also identified among poultry and humans in other countries.

Roundtable discussion on control - international perspective part 2: Non-poultry sources

Wednesday 4th November

1650-1815

O116

Assessing risk factors of sporadic Campylobacter infections: A state-wide case-control study in Arizona

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Background: Case-control studies of sporadic Campylobacter infections have predominately been conducted among non-Hispanic populations. In Arizona, rates of campylobacteriosis have been higher than the national average for the past decade, with particularly high rates among Hispanics.

Objectives: In 2010, health departments and a state University collaborated to conduct a statewide case-control study aimed to determine whether risk factors differ in a region of the United States with a more ethnically diverse case population.

Methods: Non-outbreak cases were identified through routine surveillance. Controls were recruited matched by age group, gender and residence. Ultimately, due to low matching efficiency, random effects logistic regression was used to build a multivariate model. Initial analyses found both Hispanic ethnicity and travel to be an increased risk of disease. To determine if Hispanic was a surrogate for travel or an independent risk factor, a joint variable was created.

Results: The final multivariate model found the statistically significant risk factors to be: eating cantaloupe (OR=7.64), handling raw poultry (OR=4.88) and eating queso fresco (OR=7.11). In addition, compared to Non-Hispanic/Non-Travelers, the highest risk group were Hispanic/Non-Travelers (OR=7.27), and Hispanic/Travelers (OR=5.87-not significant).

Conclusions: Results of this study suggest Hispanics have higher odds of disease, likely due to differential exposures. In addition to common risk factors, consumption of cantaloupe was identified as a significant risk factor. The results from this study will help to inform public health officials of the varying risk factors for Campylobacter in this region.

O117

Control of *Campylobacter jejuni* pathogenesis and metabolic characteristics by the post-transcriptional regulator CsrA

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Background: *Campylobacter jejuni* has a relatively small number of regulatory proteins. For several years, we have characterized the post-transcriptional regulator CsrA. CsrA is an mRNA-binding protein that typically binds at or near the ribosome binding site, modulating the translation and/or stability of target mRNAs.

Objectives: Our aim was to elucidate the regulatory mechanisms by which CsrA modulates *C. jejuni* metabolic and pathogenesis-related phenotypes, and assess the role of the putative flagellar chaperone FliW in modulating CsrA activity. Methods. We used mutation and phenotype analysis, proteomics, SELEX, pulldowns, and protein cross-linking to define the genetics and biochemistry of the CsrA/FliW regulatory system.

Results: Analysis of a *C. jejuni csrA* mutant showed that this regulator affected many pathogenesis characteristics, including motility/chemotaxis, oxidative stress resistance, biofilm formation, host cell interactions, and animal colonization. Proteome analysis of *csrA* and *fliW* mutants suggested growth-phase-dependent, coordinate regulation of >100 proteins, with predicted functions in central metabolism and the pathogenesis-related phenotypes listed above. We defined the CsrA binding site using SELEX and demonstrated specific binding of CsrA to putative target mRNAs. Biochemical analysis showed specific interactions of FliW with both FlaA flagellin and CsrA, and defined the FliW binding site near the C-terminus of CsrA. It is likely that FliW binding disrupts the second RNA binding domain of CsrA, thereby modulating its regulatory activity.

Conclusions: In total, these data suggest a model by which CsrA regulation of metabolic and pathogenesis-related targets is linked to growth phase and flagellar synthesis by means of a partner switching mechanism requiring FliW.

O118

New insights into the biogenesis and function of the electron transport chains in *Campylobacter jejuni*

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Background and Objectives: *Campylobacter jejuni* elaborates surprisingly complex electron transport chains, which are essential for growth and host colonisation. Our recent work has revealed a number of novel features in their biogenesis and function, which has implications for understanding host-pathogen interactions and the identification of antimicrobial targets.

Methods: and Results: We have recently identified several *c*-type cytochromes that transfer electrons to the high-affinity *cb*-type cytochrome c oxidase. Remarkably, deletion or site-directed mutagenesis of just one of these, CccA (cC1153) results in an almost complete loss of all other c-type cytochromes, giving rise to a highly pleiotropic respiratory phenotype. *C. jejuni* employs a membrane-bound cytochrome c synthase, CcsBA to transport and ligate haem to periplasmic apocytochromes, which must possess a reduced CXXCH haem-binding motif. In other bacteria, the cysteines in this motif are reduced by periplasmic thioredoxins, but in *C. jejuni* the thiol reductase DsbD and the thioredoxins Cj1106 and Cj1207 were found not to be essential for cytochromes c biogenesis, unlike CccA. Significantly, cytochrome c production in the *cccA* mutant could be rescued by growth with reducing agents and we showed that this mutant overproduces reactive oxygen species. We propose that in addition to being a key electron transfer protein, CccA functions to maintain apocytochrome cysteine thiols in a reduced state for haem attachment.

Conclusions: Our results have revealed an unprecedented biogenesis function for the most abundant *c*-type cytochrome in *C. jejuni*. CccA is present in all sequenced *C. jejuni* strains and might prove a therapeutic target.

O119

Glucose metabolism of Campylobacter

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Unlike most enteric bacteria, Campylobacter is considered unable to metabolize glucose due to lack of key glycolytic enzymes. However, the Entner Doudoroff pathway (ED), providing bypass of this glycolysis deficiency, is encoded by *C. jejuni* subsp. *doylei* 269.97 thus suggesting potential glucose metabolism of this strain, but this has never been investigated. In this study, the ED pathway was searched for in Campylobacter isolates and in the *C. jejuni/coli* PubMLST database, which revealed more than 60 isolates of primarily *C. coli* carrying the ED pathway. Using a phenotypic miocroarray, metabolism of glucose and glucose di-/tri-saccharides was illustrated for two ED positive strains (*C. jejuni* subsp. *doylei* 269.97 and *C. coli* B13117), but not for the ED negative *C. jejuni* NCTC11168. Glucose was not observed to affect the exponential growth rate of investigated ED positive isolates, but glucose caused significantly increased late stationary survival of the ED positive isolates. The prolonged survival correlated with substantial biofilm formation of *C. coli* B13117. Metabolomics analysis of two ED positive strains showed glucose to trigger a huge change in composition of both intracellular and extracellular metabolites. However, the metabolite alterations stimulated by glucose were quite different for the two strains, as one strain displayed higher occurrence of metabolites from the primary metabolism via the tricarboxylic acid cycle, while a distinct metabolic profile was observed for the other strain. In conclusion, this study shows the first evidence of glucose metabolism in Campylobacter, and indicate that individual ED positive isolates respond differently to glucose.

O120

Understanding the role of acetyl-lysine post-translational modifications in *Campylobacter jejuni*

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Background: Post-translational modifications (PTMs) of proteins are being increasingly reported in bacteria. Recently it has become apparent that reversible acetylation on specific lysine residues within proteins is a widespread mechanism in bacteria for control of the activity of diverse target enzymes. Research in our group has revealed some major features of the biochemistry and metabolism of *C. jejuni*. Some of these may be exploited to inhibit the colonisation of poultry and thus reduce food-chain contamination. One important aspect of metabolism that has not been investigated previously in *C. jejuni* is the control of protein activity by PTMs.

Objectives: Here we aimed to uncover the importance of protein lysine-acetylation in the global control of enzyme activity by using molecular biological and proteomic techniques.

Methods and Results: We have recently identified a novel gene in *C. jejuni* that encodes a candidate lysine deacetylase (a Sirtuin homologue). We have also found that a key metabolic enzyme, acetyl-CoA synthetase, has a conserved lysine residue that in other bacteria is known to be acetylated. Western Blotting of cell free extracts from wild-type and a deacetylase mutant with an anti-acetyl lysine antibody detected the presence of acetylated proteins. Subsequent whole cell proteomic analysis by Mass Spectrometry of wild-type compared to the deacetylase mutant revealed significant alterations in the *C. jejuni* acetylome.

Conclusions: These findings will allow us to further investigate this novel PTM system and ultimately help in understanding the role of acetylation on controlling metabolic enzyme activity and other functions within *C. jejuni*.

O121

Regulation of ggt gene expression in *Campylobacter jejuni*

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Campylobacter jejuni utilizes amino acids as carbon and energy source. Some strains of *C. jejuni* possess the enzyme gamma-glutamyltranspeptidase (GGT). This periplasmic enzyme enables growth on glutathione and glutamine and contributes to persistent colonization of the avian gut. We have previously shown that the RacRS two-component system is involved in cytoplasmic glutamate anabolism by upregulating the gltBD genes. In the present study we investigated whether the RacRS system also regulates the periplasmic glutamate anabolism by regulating the ggt gene. The combination of targeted mutagenesis, transcript analysis, growth characteristics and enzyme assays reveal that the RacRS system enhances the GGT enzyme activity by directly upregulating the transcription of ggt under low oxygen conditions (0.3%) in the presence of alternative electron acceptors. EMSA and luciferase reporter assays with variable-sized ggt promoter DNA fragments showed that RacR binds to an upstream region of the ggt promoter. In the presence of oxygen (5%), ggt expression was not regulated by RacRS. Expression of ggt peaked under these conditions around end-log phase and GGT activity is highest in stationary phase. GGT activity was repressed after addition of excess glutamine or glutamine catabolic metabolites, but not by glycolytic metabolites like serine or pyruvate. In rich medium *C. jejuni* was capable of utilizing glutamine independent of GGT, in contrast to medium with glutamine as sole carbon source. In conclusion, we show that the *C. jejuni* GGT activity is dependent on multiple factors and one of them is the RacRS two-component system.

Control strategies for Campylobacter - 2
Thursday 5th November

0910-1035

O122

Targeting *Campylobacter* in the United Kingdom: determining the level of Campylobacter species contamination in fresh whole raw chickens at retail sale

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Background: *Campylobacter*, especially *C. jejuni* and *C. coli*, are the main cause of human bacterial gastroenteritis in the developed world causing over half a million cases and 80,000 General Practitioner consultations annually in the UK. Source-attribution studies, outbreak investigations and case-control reports all incriminate chicken meat as the key food-borne transmission route for *Campylobacter* infection. In 2008, the UK Food Standards Agency identified that 27% of chicken neck skin samples from post-chill chickens had >1000 cfu/g *Campylobacter* present and set a target to reduce this to 10% by 2015.

Objective: Determine levels of *Campylobacter* spp. in neck skin samples from fresh whole raw chickens at retail sale.

Methods: Fresh whole raw chickens (n=4011) were purchased from retail sale based on market share data. Levels of *Campylobacter* were enumerated from neck skin as outlined in EC ISO/TS 10272-2 (2006) with a detection limit of 10 cfu/g. At sample collection a range of data was collected, including weight and "Use By" date.

Results: Overall, 73% of chickens tested were identified as having ≥10 cfu/g *Campylobacter* present. This included 19.4% of samples with levels >1000 cfu/g. Speciation identified that *C. jejuni* was the predominant organism, although *C. coli* was also isolated. Significant seasonal variation in levels was also observed with more contaminated chickens during the summer months.

Conclusions: The UK has not met its 2015 target and this study provides strong evidence that consumption of chicken remains a risk factor in the acquisition of campylobacteriosis.

O123

Identification of batch and process related characteristics towards a better control of Campylobacter contamination levels on broiler carcasses

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Background: Broiler carcasses contaminated with high (> 1000 cfu/g) *Campylobacter* counts play a significant role in the public health risk of campylobacteriosis. Alternatively to physical or chemical decontamination, optimization of the technical and hygiene-related factors at different points during slaughter might reduce *Campylobacter* counts on broiler carcasses. However, evidence-based guidelines are currently lacking. **Objectives:** The aim of this study was to identify factors associated with *Campylobacter* counts on carcasses during the slaughter of *Campylobacter* positive broiler batches in Belgian slaughterhouses.

Methods:*:* Quantitative data describing *Campylobacter* carcass contamination were collected during the slaughter of 28 *Campylobacter* positive broiler batches in six slaughterhouses. Additionally, batch and slaughterhouse specific information were collected and their association with *Campylobacter* counts was studied.

Results: Both the *Campylobacter* colonization level and the external contamination of incoming birds were significantly associated with high *Campylobacter* counts. However, the comparison of the routine broiler slaughter practices revealed that certain process parameter can also influence *Campylobacter* counts on broiler carcasses. Incorrect setting of the plucking, evisceration and vent cutter machines, inadequate scalding temperature, dump based unloading systems, and electrical stunning were identified as risk factors associated with higher *Campylobacter* counts.

Conclusions: Practical and economically achievable modifications of the slaughter process were identified, which may enable broiler slaughterhouses to control *Campylobacter* carcass contamination.

O124

Optimization of air chilling process to control Campylobacter contamination on broiler legs

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Most cases of campylobacteriosis are associated with eating raw or undercooked poultry meat or cross-contaminated foods by these items. Several risk assessment studies concluded that reducing *Campylobacter* load on meat would reduce significantly the number of campylobacteriosis associated with broiler meat. This work aims to define optimal air chilling conditions to reduce *Campylobacter* levels on poultry carcasses. This study was set up to investigate four major parameters in the chilling process (temperature, duration, air velocity and initial concentration of *Campylobacter*) individually and in interaction on the behaviour of Campylobacter using the Doehlert shell design. Three experimental designs were performed using a chilling prototype and a ST-45 strain isolated from poultry. Moreover, several different strains (ST, virulence) were tested according to an optimal combination of these four parameters. The maximum contamination reduction reached a rate of 63% (reduction of 1.5 log CFU/g). Duration of chilling (p=0.04), initial concentration (p=0.03) and an interaction between temperature and initial concentration (p=0.01) had significant effects. When initial concentration was fixed (10^3 CFU/g), temperature effect (p=0.0045) was confirmed. Moreover, the interaction between temperature and air velocity (p=0.007) was also significant on *Campylobacter* contamination. First results have shown no significant difference between the different strains tested. The most important result is that carcasses presenting more than 10^3 CFU/g of Campylobacter would not be significantly decontaminated during the chilling process. Moreover this work shows that a chilling process with low temperature can significantly reduce the bacterial load on chicken carcasses presenting not more than 10^3 CFU/g.

O125

Effect of feed presentation (mash vs pellets) and whole wheat addition on cecal morphology and *Campylobacter jejuni* colonization of broilers orally infected

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An experiment was conducted within project CAMPYBRO for evaluating the effect of feed presentation (FP) and whole wheat (WW) addition on cecal morphology and *Campylobacter jejuni* colonization. There were six treatments factorially arranged with two FP (Mash [M] *vs* Pellets [P]), and three levels of WW from 0-21/21-42d: 0/0, 7.5/15%, 15/30%. A total of 216 Ross 308 broilers were used (3 birds/cage, 12 cages/treatment). At 14 d, broilers were orally gavaged with 100 µl (10^5 cfu/ml of ST-45 *C. jejuni*). On days 21, 35 and 42, caeca from 12 birds/treatment were collected and *Campylobacter* counts determined (ISO 10272). At 42d, weight and pH at caeca were taken. Data were analysed by GLM procedure of SPSS. At 21d of age, M tended to show lower *C. jejuni* values than P (7.85 *vs* 8.27 $\log_{10}$ cfu/g. P=0.091). Also, the 7.5/15% inclusion rate showed less contamination than the higher level (P=0.048). Also, at 21d M with 7.5% of WW showed less *C. jejuni* population that P + 15% of WW (P=0.006). No effects on *C. jejuni* counts were detected at 35 or 42d. There was an interaction FP*WW for caeca (%BW): caeca of birds fed the 15/30%WW were the biggest in M, and 7.5/15%WW in P diets (P=0.070); and for cecal pH: WW increased the pH in M but not in P diets (P=0.016). It is concluded that M and WW at 7.5/15% showed less *C. jejuni* population than P at 21, and that WW caused different physiological effects depending on FP.

O126

A longitudinal study of Campylobacter in a dairy farm environment.

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Background: *Campylobacter* is the most common bacterial cause of diarrhoeal disease. However, in around 50% of cases, the source of infection is remains unidentified.

Objectives: To investigate the dairy farm environment as a reservoir of *Campylobacter*, both in terms of the risks to humans and as a source for infection for livestock.

Methods: A longitudinal study of a dairy farm environment was undertaken between March 2013 and October 2014 with weekly sampling of water and cattle faeces. Boot socks were used to sample fields and buildings housing cattle of different ages. *Campylobacter* was detected by culture and PCR.

Results: *Campylobacter* was identified either by culture or PCR in 49.7% of boot sock samples, 28% of water samples and 47% of field bovine faecal samples. Some areas were identified as "hotspots", with 70% of samples from one public footpath being positive by culture. The presence of *Campylobacter* in fields remained above 30% during the winter months when no livestock were present. Positivity in the samples from buildings was higher in those housing heifers (48%), compared to pregnant cows (31%) and the milking herd (15%). The most common species identified was *C. jejuni* (76%) followed by *C. coli* (15%).

Conclusions: *Campylobacter* is highly prevalent in the dairy farm environment even during the winter months, in the absence of livestock. The age of the animals and sampling location were factors of *Campylobacter* presence. The dairy farm environment could be a key source of infection of humans and livestock, even when fields are unoccupied.

H. pylori diagnosis and control
Thursday 5th November

0910-1035

O127

A screening programme to test and treat *H. pylori* infection in New Zealand: a cost-utility analysis by age, sex and ethnicity

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Background: Meta-analyses suggest that in populations with high stomach cancer incidence treating people with *H. pylori* infection reduces their subsequent risk of stomach cancer by one third. International guidelines recommend a test and treat approach for asymptomatic people in Asia-Pacific populations, and a more targeted approach for high-risk groups in Western populations. In New Zealand (NZ) the age-standardised incidence of stomach cancer among Māori males was 28 per 100,000 and 17 per 100,000 among non-Māori males (2011).

Objectives: To determine if a screening programme directed at testing asymptomatic people for *H. pylori* and treating with eradication therapy would be a cost-effective way of preventing stomach cancer in specific age, gender and ethnicity groups in NZ.

Methods: We built a NZ specific online cost-effectiveness calculator – that included stomach cancer epidemiology and NZ health system costing data (http://www.otago.ac.nz/wellington/departments/publichealth/research/bode3/otago076415.html). This Markov macrosimulation model was then further adapted to allow a more detailed cost-utility analysis for specific age, gender and ethnicity groups. Other additional features included NZ data for the proportion of stomach cancer that is non-cardia, *H. pylori* seroprevalence, and the decline in stomach cancer incidence.

Results: Preliminary results suggest that a *H. pylori* screening programme may be cost-effective particularly for Māori males. Further refinements with scenario and sensitivity analyses will be presented.

Conclusions: Rapid cost-utility analysis can provide valuable cost-effectiveness information for a *H. pylori* screening programme in targeted age, gender and ethnicity groups. Results will inform whether targeted *H. pylori* screening is cost-effective in NZ.

O127

Structure and specificity of *Helicobacter pylori* aminopeptidase

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Background: The standard H. pylori eradication therapy has lost its efficacy, with an eradication rate dropping to as low as 60% in Western Europe. Aiming to develop an alternative therapy, we have performed initial characterisation of *H. pylori* M17 aminopeptidase (HpM17AP).

Objectives: To address the structural basis of catalysis and inhibition of this enzyme, we have established its specificity towards an N-terminal amino acid of the substrate and determined the crystal structures of HpM17AP and its complex with the inhibitor bestatin.

Methods: We have analysed the diffraction data sets for HpM17AP and its bestatin complex collected to 2.0 Å and 1.9 Å, respectively. HpM17AP activity was screened against a fluorogenic substrate library containing 20 natural and 94 unnatural amino acids.

Results: The position of phenylalanine moiety of the inhibitor with respect to the active-site residues and with respect to other M17 aminopeptidases suggested that it represents the S1 subsite. In contrast to most characterized M17 aminopeptidases, HpM17AP displays preference to L-Arg over L-Leu.

Conclusions: A close similarity between the structures of HpM17AP and its homologues from other bacteria has allowed the structural features that determine differences in their substrate specificity to be analysed.

O129

Stearylamine-containing liposomes have bactericidal activity against *Helicobacter pylori*

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Background: *Helicobacter pylori* is a causative agent of gastritis, peptic ulcers and gastric cancer. Prevalence of *H. pylori* infection is 60% in Aboriginal communities in Arctic Canada. Approximately 30-40% of community members fail standard therapy, possibly due to antimicrobial resistance or problems with dosing regime compliance. Recent unpublished observations (Mahmoud 2014) suggest a single dose of phosphatidylcholine (PC) : cholesterol (CH) : stearylamine (SA) liposomes (7:3:2) impairs *H. pylori* growth in vitro.

Objective: To evaluate *H. pylori* growth and gastric epithelial cell viability after *in vitro* exposure to increasing concentrations of SA-liposomes.

Methods: Three liposome formulations were prepared with the following ratios of PC:CH:SA (F1=7:3:1, F2=7:3:2, F3=7:3:3). At time 0 h and after 24 h incubation with liposomes ranging in concentration from 0 to 200 µg/mL, *H. pylori* growth was evaluated macroscopically and spectrophotometrically to determine the minimal inhibitory concentration (MIC), and quantified by performing colony counts to determine the minimal bactericidal concentration (MBC). NCI-N87 gastric carcinoma cells were cultured until confluent, exposed to F1, F2 and F3 liposomes at MBC values for 24 hours, and cell viability determined by dye exclusion assay.

Results: Exposure to F1 liposomes had no inhibitory or bactericidal activity for *H. pylori.* For F2 liposomes, the MIC was 100 µg/mL and MBC was 200 µg/mL, versus 50 µg/mL and 100 µg/mL, respectively, for F3 liposomes. Gastric cell viability was unaffected by exposure to F1, F2, F3 liposomes and unchanged from untreated controls.

Conclusions: Increasing liposome stearylamine content elicits antimicrobial activity but is non-toxic for cultured gastric cells, suggesting a potential alternative for *H. pylori* treatment.

O130

Helicobacter spp. with flexispira morphology: an infectious cause of abortion in sheep

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In New Zealand the agents most commonly diagnosed in sheep abortion are campylobacters, toxoplasmas and *Salmonella* Brandenburg, accounting for approximately 80% of sheep abortions where a causal agent is identified. However the agents causing abortion are often not determined. An aetiological agent was pursued during a large abortion outbreak, affecting a Southland farm in 2009, where endemic agents had been excluded. Moderate numbers of a slightly curved rod with a spiral periplasmic membrane were identified within the biliary caniculi of diseased liver tissue by electron microscopy. This ultrastructure was consistent with that of the bacterium *Flexispira rappini* (subsequently *Helicobacter* spp. with flexispira morphology), and a provisional diagnosis of abortion associated with this agent was made. To assess the association of Helicobacter with sheep abortion outbreaks we conducted a survey of abortion submissions from the 2012 lambing season, in the southern South Island of New Zealand. A comparison was made of the proportion of laboratory submissions culture or PCR positive for Helicobacter, from flocks in which no other agent had been identified, compared to flocks that had a known cause of abortion. Where no diagnosis had been made aborted material was positive in 8 submissions (20%, 8/40) from five of the 31 survey farms (16%, 5/31). Helicobacters were not detected in any of the 18 submissions from the 17 control farms. The *Helicobacter* spp. were identified as *Helicobacter trogontum* (Flexispira taxon 5 genotype) and *Helicobacter bilis* (Flexispira taxon 8 genotype). Findings support *Helicobacter* spp. with flexispira morphology as a likely causative agent of abortion outbreaks in southern New Zealand.

O131

Structural basis for the inhibition of *Helicobacter pylori* alpha-carbonic anhydrase by sulfonamides

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Background: Periplasmic α-carbonic anhydrase of *Helicobacter pylori* (HpαCA), an oncogenic bacterium in the human stomach, is essential for its acclimation to low pH. It catalyses the conversion of carbon dioxide to bicarbonate using Zn(II) as the cofactor. In *H. pylori*, *Neisseria* spp., *Brucella suis* and *Streptococcus pneumoniae* this enzyme is the target for sulfonamide antibacterial agents.

Objectives: Structural and functional studies of HpαCA to understand the mechanisms of *H. pylori* pathogenesis and enable their assessment as targets for drug design.

Methods: HpαCA has been purified and crystallized by the hanging drop vapour-diffusion method. Diffraction data sets for HpαCA complex with acetazolamide and methazolamide have been collected to 2.0 Å and 2.2 Å, respectively, using the MX1 & MX2 beamlines of the Australian synchrotron.

Results: We present structural analyses correlated with inhibition data, on the complexes of HpαCA with sulfonamides acetazolamide and methazolamide which reveal that two sulfonamide oxygen atoms of the inhibitors are positioned proximal to the putative location of the oxygens of the CO2 substrate in the Michaelis complex, whilst the zinc-coordinating sulfonamide nitrogen occupies the position of the catalytic water. The structures are consistent with acetazolamide acting as site-directed, nanomolar inhibitors of the enzyme by mimicking its reaction transition state. Additionally, inhibitor binding provides insights into the channel for substrate entry and product exit.

Conclusion: This analysis has implications for the structure-based design of inhibitors of bacterial carbonic anhydrases.

1110-1230
O132

Using whole genome sequence data to predict the antimicrobial resistance profiles of human campylobacteriosis isolates

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Background: The determination of antimicrobial susceptibility and resistance in *Campylobacter* currently relies on phenotypic approaches that have variable reliability and reproducibility. The analysis of whole genome sequence (WGS) provides an alternative paradigm for investigating and reporting such data.

Objectives: To predict antibiotic resistance and susceptibility from WGS and describe inheritance patterns of resistance-conferring alleles in a population of clinical *Campylobacter* isolates collected over a three year period, from Oxfordshire, UK.

Methods: Using BIGSdb analysis tools, multi-locus sequence typing (MLST), core-genome MLST (cgMLST), and antibiotic resistance-associated coding sequences were identified in WGS data from 2,556 human campylobacteriosis isolates within the publicly available PubMLST.org/campylobacter database.

Results: Ciprofloxacin resistance-associated *gyrA* mutations were identified in 40.2% of isolates, and were significantly associated with ST-353, ST-354, ST-464, ST-607 clonal complexes and unassigned *C. jejuni* isolates (P>0.05). The *tetO* locus, identified in 40.5% of isolates, was found located on either chromosomal or plasmid contigs, and was significantly associated with ST-206, ST-354, ST-443, ST-464, ST-574 complexes and unassigned *C. jejuni* (P>0.05). Phylogenetic analyses using cgMLST data demonstrated likely clonal expansion of isolates belonging to ST-354 and ST-464 complexes, between which the position of the chromosomally encoded *tetO* alleles differed. Genes conferring aminoglycoside resistance were identified in fewer than 1.0% of isolates.

Conclusions: This methodology successfully identified ciprofloxacin and tetracycline resistance-associated genotypes among *Campylobacter* isolates and confirmed high levels of aminoglycoside susceptibility. Identification of clonal expansion events in multiple clonal complexes provided evidence of continued convergent evolution in antibiotic resistant clinical campylobacteriosis isolates.

O133

Prevalence of Campylobacter and their antimicrobial resistance in broilers at slaughter in Ecuador

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Background: *Campylobacter coli* and *Campylobacter jejuni* are found in poultry and represent an important source for human gastrointestinal infections worldwide. Despite of its importance, prevalence and drug resistance of these bacteria are still unknown or poorly studied in developing countries.

Objectives: The aim of this study was to estimate the prevalence and antimicrobial resistance of *C. coli* and *C. jejuni* in broilers at slaughter in Ecuador.

Methods: Caeca content were collected from 379 batches at 6 slaughterhouses during 1 year. After bacteriological isolation on MCCDA Agar, speciation was performed with a multiplex PCR. MIC values for ciprofloxacin, nalidixic acid, tetracycline, erythromycin, streptomycin, chloramphenicol and gentamicin were obtained using a EUCAMP kit.

Results: Bacteriological isolation showed *Campylobacter spp*. in 249 samples (65.7%). 158 (67.5%) samples contained *C. coli,* 46 (19.7%) *C. jejuni*, 30 (12.8%) *C. coli* and *C. jejuni*. From 15 samples *Campylobacter* isolates were not speciated. All tested strains showed resistance to at least 1 antibiotic. Rates of antimicrobial resistance were: 98.5 % (ciprofloxacin), 98.5% (nalidixic acid), 74.2% (tetracycline), 16.7% (erythromycin), 7.6% (streptomycin), 3% (chloramphenicol) and 0% (gentamicin).

Conclusions: The high prevalence of *Campylobacter* in broilers and their drug resistance profile may represent a major public health concern in Ecuador.

O134

Evolution of Campylobacter in a persistently infected human host

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Campylobacter jejuni's presence in the gastrointestinal tract of humans is usually associated with acute gastroenteritis. However, *C. jejuni* has been isolated from patients with prolonged gastrointestinal problems. Whether *Campylobacter* contributes to these gastrointestinal problems remains to be determined. The aim of this study was to investigate an individual who has been diagnosed with campylobacteriosis intermittently for 8 years, and examine the evolution of the excreted *Campylobacter*. We performed whole genome sequencing, antimicrobial susceptibility testing and other phenotypic tests on six *C. jejuni* ST45 isolates collected from the patient over 8 years. We found that the isolates were closely related, sharing a common ancestor at approximately the same time that *Campylobacter* excretion began. We also found that the isolates displayed gradual changes in antimicrobial susceptibilities and other phenotypes, and that these phenotypic changes resulted from the accumulation of point mutations. Our results demonstrate how antibiotic therapy may select for resistance in *Campylobacter*, how small genotypic changes can have significant effects on the phenotype, and how constant or repeated colonisation can select for certain *Campylobacter* phenotypes. This study should promote further research into long-term *Campylobacter* excretion, its effect on *Campylobacter*'s genotype and phenotype, and the factors that influence *Campylobacter* excretion and colonisation.

O135

Rapid emergence of tetracycline and fluroquinolone resistant *Campylobacter jejuni* ST-6964 in poultry and humans in New Zealand

French, NP[1]; Biggs, R[2]; Biggs, PJ[3]; Bloomfield, S[3]; Dyet, K[4]; Gilpin, BJ[4]; Heffernan, H[4]; Midwinter, AC[3]; Mulqueen, K[5]; Rogers, LE[3]; Williamson, DA[4];

mEpiLab, Massey University[1]Tegel Foods Ltd[2]Massey University[3]Institute of Environmental Science and Research Limited[4]Poultry Industry Association of New Zealand[5]

Until recently New Zealand recorded relatively low levels of antimicrobial resistance in Campylobacter spp. In May 2014 a previously unreported antimicrobial resistant C. jejuni (tet/FQ ST-6964) was isolated from poultry in the Manawatu sentinel site in the North Island; followed by human cases from August 2014 onwards in the Manawatu and Auckland, and a foodborne outbreak in Wellington. Cross-sectional studies conducted in 2015 identified a relatively high prevalence of tet/FQ ST-6964 in three of the four major poultry suppliers in the North Island (between 27 and 62% of pooled caecal samples from one week of slaughter) and in approximately one third of human campylobacteriosis cases in Auckland. Subsequent isolation of ST-6964 in a breeder (parent) flock suggests a transmission route common to multiple poultry suppliers. Whole genome sequencing revealed a high degree of genetic relatedness between poultry and human isolates. FQ resistance was associated with the gyrA C257T mutation, and tetracycline resistance with the tet(O) gene. Relatively high diversity was observed (>500 SNPs between 21 isolates), with many of the SNPs located in integrated element 1, the well-described mu-like phage insertion, with evidence of gene deletion in this region in approximately 50% of isolates. Government, industry and academia are working collaboratively to understand the emergence and persistence of this strain given no evidence of fluroquinolone use in poultry production, and to identify potential control measures. The rapid emergence and wide geographic spread of this strain has implications for food safety, public health, and biosecurity in the New Zealand poultry industry.

O136

Molecular detection and antimicrobial resistance pattern of Campylobacter species isolated from retail meat shops in Lahore, Pakistan

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Background: Campylobacter, one of the emerging zoonoses, is worldwide in distribution. This thermo-tolerant pathogen is more common in poultry and is one of the leading causes of diarrhea and gastroenteritis in humans. **Objectives:** Estimation of Campylobacter burden in various meat sources of Pakistan and their resistance to commonly used antibiotics in human and veterinary practices.

Materials and Methods: A cross-sectional study was conducted to estimate the prevalence of Campylobacter in retail meat in the district of Lahore (Pakistan) during January 2014 to September 2014. A total of 240 samples 80 each of beef, mutton and poultry, were collected from retail shops through systematic sampling. The ISO 10272-1:2006 (E) method was used to detect *Campylobacter* spp. followed by PCR for confirmation. Antimicrobial resistance against commonly used antibiotics was studied by the disc diffusion method.

Results: Of the 240 meat samples, Campylobacter was isolated from 32(13.3%). The prevalence of Campylobacter in beef was 7.5% (6/80), in mutton 11.2% (9/80) and in poultry 21.2% (17/80). *Campylobacter jejuni* (84.3%) was most frequently isolated followed by *C. coli* (15.7%). Highest resistance in Campylobacter was found against Enrofloxacin 81.2% (26/32), followed by Ciprofloxacin 71.2% (23/32), Amoxicillin 68.7% (22/32), Colistin 68.7% (22/32), Neomycin 40.6% (13/32) and Nalidixic Acid 31.2% (10/32) and Gentamicin 25% (8/32).

Conclusions: Campylobacter species is prevalent in all three types of meat in Lahore. The highest prevalence was recorded in chicken meat followed by mutton and beef, respectively. In addition, the isolates were resistant to frequently used antibiotics in human practice thus posing potential health risk to public health.

O137

Is the immune response to Campylobacter in the chicken directed at prevention of extra-intestinal spread at the expense of gastrointestinal persistence?

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University of Liverpool[1]Roslin Institute[2]University of Newcastle[3]University of Swansea[45]

Background and Objectives: Previously we have shown that *Campylobacter* elicits an early, and sometimes damaging, innate immune response in the chicken intestinal tract (Humphrey et al, MBio 5(4):e01364-14. 2014). Here we further characterise this mucosal immune response.

Methods: RNA was isolated from intestinal tissue from 2 broiler chicken breeds (A1 and B2) infected orally with *C. jejuni* M1 preserved in RNAlater at -70°C . Relative expression of cytokines IL-13 (Th2), IL-19, TGF-β4 (regulatory) and IL-17A and IL-17F (Th17) in infected and control birds was made on caecal, caecal tonsil and ileum samples at 2, 5, and 12 dpi through qRT-PCR.

Results: Expression of IL-13 was found in both breeds, consistent with production of specific antibody. The slower-growing breed B2 expressed higher levels of IL-19 at 2 and 5 dpi consistent with expression of IL-10 previously described though both breeds expressed TGF-β4 following infection at all time points. IL-17A and IL-17F were expressed in both breeds at 2 and 5 days post infection and IL-17F only at 12 days post infection though expression was generally greater in the B2 breed.

Conclusions: *C. jejuni* infection not only elicits an inflammatory and antibody response in the chicken intestinal mucosa, but also leads to significant expression of regulatory cytokines, Interestingly breeds differ significantly in IL-10 family cytokines associated with induced T regulatory cells, but show similar expression of TGF-β4 associated with natural Tregs and Th3 responses. Significant expression of IL-17 in the gut suggests a strong Th17 response. Although Th17 cells are often associated with inflammation, they also act as sentinels in the intestinal tract preventing pathogen invasion by maintaining tight junctions in the epithelial cell layer and eliciting the production of antimicrobial peptides. These findings suggest the 'natural' immune response in the chicken protects against disease by limiting *Campylobacter* to the gut something that may be difficult to overcome by vaccination.

O138

Applications of recombinant attenuated Salmonella vaccines expressing various *Campylobacter jejuni* antigens for the reduction of *C. jejuni* colonization in poultry

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The most substantial risk factor for campylobacteriosis, a disease that sickens an estimated 1.3 million people annually in the U.S. alone at a cost of more than $1.7 billion, is the handling and consumption of raw and undercooked poultry. Based on mathematical modeling, it is believed that even a modest reduction of *Campylobacter* in broilers will reduce human disease substantially. Current strategies are inadequate in the management of this pathogen within poultry, which are commonly colonized to high levels without identifiable pathology. To this end, we have examined the ability of immunogenic *C. jejuni* proteins to reduce colonization when administered via an attenuated *Salmonella* Typhimurium vaccine prior to challenge with *C. jejuni*. For all studies, day-old *Salmonella* and *C. jejuni*-free Cornish X Rock broiler chicks obtained from a commercial hatchery were housed in isolated groups. Initially, chicks were vaccinated twice, by oral gavage, 10 days prior to challenge with *C. jejuni*. Previously, we have presented data indicating reduction of colonization at day 36 post-hatch, following vaccination at days 10 and 16 with RASVs expressing two novel *C. jejuni* genes. Here we present the progress of this work, including the efficacy of a previously tested vaccine at reduced, industry-compatible doses; efficacy of administration by industry-compatible techniques including spray cabinet or drinking water; and the efficacy of an additional gene previously associated with poultry colonization, *cj0248*, expressed in the RASV system, for which trials are currently underway.

O139

Sequential optimization of an avian vaccine protocol against Campylobacter

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ANSES, Ploufragan/Plouzané laboratory[1]CZVeterinaria[2]

Campylobacteriosis is a major public health concern with nine million cases each year in Europe. Poultry constitutes the main reservoir of *Campylobacter* and poultry meat the main source of human contamination. Poultry vaccination could be a potential way to reduce *Campylobacter* intestinal loads and therefore impact human disease incidence. However, despite many studies, no vaccine is available yet. We suggest here the development of an optimal protocol of immunization based on three sequential in vivo trials. Each trial consists in two immunizations followed by *Campylobacter* infection. Flagellin and/or FliD proteins are used as antigen. The first trial, dedicated to DNA/DNA vaccination with and without adjuvant (CpG and IL-2) did not allow significant reduction of *Campylobacter* loads in vaccinated groups compared to the control one. Nevertheless, significant differences between CpG and IL-2 groups suggested a negative effect of IL-2 on immunizations. The assessment of the systemic humoral immune response showed a slight increase of antibodies levels after the oral challenge. However, the high variations between chickens did not lead to statistical differences between experimental groups. Moreover, no difference could be observed among groups for the mucosal immune response. In the second trial, DNA/DNA and DNA prime/protein boost vaccinations will be compared. According to the first in vivo results, experimental timing for immunizations and challenge will be performed earlier, which could improve chickens' immunity at the end of the experiment. These trials will allow to define an optimal protocol that could be used to test new vaccines against *Campylobacter* in live animals.

O140

An important structural motif in the bacterial oligosaccharyltransferase for N-linked protein glycosylation in Campylobacter

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Bacterial N-linked protein glycosylation was first described in *Campylobacter jejuni*. The oligosaccharyltransferase, PglB, transfers a conserved heptasaccharide to asparagine residues within the D/E-X1-N-X2-S/T sequon ($X_{1/2} \neq P$) of acceptor proteins and releases free oligosaccharides (fOS) into the periplasm. The *C. jejuni* protein glycosylation machinery can also be functionally transferred into *Escherichia coli* to engineer novel glycoproteins. It is known that a conserved WWDXG motif located within the soluble C-terminal domain of PglB is necessary for N-glycan transfer and fOS release. We identified another conserved PglB sequence, the DXXK motif close to WWDXG. Functional analyses of the D and K residues through comparison of single and double point mutations resulted in the loss or reduction of protein glycosylation when these PglB mutant proteins were co-expressed with an N-glycan acceptor protein in *E. coli*, indicating that both amino acids are important for activity. Similarly, complementation of a *C. jejuni pglB* mutant with *pglB* DXXK mutant alleles showed a reduction in N-linked protein glycosylation and fOS release. Analysis of the available crystal structures of *C. jejuni* PglB revealed that D and E within DXXK form two salt bridges with adjacent amino acids when PglB is in its active conformation. Interestingly, certain *Campylobacter* species contain two PglB orthologues. In PglB complementation studies using either the *E. coli* system or a *C. jejuni pglB* mutant, we demonstrate that only the orthologue containing a DXXK motif is active. Analysis of the structure-function of bacterial oligosaccharyltransferases will assist in more efficient production of glycoconjugate vaccines in *E. coli*.

O141

Antibody has no effect on caecal colonization in experimental *Campylobacter jejuni* infection of broiler chickens

Lacharme-Lora, L[1]; Wright, E[1]; Wattret, G[1]; Jopson, S[1]; Williams, N[1]; Wigley, P[1];
University of Liverpool[1]

Background: Understanding the protective role of antibody is regarded as a key in development of poultry vaccines for *Campylobacter* but few functional immunological studies have been performed.

Objectives: In this work we have used depletion of the Bursa of Fabricius (the primary lymphoid organ associated with B lymphocyte development in birds) to deplete the antibody response to determine the role antibody plays in primary *Campylobacter jejuni* infection of the chicken.

Methods: Depletion of the Bursa (bursectomy) of Ross 308 broiler chicks was performed by intramuscular injection of cyclophosphamide for the first 4 days of life. Circulating lymphocyte populations were monitored by flow cytometry for B and T lymphocyte markers. At 28 days of age bursectomised and control birds were challenged orally with 10^8 CFU *C. jejuni* M1. Post-infection quantification of colonisation in the jejunum, ileum, caeca and colon, and specific antibody levels in serum were determined at 14 and 28 days post infection. **Results:** Bursectomised birds fail to produce specific circulating IgG or IgM antibodies in response to infection following depletion of over 90% of the B Lymphocyte population. Colonisation of the caeca was found at similar levels in both control and bursectomised birds at both time points but levels in the ileum and jejunum were lower in control animals that produce a specific antibody response at 28 days post infection.

Conclusions: Antibody plays little role in clearance of *C. jejuni* from the caeca, the major site of colonization, but plays a role in reducing levels in the small intestine.

Control strategies for Campylobacter - 3
Thursday 5th November

1315-1405

O142

Effect of feeding a combination of a yeast product and a probiotic, alone or in combination with a blend of mono-glycerides, on Campylobacter colonization in broilers

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An experiment was conducted within the EU-FP7 project CAMPYBRO in order to evaluate the effect of a combination of a yeast extract (XPC) with a multispecies probiotic (PS) alone or together with a blend of mono-glycerides (MG) added to the feed on *Campylobacter* counts in broilers. There were three treatments applied from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives), T2: T1 + XPC at 1,250 g/t + PS at 1,000 g/t and T3: T2 + MG at 8,000 g/t. A total of 126 one-day-old Ross 308 broilers were divided into the experimental treatments. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1 x 10^5 CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca from 12 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). Data expressed as $\log_{10}$ CFU/g caeca content analysed by the nonparametric test of Kruskall-Wallis, followed by the Dunn's test (SPSS v.19.0). No significant differences in the *Campylobacter* counts were observed between the two products tested and the control treatment at 21 and 35 d of age. At the end of trial, both combinations significantly reduced *Campylobacter* colonization when compared with non-treated broilers (8.39^a, 6.86^b and 7.51^b $\log_{10}$ CFU/g, for T1, T2 and T3, respectively). It is concluded that the combination of a multispecies probiotic with a prebiotic is effective and reduces *Campylobacter jejuni* at cecal level.

O143

Picking up Campylobacter with the weekly shop: A United Kingdom study to establish the levels of campylobacter on the packaging of fresh raw whole chicken at retail sale over a 12 month period

Jorgensen, F[1]; Madden, R H[2]; Rowswell, L[3]; Arnold, E[1]; Charlett, A[1]; Elviss, N C[1];

Public Health England[1]Agri-Food and Biosciences Institute[2]Food Standards Agency[3]

Background: Source-attribution studies, outbreak investigations and case-control reports all incriminate chicken meat as the key food-borne transmission route for *Campylobacter* infection. Handling chicken in the kitchen has been linked to infection, but the role of packaging of meat at retail sale has received limited attention as a potential source of infection.

Objective: To establish the risk of exposure to *Campylobacter* species to consumers from handling packaged fresh whole raw chicken at retail sale.

Methods: The outer packaging fresh whole retail chickens (n=4005) were swabbed to establish the levels of *Campylobacter* species present. Samples were collected in sterile plastic bags, based on market share data, which allocated the majority of samples to the major supermarkets in the UK although a variety of retailers were represented. Packaging included plastic bags used by butchers shops through to modified atmosphere packaging in plastic film by the supermarket brands. On receipt in the laboratory, the packing was swabbed and analysed to determine carriage of *Campylobacter* on the external surface.

Results: Overall 6.8 % of chicken packaging was contaminated with *Campylobacter*. The majority, 5.2% (n=209), were contaminated with between 10–99 cfu per swab but 1.4% (n=58) had between 100–1000 cfu per swab. At the higher level of contamination, 0.1% (n=5) of chicken packaging tested had >1000 cfu (up to 4500) per swab. *Campylobacter jejuni* and *C. coli* were detected.

Conclusions: This work shows that handling fresh whole raw chicken at retail sale presents some risk to consumers, despite the pre-packaged approach many retailers employ.

O144

Lessons from New Zealand's prolonged campylobacteriosis epidemic

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University of Otago, Wellington[1]

Background: For more than 30 years campylobacteriosis has been the most commonly notified disease in NZ. Its incidence rose markedly from the mid-1980s to the mid-2000s. Despite good evidence linking this epidemic to contaminated chicken meat, control action was slow.

Objectives: To identify lessons from efforts to manage New Zealand's prolonged national campylobacteriosis epidemic from contaminated poultry meat.

Methods: Summarise epidemiological data on campylobacteriosis in NZ and policy responses. Review wider policy and practice implications.

Results: Campylobacteriosis incidence peaked in 2006 with 15,873 notifications, suggesting a population incidence of 148,000 or 3.5% per annum, including about 1,000 hospitalisations. Major regulatory interventions to reduce contamination levels in fresh poultry were implemented in April 2007 followed by a rapid 50% reduction in human disease.

Conclusions: Fresh poultry meat contaminated during processing causes the majority of human cases of campylobacteriosis. The success of the NZ intervention illustrates the value of a strong regulatory response, supported by high quality surveillance & public health advocacy. More work is needed to control the largest 'common source outbreak' in NZ's history Wider lessons about food safety: Slowly evolving epidemics may get less attention than they deserve; Robust epidemiological evidence is not always enough; Regulating producers is usually more effective than educating consumers; Effective public health surveillance can drive improved food safety; We need a highly effective food safety regulator; We need informed, independent food safety resarchers & advocates; Food safety concerns will continue & evolve. Some of these lessons are applicable to other areas of public health.

Late breakers session
Thursday 5th November

1315-1405

O145

Effects of D-Tryptophan on *Campylobacter jejuni* biofilm formation

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University of Leicester[1]

D-amino acids (DAAs) are promising therapeutic agents that trigger the dispersal of biofilm of several bacteria and the proposed mechanism is interference with the exopolysaccharide component of the matrix. We hypothesised that D-Trp disrupts the biofilm of *C. jejuni* by inducing the degradation of the extracellular matrix (ESM).To investigate this hypothesis wild type *C. jejuni* NCTC 11168, a dispersion-deficient mutant and the complemented mutant were used. Biofilms, with and without treatment with D-Trp, were stained using crystal violet and ESM was visualised by confocal laser scan microscopy (CLSM) with a conjugate of the carbohydrate-binding protein Concanavalin A with SYTO green 9 fluorescent dyes. We found that in comparison with untreated control, wild type biofilm was significantly reduced by 53.67% with 0.1μM of D-Trp and the complemented mutant showed a comparable 45.89% reduction of biofilm with 0.1μM of D-Trp compared to the untreated control. In contrast, the dispersion-deficient mutant showed no reduction response with D-Trp treatment. We observed that ESM was increased in the dispersion-deficient mutant after treatment with D-Trp, which may suggest that D-Trp could induce the detachment of biofilm due to degradation of the ESM matrix around the biofilm thus releasing seed cells. We also found that D-Trp promotes chemotactic motility as judged by a plate-based swarm assay. These results found that D-Trp can inhibit existing biofilms and also inhibited further biofilm growth and caused partial biofilm disassembly. Our data provides evidence that the inhibitory effect of D-Trp on *C. jejuni* biofilms at least in part involves the degradation of the biofilm matrix, promoting the transition from biofilm to planktonic cells.

O146

Selected antimicrobial peptides inhibit *in vitro* growth of *Campylobacter jejuni*

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Novel alternatives to traditional antibiotics are urgently needed for food-animal production. A goal of our laboratory is to develop and evaluate antimicrobial peptides (AMP) to control and reduce foodborne pathogens in poultry. AMP have been found in most every class of living organism where they have evolved as a host defense mechanism against invading microorganisms. Our working hypothesis is that AMP can be identified that inhibit the growth of *Campylobacter jejuni* and subsequently can be utilized to reduce the Campylobacter load among commercially produced chickens. Because of their modes of action, these AMP are much less likely to engender antimicrobial resistance. We chemically synthesized a set of 11 unique AMP and evaluated them for ability to inhibit growth of two strains of *C. jejuni*. Six of the AMP we tested produced zones of inhibition on lawns of *C. jejuni*. These AMP included: NRC-13, a variant of Pleurocidin isolated from the American plaice-flounder; RL-37, a 37-residue AMP of the cathelicidin family which is expressed in bone marrow of the rhesus monkey; Temporin, from the frog, *Rana temporaria*; a potent hybrid AMP composed of residues 1-8 of Cecropin A (from the Cecropia moth) fused to residues 1-12 of Magainin 2 (from the African clawed frog *Xenopus laevis*); Dermaseptin from the skin of frogs of the genus *Phyllomedusa*; and the synthetic OAK, C12K-2 beta 12. Our next steps are to express AMP in yeast and explore encapsulation technologies to stabilize the AMP for trials involving oral delivery to chickens.

O147

Characterization of worldwide Helicobacter pylori strains reveals genetic conservation and essentiality of serine protease HtrA

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HtrA proteases and chaperones exhibit important roles in periplasmic protein quality control and stress responses. The genetic inactivation of htrA has been described for many bacterial pathogens. However, in some cases such as the gastric pathogen Helicobacter pylori, HtrA is secreted where it cleaves the tumor-suppressor E-cadherin interfering with gastric disease development, but the generation of htrA mutants is still lacking. Here, we systematically analyzed htrA and show that the corresponding gene locus is highly conserved in worldwide H. pylori strains. The presence of htrA was confirmed in 992 gastric patients. Differential RNA-sequencing (dRNA-seq) indicated that htrA is encoded in an operon with two subsequent genes, HP1020 and HP1021. Genetic mutagenesis and complementation studies revealed that HP1020 and HP1021, but not htrA, can be mutated. In addition, we demonstrate that suppression of HtrA proteolytic activity with a newly developed inhibitor is sufficient to effectively kill *H. pylori*, but not other bacteria. We show that *Helicobacter* htrA is an essential bifunctional gene with crucial intracellular and extracellular functions. Thus, we describe here the first microbe in which htrA is an indispensable gene, a situation unique in the bacterial kingdom. HtrA can therefore be considered a promising new target for anti-bacterial therapy.

O148

Session: Comparative 'omics: Campy
Tuesday 3rd November, 1110-1230

A co-transformation strategy identified Cj1501c as an important player involved in conjugation in *Campylobacter jejuni*

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The University of Tennessee[1]Iowa State University[2]

Campylobacter jejuni displays significant strain diversity due to horizontal gene transfer. Conjugation is an important horizontal gene transfer mechanism contributing to the evolution of bacterial pathogenesis and antimicrobial resistance. *C. jejuni* strains display great variation in conjugation efficiency; however, the underlying mechanisms are still unknown. In this study, a co-transformation strategy was developed to obtain high frequency conjugation (HFC) derivatives of *C. jejuni* NCTC 11168, a standard strain with extremely low conjugation efficiency (6.3 × 10E-8 CFU/recipient). Specifically, erythromycin resistance marker *(erm)* was first introduced into a HFC *C. jejuni* strain (2.2 x 10E-4CFU/recipient) and genomic DNA from this strain was introduced into *C. jejuni* NCTC 11168 via natural transformation. Given that multiple recombination loci could occur within one recipient cell independently during natural transformation, the genetic components involved in HFC may be co-transformed and enriched with the erythromycin selection marker. All transformants were then pooled and harvested for conjugation. Nine transformants were identified and demonstrated to display HFC phenotype. The genome of six HFC derivatives and two low frequency derivatives were subjected to whole genome sequencing using MiSeq. Comparative genomics analysis and genetic manipulation indicated that the Cj1051c, which encodes a putative restriction-modification enzyme, plays a significant role in conjugation in ***C. jejuni*** NCTC 11168. Together, this study successfully developed and utilized a unique co-transformation strategy to identify Cj1051c as an important component involved in conjugation in *C. jejuni*.

O149

Session: Roundtable discussion on poultry control
Wednesday 4th November, 1415-1530

A longitudinal study of interventions and Campylobacter genotypes in British broiler farms

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University of Aberdeen[1]Avian Science Research Centre, Scotlands Rural College[2]

Background: Colonisation of broiler flocks on farm is a major challenge for the poultry industry. Objectives: To determine the efficacy of interventions (biosecurity barrier, organic acid treatment of drinking water and fly-screen). To utilise genotyping data to detect carry over between crops and identify potential sources of flock colonisation. Methods: Broiler farms (n=24) were sampled prior to thinning and at clearance for 8 crops. *Campylobacter* was isolated, whole genome sequenced and MLST genotypes obtained. Results: Prevalence was higher (P0.05). At clear 6 farms were always positive (P=0.005). There were 64 out of 168 flocks that were positive at clear and also positive pre-thin in the following crop. Of these 64 flocks 6 had the same genotype at clear and at the following pre-thin (higher than by chance, P=0.04). Of the 6 positive flocks, two farms have two carry overs each (P=0.009). However, carry-over of the same genotype only explains 4% of overall flock positivity prevalence. STRUCTURE inferred putative source pre-thin as cattle (0.315), sheep (0.217), wild birds (0.381) and pigs (0.086). Two sequence types (ST814 and ST257) were more common at clear and flocks were more likely to change from C. jejuni to C. coli (P=0.03). Conclusions: Interventions did not reduce flock prevalence, carry over between flocks explains only a small amount of positivity, some farms are continually positive and a number of external source reservoirs contribute to flock prevalence.

Posters

P001

Relationship between *Helicobacter pylori* virulence genes and clinical outcomes

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King Saud University[1]

Helicobacter pylori has been strongly associated with gastritis, gastric and duodenal ulcers,and it is a risk factor for gastric cancer. Two major virulence factors of *H. pylori* have beendescribed: the cytotoxin-associated gene product (cagA) and the vacuolating toxin (vacA).Since considerable geographic diversity in the prevalence of *H. pylori* virulence factors hasbeen reported, the aim of this work was to determine if there is a significant correlationbetween different *H. pylori* virulence genes (cagA and vacA) in 68 patients, from SaudiArabia, and gastric clinical outcomes. *H. pylori* was recognized in cultures of gastricbiopsies. vacA and cagA genes were detected by polymerase chain reaction (PCR). ThecagA gene was obtained with 42 isolates (61.8%). The vacA s- and m- region genotypeswere determined in all strains studied. Three genotypes were found: s1/m1 (28%), s1/m2(40%) and s2/m2 (26%). The s2/m1 genotype was not found in this study. The relation ofthe presence of cagA and the development of cases to gastritis and ulcer was statisticallysignificant ($P < 0.05$). The study showed a significant correlation between the vacA s1/m2genotype and gastritis cases, and a significant correlation between vacA s1/m1 genotypeand peptic ulcer cases. The results of this study might be used for the identification of high-risk patients who are infected by vacA s1/m1 genotype of *H. pylori* strains. In conclusion, *H. pylori* strains of vacA type s1 and the combination of s1/m1 were associatedwith peptic ulceration and the presence of cagA gene

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P002

MALDI-TOF and rpoB sequencing reveals previously undetected case of Arcobacter butzleri from human diarrhoea.

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Universitat Rovira i Virgili, Reus, Spain[1]Hospital Universitari Joan XXIII, Tarragona, Spain[2]Hospital Universitari Sant Joan, Reus, Spain.[3]

Background: The genus *Arcobacter* includes aerotolerant gram-negative bacteria and was the fourth most common pathogenic bacterial genus isolated from stool specimens of patients with acute enteritis in Belgium when using a specific culture media. However, this is not the case in many other studies where Arcobacter is identified by chance from media used for Campylobacter or other enterobacteria.

Objectives: To characterize the sequence types and virulence genes present in *Arcobacter butzleri* strains associated to cases of human diarrhea.

Methods: Four *A. butzleri* strains, 3 recovered from CIN agar (BD) and identified by MALDI-TOF and 1 isolated from Campylosel agar (bioMérieux) and identified by sequencing the rpoB gene were investigated. Sequence types were determined after sequencing the aspA, atpA, glnA, gltA, glyA, pgm and tkt genes by comparison with the MLST database. The presence by PCR of 5 virulence genes (ciaB, cadF, cj1349, hecA and irgA) was determined using primers described previously.

Results: The 4 *A. butzleri* strains were all confirmed as this species with the sequences of housekeeping genes. All strains showed to belong to new sequence types on the basis of the new alleles found for the genes glyA and tkt despite alleles found for atpA and glnA were already present at the database. At least 3 virulence genes were present in all the strains.

Conclusions: Isolation of new sequence types of *Arcobacter butzleri* form CIN agar seems to be common and MALDI-TOF enabled their fast and reliable identification.

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P003

Discriminative power of phenotypic and genotypic Campylobacter typing methods

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Background: Campylobacteriosis is the predominant human zoonosis in Europe. Broiler meat is considered the principal transmission vehicle of *Campylobacter* to humans. The challenge is to unravel the epidemiology of this transmission. Therefore, powerful discriminative typing techniques for the *Campylobacter* strains are needed.

Objectives: Compare the discriminatory power of phenotypic and genotypic typing methods on *Campylobacter* isolates from broiler carcasses.

Methods: *C. jejuni* and *C. coli* (n = 94 and 52) broiler carcass isolates were characterized by multi-locus sequence typing (MLST), antibiotic microbiological resistance (AMR), presence / absence of 5 putative virulence genes, and exclusively for *C. jejuni*, determination of A to E lipooligosaccharide (LOS) class. Discriminatory power was calculated by the Simpson's index. Results The predominant MLST clonal complex (CC) for *C. coli* was CC-828 (84%), which contained the predominant sequence type (ST) ST-854 (13%). For *C. jejuni*, the predominant CCs were CC-21 (20%) and CC-45 (11%) and the predominant STs, ST-464 (6%) and ST-5970 (6%). The combined ciprofloxacin, nalidixic acid and tetracycline resistance was the most frequent resistance profile (*C. jejuni* 26% and *C. coli* 30%). The presence of all 5 putative virulence genes was the most frequent profile (C. jejuni 95% and C. coli 65%). All but the LOS class A, were equally found. MLST ST was the most discriminative typing method (*C. jejuni* 0.981 and *C. coli* 0.957). MLST ST combined with AMR profiling was the most discriminative combination (*C. jejuni* 0.988 and *C. coli* 0.983).

Conclusions: Individually, the typing by MLST ST showed the highest discrimination, this discrimination power was increased by the addition of AMR profile determination.

P004

Frequency of Campylobacter species isolated from dogs, broilers, chicken meat, wild boars, red deer, fallow deer and mouflons from Southern Spain

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Four different studies were performed to identify *Campylobacter* species in Southern Spain. A total of 3350 samples were collected: 290 rectal swabs were obtained from dogs in veterinary clinics, 2,221 cloacal swabs from broilers in farms, 476 chicken meat swabs were taken at slaughterhouse and 363 faecal samples from wild ungulates (126 from wild boar, 179 from red deer, 45 from fallow deer and 13 from mouflon). Samples were cultured in selective medium, being *Campylobacter* species identified by multiplex PCR. The average prevalence of *Campylobacter* in Southern Spain was 39.9% (35.2% in dogs, 38.1% in broilers, 69.7% in chicken meat, 38.9% in wild boar, 2.8% in red deer and 7.7% in mouflon. No positive samples were detected in fallow deer). *Campylobacter jejuni* was the species with the highest frequency in the studied area (52.7%), followed by *C. coli* (26.8%). Significant differences in *Campylobacter* species were found between hosts (p<0.001). In this sense, *C. jejuni* was the most frequent species in broilers (512/846; 60.5%), *C. coli* was the most frequent species in chicken meat (166/332; 50%) and in red deer (3/5; 60%), *C. upsaliensis* was the most frequent species in dogs (60/102; 58.8%) and *C. lanienae* was the only species isolated in mouflon (1/1; 100%) and the most frequent in wild boar (34/49; 69.4%).

P005

Defining and implementing a core genome multi-locus sequence typing (cgMLST) scheme for *Campylobacter jejuni* and *C. coli*.

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Background: The increasing availability of large *Campylobacter* collections (thousands of isolates) that have been characterised by whole-genome sequencing (WGS) has created a need for structured approaches that can effectively summarise and analyse these extensive data. The hierarchical gene-by-gene comparison of genomes enables the study of isolates from "domain to strain" and a widely accepted set of core loci common to *Campylobacter* isolates (cgMLST) is one means of facilitating such analyses.

Objectives: The definition of a core genome (v1.0) for comparative genomic analyses of *Campylobacter jejuni* and *C. coli* based on human clinical disease isolates.

Methods: From the 1,643 loci defined by the re-annotation of the NCTC11168 genome, those appearing in 95% or more of 2,742 Oxfordshire human surveillance draft genomes were identified, using the Genome Comparator (GC) function of BIGSdb. Potential paralogues were identified and excluded from the set. Sequence similarities among cgMLST loci were identified using GeneDB. Results Analysis of 2,742 genomes from 2,449 (89.3%) *C. jejuni* and 293 (10.7%) *C. coli* isolates, identified 1,365 shared loci present in 95% of isolates. A total of 22 potential paralogous loci were identified and removed from the core list to provide a core genome multi-locus sequence typing (cgMLST) scheme of 1,343 loci.

Conclusions: The cgMLST scheme (v1.0) has been has been defined for comparative analyses of human *Campylobacter* disease isolates, by public health and research communities worldwide and is available at http://pubmlst.org/campylobacter/.

P006

Histological examination of *Campylobacter fetus* subsp. *venerealis* strain variation in a small animal model

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Infection with *Campylobacter fetus* subsp. *venerealis* in cattle is a leading cause of abortion and infertility. A small animal model can be used to define abortive characteristics of strains, and bacterial infection response. The aim of this study was to compare abortifacient properties between *C. fetus* subsp. *venerealis* strains in a pregnant guinea pig model using vaccination as a virulence parameter. Four groups of 10 pregnant guinea pigs (four vaccinated; vac) and 6 non-vaccinated; non-vac) were challenged intra-peritoneally at week five of gestation with one of four different strains at a concentration of 10^7 CFU/ml. Tissues from dams and foetuses were examined by culture, histologically graded on an empirical scale, and PCR. Proportions were compared using Fisher's Exact test. Strain 76223 resulted in 8/10 abortions (6/6 non-vac, 2/4 vac), strain 924; 2/10 (2/6 non-vac, 0/4 vac), strain 635; 1/10 (1/6 non-vac, 0/4 vac), and strain 600; 2/10 (1/6 non-vac, 1/4 vac), within 12 days (p=0.007). *C. fetus* subsp. *venerealis* induced variably severe fibrinosuppurative placentitis, endometritis and neutrophilic vasculitis, with severity dependent on strain and vaccination status. Changes were most severe and consistent within the junctional zone, however, in highly abortive strains like 76233 neutrophilic inflammation was seen in most compartments of the placental unit. Histological findings correlated with *Campylobacter* reisolations, and were most severe in later aborting dams. Thus, duration of infection, not abortion, correlated with severity of pathology. The pregnant guinea pig model could be used to further investigate the pathogenesis and immunity of *C. fetus* subsp. *venerealis*.

P007

Exploring porA allele diversity among *Campylobacter jejuni* isolated from the environment on a dairy farm.

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Background: The *porA* gene encodes a major outer membrane protein with hyper-variable and conserved regions and which has been proposed as a sub-typing method informing on the diversity of *Campylobacter* isolates subsequent to whole genome sequencing.

Objectives: To investigate the diversity of *C. jejuni* isolated from throughout a UK dairy farm through comparison of porA gene sequences.

Methods: The *porA* gene sequence was determined for 744 isolates of *Campylobacter* from 200 environmental boot sock, water and bovine faecal samples collected from the dairy farm April-October 2013. SplitsTree and phylogenetic analysis were used to investigate the level of recombination and familiarity between the *porA* sequences of the isolates.

Results: A total of 61 *porA* alleles were identified with mixed populations in 44 samples. Sixteen *porA* alleles were only identified in water and thirteen only in faeces. Six *porA* alleles were identified in all three of the sample types, whilst four alleles are associated with persistence, being identified in samples from over half of the duration of sampling. Furthermore, a high rate of turnover of alleles was identified, with 51 (83%) being identified in only one or two months. SplitsTree analysis indicated a significant (P=0.003) amount of recombination events, while phylogenetic analysis showed four main clusters.

Conclusions: This suggests an association between sequence of the *porA* gene and persistence within certain environments on the farm. In addition, based on the phylogenetic trees, we could speculate that the diversity of the isolates is likely due to recombination among *C. jejuni* rather than new *Campylobacter* being introduced onto the farm.

P008

Short-term evolutionary dynamics of *Campylobacter jejuni* in chickens

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Chickens are an important source of human infection with *Campylobacter jejuni* and, although many studies have examined the epidemiology and evolution of this bacterium, few have considered the short-term bacterial evolutionary dynamics following challenge of chickens with fully characterised strains. Chickens were challenged with two *C. jejuni* sequence types, ST-474 and ST-45 previously isolated from chickens. Isolates collected on different kill days were multilocus sequence typed, and 27 of the 168 isolates cultured from the caeca of 12 birds (plus the two inoculum strains) were whole genome sequenced and analysed. Only ST-474 isolates were recovered. No evidence of recombination between the ST-45 and ST-474 strains was observed. We found 15 core single nucleotide polymorphisms (SNPs) and three non-core SNPs across the ST-474 isolates (compared to the inoculum ST-474 isolate). Fourteen of the core SNPs were non-synonymous point mutations confined to nine genes which were all associated with cell shape, chemotaxis or motility of the bacteria. We identified six independent SNPs in a single gene, mreB, encoding a homologue of actin. We identified alterations in the motility of the bacteria which may be associated with the detected SNPs. ST-45 was out-competed by ST-474 in the chicken gut. Non-synonymous mutations were detected, some of which may have had functional effects on the bacteria.

P009

Characterization of Campylobacter free broiler cecal microbiota in an industrial setup

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Campylobacter presence/absence is currently reported in poultry houses by using boot-swab methodology. Modelling of host factors and host/microbe and microbe/microbe interactions in the gastrointestinal tract will be important to identify which members of the complex matrix of the gastrointestinal ecosystem serve as key elements that function as an overall barrier to prevent pathogens from penetrating this ecosystem. We aim to identify the difference in microbiota composition and implement an in house strategy to reduce campylobacter presence in broilers intestine/broiler house using a cost efficient technology. The entire cecum (containing both adherent and luminal bacteria) was harvested from broilers for the purpose of microbiota analysis. The broiler ceca were dissected aseptically and immediately snap frozen (liquid nitrogen) until processing. Cecal DNA was extracted using the QIAamp DNA Stool Mini Kit according to the manufacturer's instructions. Genomic DNA concentration was determined using a NanoDrop. For qPCR analysis, DNA samples were diluted in sterile water to a concentration of 1ng/µl. Our results suggest that poultry diets can be used efficiently to manipulate their microbiota composition and to reduce *Campylobacter* load in farmed poultry.

P010

Detection and management of human Arcobacter infections: still a slow cooking show?

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Background: During a five year single center study in Belgium, *Arcobacter* species were the fourth most common gastrointestinal (GI) pathogen with a prevalence of 2,1%[1]. Detection, identification and antibiotic susceptibility testing is warranted in infants and immunocompromised patients.

Objectives: After completion of the study, culture of *Arcobacter* was incorporated into the routine GI pathogen screening in stool samples. Efficiency of culture, mean turnaround times (TAT) for detection and identification and the need for and performance of antibiotic susceptibility testing (AST) were investigated.

Methods: Culture was performed by plating 48 hours incubated *Arcobacter* enrichment broth onto a solid *Arcobacter* selective medium for 72 hours. Identification was executed by mass spectrometry (MALDI TOF MS, Bruker, Germany), AST by gradient diffusion strip testing (E-test, bioMérieux, France). During a validation period of 16 months, prevalence of *Arcobacter* and TAT's for identification and susceptibility testing were registered.

Results: Prevalence (2.5 %, n=45) of *Arcobacter* was comparable to previous study results. Mean TAT to positivity (4 days) was significantly longer compared to the top ranking pathogens *Campylobacter* (1 day), *Salmonella* (2 days) and *Clostridium difficile* (<1day). Identification with MALDI was fast and accurate. For the patients (15%, n=7) who needed antibiotic treatment, AST result was available within 24 hours after detection.

Conclusions: *Arcobacter* detection in routine setting proved efficient but very slow. Identification using (combined) molecular reference methods is not feasible. MALDI could be a promising but not yet validated tool. AST results remain mandatory in a defined population. E-test methodology is preferred. [1] Prevalence of *Arcobacter* Species among Humans, Belgium, 2008-2013, Emerg Infect Dis.2014; 20:1731-34.

P011

Arcobacter identification: thank heaven there is MALDI?

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Background: Biochemical tests, used for the identification of *Arcobacter* spp., often yield negative or variable results. Molecular methods are considered more reliable but are time consuming and can lack specificity. Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a fast, cheap and robust technique that has recently revolutionized genus and species identification in clinical microbiology. Objective: To validate MALDI-TOF MS Microflex (Brucker Daltonics, Germany) for the identification of human clinically relevant *Arcobacter* isolates. The performance of the *in vitro* diagnostic (IVD) spectrum database was compared to an in house created reference spectrum library.

Methods: The new library (NL) consists of 30 reference strains of four human clinically relevant *Arcobacter* species. A standard score cut-off >2 for species identification was used. A challenge panel of human and veterinary clinical strains of *Campylobacteraceae* as well as reference strains of recently described environment-related *Arcobacter* spp. were included to test sensitivity and specificity. All strains were additionally identified with biochemical tests, m-PCR and PCR-RFLP.

Results: Sensitivity of NL was significantly better than IVD for *A. butzleri* and *A. cryaerophilus* identification (100% and 96% versus 85% and 7.5%). Specificity was excellent without any misidentifications of human clinical strains of *Campylobacter fetus* and *Campylobacter jejuni*.

Conclusions: The IVD spectra database is not fully capable of identifying *Arcobacter* to species level. Introduction of a NL with a more representative set of strains can significantly improve sensitivity of identification with conservation of excellent specificity.

P012

Molecular epidemiology of *C. coli* isolated from different sources in New Zealand between 2005 and 2014

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Background: Campylobacteriosis is one of the most important foodborne diseases worldwide and is a significant health burden in New Zealand. *C. jejuni* and *C. coli* are the most important human pathogens of the genus accounting for approximately 90 - 95% of human cases of campylobacteriosis. Due to the prioritization of the study of *C. jejuni* as a human pathogen, the influence of *C. coli* strains on human health in New Zealand is not well understood and its impact likely to be underestimated.

Objectives: To identify *C. coli* isolates collected in the Manawatu region of New Zealand from human, poultry, environmental water and ruminant faeces sources during the last decade and study their genetic relatedness to measure the contribution of those sources to the burden of human disease.

Methods: Campylobacter isolates were speciated by PCR to detect the ceuE gene associated with *C. coli* and then typed by Multi Locus Sequence Typing (MLST).

Results: A total of 1596 Campylobacter were isolated from human campylobacteriosis cases in the last 10 years with *C. coli* isolates being 2.75% (n=44/1596). MLST revealed 24 sequence types (STs) with ST-1581 as the dominant *C. coli* ST isolated from both human samples (n=11/44) and poultry (n=44/109). ST-1590 was only isolated from human samples and poultry whereas ST-3232 was only isolated from human samples and sheep faeces.

Conclusion: *C. coli* isolates tested in this study especially from poultry meat, and to less extent from sheep faeces, showed relatedness to STs associated with human illness.

P013

The effect of supercoiling on *C. jejuni* physiology

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Supercoiling of bacterial cells is determined by the presence of nucleoid-associated proteins and by the opposing activities of DNA gyrase and Topoisomerase I, introducing and relaxing negative supercoils respectively. For some bacterial species, DNA topology has been shown to change by environmental stimuli and serve as a mechanism of global regulation. Moreover, variation of DNA supercoiling levels of individual cells may cause variation in gene expression and give rise to phenotypic heterogeneity. The focus of this study is to investigate the effect of supercoiling on *C. jejuni* physiology and phenotypic heterogeneity in comparison to *S.* typhimurium, a well-studied organism regarding DNA supercoiling. Decreased supercoiling was imposed by treatment with sub-inhibitory concentrations of the gyrase inhibitor novobiocin. Subsequently, cells were stained with either the fluorescent redoxsensor dye, CTC, or the membrane-potential indicator dye, DiBAC4; the distribution of single-cell responses to the decreased supercoiling analyzed using flow cytometry. This showed the respiratory activity and membrane potential of C. jejuni not to be noteworthy affected by the novobiocin treatment. In contrast, significant increased population diversity of both metabolic activity and membrane potential was observed following novobiocin treatment of Salmonella, thus indicating that supercoiling of Campylobacter does not impose an equal global regulatory role as in Salmonella. On the other hand, it was observed that *C. jejuni* cells without novobiocin treatment displayed greater single-cell diversities of metabolic activity and membrane potential compared to untreated Salmonella cells. Studies are in progress to further characterize the impact of supercoiling levels on the physiology of *C. jejuni*.

P014

CjICE1: a novel, conserved integrative conjugative element in *Campylobacter jejuni* and *Campylobacter coli*

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Background: Mobile genetic elements play important roles in horizontal and lateral gene transfer in bacteria, and are an important agent of genetic transfer in bacteria, and have been responsible for transfer of toxin genes, efflux systems, antibitiotic resistance and virulence properties within and between bacterial species. Four insertion elements (CJIE1-CJIE4) were described previously in *C. jejuni* and *C. coli*, and these show significant genetic variability in gene content and distribution. Objective: To assess the distribution of existing insertion elements and identification of novel insertion elements in *C. jejuni* and *C. coli*.

Methods: A total of 4,232 genome sequences were genotyped in silico for presence of known and novel insertion elements.

Results: The CJIE1-CJIE4 insertion elements show differential distribution patterns, with CJIE3 only found in specific MLST-clonal complexes such as ST-464 and ST-257, with the latter lacking the CJIE3-associated Type VI secretion system. In contrast, CJIE1, CJIE2 and CJIE4 are not associated with specific MLST-types. The genotyping confirmed the genetic heterogeneity of the CJIE1-CJIE4 elements. A new 70-80 kb putative mobile element was also identified in 114/4232 genomes, which has been tentatively named CampyICE1, and is found in both *C. jejuni* and *C. coli*. Unlike the other CJIEs, it is highly conserved and contains tra genes encoding Type IV conjugative pili, a gene encoding an extracellular DNase, and a Type II CRISPR-Cas system, and in some cases genes encoding aminoglycoside resistance.

Conclusion: The Campylobacter genome contains several mobile genetic elements, which could mediate transfer of virulence potential and antimicrobial resistance between Campylobacter isolates.

P015

Genomotyping of *Campylobacter jejuni* and *Campylobacter coli* reveals lineage-specific clustering of metabolic and virulence markers

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Background: The thermophilic Campylobacter species *C. jejuni* and *C. coli* are genetically variable, and genomic differences are thought to contribute to differences in virulence and biology of these important pathogens. International genome sequencing initiatives have ensured that thousands of *C. jejuni* and *C. coli* genome sequences are now available for comparative and functional genomics studies. Objective: To identify candidate genes that may explain differences in biology, transmission and virulence of *C. jejuni* and *C. coli*.

Methods: A total of 4,232 genome sequences were obtained from Genbank, PATRIC and the Campylobacter pubMLST databases. Comparative genomics analyses focused on the identification of lineage-specific genes, and on distribution patterns of metabolic and virulence markers in Campylobacter clonal complexes.

Results: *C. jejuni* and *C. coli* genomes clustered mostly according to MLST-clonal complexes, with livestock-associated sequence types clustering separately from water/wildlife-associated sequence types. In silico genotyping showed distinct distribtion of metabolic markers such as GGT, asparaginase, DMSO reductase, fucose utilisation and vitamine B5 biosynthesis genes. Similarly, putative virulence markers like the Type VI secretion system are limited to related subgroups. Novel lineage-specific genes include iron acquisition systems and a CRISPR system in riparian *C. coli* which differs from the one found in *C. jejuni* and agricultural *C. coli*, consistent with a lack of genetic exchange between riparian and agricultural Campylobacters.

Conclusions: The distinct genetic population structure of *C. jejuni* and *C. coli* is accompanied by lineage-specific distribution patterns of virulence and metabolic markers, which may explain the differences in host range, pathogenicity and transmission potential.

P016

Growth of Campylobacter incubated aerobically in media supplemented with peptones

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Growth of Campylobacter cultures incubated aerobically in media supplemented with peptones was studied, and additional experiments were conducted to compare growth of the bacteria in media supplemented with peptones to growth in media supplemented with fumarate-pyruvate-minerals-vitamins (FPMV). A basal medium composed of (g/l) tryptose, 10; yeast extract, 5; agar, 1.5; and NaHCO3, 0.05 was prepared, then supplemented with 5% (w/v) peptones (beef extract, lactalbumin hydrolysate, or soytone). Campylobacter growth in peptone media was determined by inoculating media with log 4 cfu/ml of *Campylobacter coli, Campylobacter fetus, Campylobacter jejuni,* or *Campylobacter lari* and measuring culture optical density (OD) during aerobic incubation for 48 h at 37C. Additional experiments compared cfu/ml of Campylobacter recovered from inoculated peptone or FPMV media incubated aerobically for 48 h at 37C in culture flasks. After aerobic incubation, cfu/ml were enumerated on selective Campylobacter agar incubated microaerophilically for 48 h at 37C. Results indicated significant ($p < 0.05$) increases in OD of all isolates, except *C. jejuni* 33560, after 48 h of aerobic incubation in media supplemented with beef extract or soytone. Additionally, there was a 4-5 log cfu/ml increase in all isolates cultured aerobically for 48 h in media supplemented with beef extract or soytone, and a 4-5 log increase in *C. fetus, C. coli*, and *C. jejuni* 33560 cultured in media supplemented with FPMV. No Campylobacter were recovered from media that was not supplemented with peptones or FPMV. Findings indicate that beef extract and soytone contain metabolites that support growth of Campylobacter incubated aerobically. These media might provide a less expensive, simplified alternative for culturing Campylobacter.

P017

A capsular methyltransferase and an aminotransferase are associated with serum complement resistance in *Campylobacter jejuni*

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Background: The outer surface capsule of *Campylobacter jejuni* protects the bacterium from complement-mediated killing. However, complement sensitivity often occurs within strains that possess identical capsular serotypes. The objective of this study was to determine whether phase-variable genes involved in capsule biosynthesis steer complement-mediated immunity.

Methods: Knock-out mutants of five phase-variable capsular genes generated in *C. jejuni* strain 11168H (serotype HS:2), were tested in complement killing assays. Serum IgG deposition was measured and the presence of the capsule was assessed using Alcian blue staining. Capsule loci of seven clinical HS:2 isolates were sequenced to assess these phase variable genes.

Results: Knock-out mutants of gene cj1426, a methyltransferase and gene cj1437, an aminotransferase, resulted in clear zones due to complement-mediated killing when bacteria were exposed to 50%, 25% or 12.5% serum. Already at 50% serum, overgrown zones were observed for the remaining knock-out mutants and the wild type strain. Enhanced IgG deposition was observed for strain Δcj1437, but not strain Δcj1426, suggesting that different complement pathways are affected by phase-variation in different *C. jejuni* capsule genes. Alcian blue staining demonstrated that a capsule was produced by all five knock-out mutants. Sequencing revealed that 4/7 and 3/7 clinical *C. jejuni* isolates had a predominant 'phase off' state for genes cj1426 and Cj1437 respectively.

Conclusions: Disruption of a capsular methyltransferase and aminotransferase gene in *C. jejuni* strain 11168H resulted in a phenotypic switch from complement resistance to complement sensitive. However, strain variants that do not express the methyltransferase or aminotransferase may still cause disease.

P018

Pan-genomic comparison of closely related Australasian Rail Campylobacter spp. isolates.

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Background: New Zealand has been isolated for a long time, resulting in many unique endemic species, and this isolation may have affected the microorganisms they host. Within the NZ Rail family is the pukeko (*Porphyrio porphyrio melanotus*), believed to be a recent arrival (~500 years ago) probably from the Australian purple swamphen (APS) (*Porphyrio porphyrio melanotus*) population. A more ancient arrival (~2.5 million years ago) is the takahe (*Porphyrio hochstetteri*). Both of these NZ Rails today share many of the same *Campylobacter jejuni* sequence types, but it is unknown how they relate to the Australian purple swamphens.

Objectives: Make a comparison of genomes from *Campylobacter* spp. isolates from the APS with isolates associated with the pukeko, takahe and a range of other NZ isolates to identify if the pattern of evolutionary divergence is consistent with the hosts historical geographical isolation.

Methods: After next generation sequencing, the draft genomes were sequenced and assembled using a customised pipeline. To represent the core genome we compared 16S and ribosomal Multilocus sequence typing (rMLST). The pan-genome was analysed using a Dollo distance matrix create from a presence absence matrix of homologues.

Results: A consistent pattern of relationships between the isolates was found in the core genome and pan-genome. A putative new *Campylobacter* spp. was identified.

Conclusions: The close relationships between *C. jejuni* from the APS, pukeko and takahe suggest a recent common ancestor followed by divergence after geographical separation. The relationships between genomes from both wild birds and livestock was consistent between the core genome and the pan-genome.

P019

Improved assays to determine the chemotactic behaviour of *Campylobacter jejuni*

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Chemotaxis in *Campylobacter jejuni* enables movement toward favourable conditions and away from hazardous ones and has been shown to be involved in cell invasion and colonisation of the gastrointestinal tract. The Hard agar plug (HAP) technique is one method used to measure chemotactic response of *C. jejuni* toward attractants and repellents. However, this assay does not provide truly quantitative measurements and is not effective for the observation of chemotaxis over short time periods. In addition, measurement of migration by chemotaxis can be complicated because consumption of chemoeffector may create a secondary gradient that the bacteria also sense. The aim of this work was to establish a convenient method to measure chemotactic responses of *C. jejuni* quantitatively over a short time period. We have modified the HAP assay (t-HAP) and included the addition of Triphenyltetrazolium chloride (TTC), to enable quantification. Results showed that modified t-HAP can be quantitatively used to compare different concentrations of chemoattractants over time periods of 30 minutes to 3 hours, with TTC acting as an indicator of viable metabolising chemotactic *C. jejuni* due to enzymatically reduction of TTC- to red TFP (1,3,5-Triphenylformazan). A second assay was developed based on the µ-slide chemotaxis chamber (3D chemotaxis µ-slide , Ibidi GmbH, Martinsried, Germany), a two reservoir system where migration in response to a chemoattractant gradient is directly monitored by microscopy in addition to viable counts. In addition, the µ-slide allowed video tracking of cells to investigate swim/turn patterns.Using these assays, our data confirm that *C. jejuni* chemotaxis depends on amino acid type and concentration. *C. jejuni* has preferential chemotactic patterns, where l-serine was preferred to l-proline, l-glutamate and l-aspartate. To conclude, both methods show an acceptable degree of reproducibility and allow the assessment of attractant specificity in *C. jejuni* and the µ-slide chemotaxis chamber enables tracking individual cell to study motility patterns.

P020

Determination of the cellular localisation of transducer-like proteins in *Campylobacter jejuni* using a fluorescent reporter (iLOV)

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The flavin mononucleotide (FMN)-based fluorescent protein (FbFP) exhibits bright cyan-green fluorescence under both aerobic and anaerobic conditions and can be used to label facultative and strict anaerobic bacteria. We have found that an iLOV reporter, created from the *Arabidopsis thaliana* LOV2 Phototropin domain, Light, Oxygen, or Voltage-sensing (LOV), is a promising reporter in *Campylobacter jejuni*. We investigated the localization of two chemotaxis proteins in *C. jejuni*, which are not predicted to be membrane associated, through fluorescent microscopy (FM) and confocal laser scan microscopy (CLSM) of strains containing gene fusions with the fluorescent reporter (iLOV). A gene splicing by overlap extension PCR cloning strategy was used to fuse the iLOV reporter to tlp5 and tlp8. The coding sequences under the control of either the pfdxA or pPorA promoters were translationally fused to the iLOV domain and this was inserted into the chromosomal Cj0046 locus by allelic exchange. Microscopy of the iLOV fusion mutants indicated that Tlp5 and Tlp8 show a pattern of polar accumulation. Tlp5 clusters were predominately positioned at one pole whereas Tlp8 was found to accumulate at both ends of the cell. These observations show that the iLOV reporter system is a promising tool for monitoring bacteria in situ and for further understanding of biofilm formation.

P021

Local genes, for local bacteria: Geographical structuring in Campylobacter populations arising from local recombination

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Background: Geographical clustering is well documented in bacteria. The historical ancestry *Helicobacter pylori* can be reconstructed in concordance with human patterns of migration and *Mycobacterium tuberculosis* show enough allopatric clustering to successfully manage clinical outbreaks. In *Campylobacter*, source attribution from genomic data has been successful in distinguishing the host source of infection, but not geographical origin.

Objectives: We investigate biogeographical signals in genes that recombine rapidly to determine the extent of clustering in geographically distinct *Campylobacter* population genomes.

Methods: Whole genome sequences from 315 *Campylobacter* isolates from North America and the UK were analysed and the genetic inheritance of 15 closely matched pairs of isolates was quantified.

Results: Isolates from within the same country shared more DNA than isolates from different countries. Using a pairwise approach we identify regions of high diversity and test their correlation with geographical signal. The seven genes that demonstrated the greatest clustering by geography were used to attribute putative source using STRUCTURE. A further 383 UK clinical isolates were used to detect signals of foreign travel. Patient records indicated that 46 cases had travelled abroad less than two weeks prior to sampling and 34 (74%) of those *Campylobacter* genomes were deemed to be from a non-UK origin.

Conclusions: Detection of signals of biogeographical differences in *Campylobacter* genomes will contribute to improved source attribution of clinical Campylobacter infection and inform public policy and contribute to intervention strategies to reduce campylobacteriosis.

P022

Farm specific risk factors for Campylobacter colonization of broilers in six European countries

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This study was part of the EU financed project CamCon. The objective of the study was to identify on-farm risk factors for *Campylobacter* colonization of broiler flocks based on comparable data from six European countries. The data included explanatory variables from a large questionnaire concerning production, farm management procedures and conditions, climate data on mean temperature, sunshine hours, precipitation, as well as data on *Campylobacter* status of broiler flocks. Overall, the study comprised data from more than 6000 flocks. The data were analyzed using a generalized linear model using backwards elimination and forward selection. Due to the structure of the data, several models were explored, by applying different strategies for categorizing explanatory variables and for selection and elimination of variables in the model. The risk of broiler flocks becoming colonized with *Campylobacter* was clearly affected by country. In descending order; broiler flocks were more likely to be colonized in Poland, the UK, Spain, the Netherlands, Denmark and Norway due to country specific factors that could not be explained by the management and climate variables in the explored models. The seasonality in the prevalence of *Campylobacter* was described nicely by temperature, i.e. the number of positive flock increased with increasing temperatures. The age of broiler houses, presence of anterooms and barriers in all houses, designated tools for each house as well as length of downtime and the type of drinker systems were found to affect the risk of the broiler flocks becoming colonized by *Campylobacter*.

P023

Association of H. pylori eradication with alcohol and smoking

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Background: *Helicobacter pylori* (*H. pylori*) infection is very frequent world-wide and causes gastritis, ulcers, MALToma, cancer, dyspepsia, etc. Eradication of *H. pylori* plays an important role in treatment of those diseases. Objectives; We hypothesized patient's life style, co-morbidities such as history of alcohol, smoking, diabetes mellitus, hypertension would affect eradication of *H. pylori*.

Method: Total of 156 patients were analyzed who had Urea breath test (UBT) between February, 2012 and February, 2015. Standard triple therapy was used in all cases. Eradication result after each therapy was checked with parameters that could affect the outcome such as age, sex, smoking, alcohol, diabetes mellitus, hypertension.

Results: Among 156 patients, 18 patients were excluded due to missed UBT result. 102 (73.9%) patients showed successful eradication outcome and 36 (26.1%) did not. The eradication rate was 82.3% (n=28) among smokers (n=34) and 71.4% (n=50) among non-smokers (n=70), 77.3% (n=34) among alcoholics (n=44) and 70.5% (n=43) among non-alcoholics (n=61), but results were not statistically meaningful.

Conclusions: In this study, the differential eradication rates of *H. pylori* in alcoholics and smokers were not significant. However, to determine the factors affecting UBT outcomes more precisely, prospective studies should be considered.

P024

In ovo vaccination to prevent colonization of broilers by *C. jejuni*

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Colonization of poultry by *Campylobacter jejuni* is a significant food safety risk. *C. jejuni*, a harmless commensal in poultry, causes disease once transferred to humans, usually via handling or consumption of insufficiently cooked poultry products. Vaccination to prevent colonization of the poultry is one strategy to reduce the risk of contaminated food. Vaccination against avian viral diseases by the in ovo route is routinely practiced by the broiler industry and is the preferred route of delivery for vaccines as it allows the development of early immunity, uniform and rapid delivery of vaccine, reduced stress to the birds and reduced labour costs. Since newly hatched chicks are not routinely colonized by *Campylobacter* but are readily colonized by two weeks of age, the development of early immunity is especially attractive. We have tested the use of subunit vaccines given by in ovo injection. The use of subunit vaccines is a new approach for this route of vaccination. We have cloned and expressed selected *C. jejuni* proteins in *E. coli*. We have determined that in ovo vaccination using subunit proteins or a whole cell bacterin given without adjuvant does not result in a significant immune response. In addition no reduction in level of colonization by *C. jejuni* was observed after challenge of the birds. It appears that for this approach to be successful an appropriate adjuvant will be needed. Our investigation is now focused on the selection of additional subunit proteins for use and on the investigation of the effects of various adjuvants.

P025

In vitro phase variable genes of *Campylobacter jejuni* display evidence of inter-gene dependencies and selection

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In vitro data of the *Campylobacter jejuni* strain NCTC11168 with an inoculum and five subsequent passages comprises of 28 genes assumed to have the property of phase variation. In this process, the protein expression of a gene experiences a reversible change between an ON or OFF classification of phenotypes due to high frequency mutations of its poly-G/poly-C tracts. The genes were subject to pairwise Fisher's exact test, under the null hypothesis that the genes can be treated as non-interacting (independent) when mutations occur through cell reproduction between the passages. Inter-gene dependencies are seen with strong significance for the three genes cj0617, cj0685 and cj1437. Simulation of a stochastic mutation only model shows that for these three genes, with experimentally estimated mutation rates and number of generations, the final distribution of observed phenotypes is different to the estimated distribution. These observed distributions fall outside a 95% confidence interval of acceptable distributions in which the mutation only model would work. By utilising a stochastic model instead with both mutation and selection, the model estimates now closely resemble the observed final distributions. Approximate Bayesian Computation (ABC) finds fitness parameters from the model, with selective advantages for the three genes between 0.5-2% (1.005-1.02). The evolution of gene cj1437 is clearly governed by selection, because the two-nucleotide switch from tract length 9G in the inoculum (ON state) to tract length 11G in the final distribution (OFF state) is unlikely to occur due to mutation only, whereas the other 27 genes only exhibit either a 0G or 1G switch.

P026

Campylobacter contamination of carcasses during the slaughter of broilers in Ecuador.

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Background: *Campylobacter* are found in poultry and represent an important cause of gastrointestinal infections worldwide. Contamination of broiler carcasses occurs mainly during the slaughtering process and the public health risk is closely related to the number of *Campylobacter* present on carcasses. In contrast to developed countries dynamics of *Campylobacter* contamination of broiler carcasses during slaughter in developing countries are rarely reported.

Objectives: The aim of this study was to provide quantitative data about *Campylobacter* contamination during slaughter in Ecuador.

Methods: Ten *Campylobacter* positive flocks slaughtered in two slaughterhouses (5 flocks each) using manual evisceration were sampled. From each flock 5 samples of breast skin were aseptically collected after plucking, after evisceration, before chilling (final washing) and after immersion chilling. *Campylobacter* counting was performed using Rapid Campylobacter Agar.

Results: Obtained results indicated that during the whole slaughter process *Campylobacter* counts differed considerably for flock to flock in both slaughterhouses. In both slaughterhouses evisceration and final washing step did not lead to a significant change in *Campylobacter* load ($p>0.05$) compared to the load after plucking. However immersion chilling caused a significant decrease in the *Campylobacter* counts on carcasses in both slaughterhouses ($p<0.05$).

Conclusions: *Campylobacter* counts after plucking were not influenced by manual evisceration and final washing. In contrast immersion chilling of carcasses reduced considerably the counts on carcasses leading in most case to a contamination of the breast skin of least than 1000cfu/g probably due to the addition of chlorine.

P027

Population SNP profiling (PSP) of *Campylobacter jejuni* from the poultry and dairy farm environment

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Background: Although the main route of transmission of *Campylobacter* to humans is thought to be foodborne, the role of the environment is poorly understood. Furthermore, current knowledge is derived from cultured bacteria. Given variations in culturability and the likelihood for intra-species diversity, it is likely that data based on culture methods alone do not reflect the true population composition in the environment.

Objectives: Our aim was to develop a novel sequencing-based technique to define *Campylobacter* population profiles in samples from the poultry and dairy farm environment in order to track temporal-spatial changes in populations.

Methods: An Illumina amplicon sequencing-based population SNP profiling (PSP) approach was developed utilizing the MLST loci of *C. jejuni*. The technique was tested on mock communities composed of various combinations of known sequence types and then applied to bacterial DNA extracted directly from poultry and dairy farm bootsock and water samples, enriched in Exeter broth.

Results: The mock communities designed to mimic natural *Campylobacter* populations with varying diversity and complexity demonstrated that PSP is able to differentiate well between sequence types, with rare alleles (1% of total composition) easily detectable. There was statistically significant correlation between the predicted and detected (by sequencing) SNP profiles in mock communities.

Conclusions: We present a novel amplicon sequencing based approach for analyzing and comparing *Campylobacter* population structures. This approach indicates considerable intra-species diversity in some environmental samples. Our findings will give an insight into the true distribution and transmission routes of *Campylobacter* populations in the farm environment.

P028

Cycling of *Campylobacter jejuni* in a dairy farm environment

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Campylobacteriosis, the most frequently reported bacterial gastrointestinal disease in NZ, is primarily caused by *Campylobacter jejuni*. Dairy cows are recognised as an important reservoir from which transmission of *Campylobacter* to humans can occur. The aim of this study was to explore the mechanisms underlying the excretion of *C. jejuni* in a dairy herd by investigating potential on-farm sources for cow contamination. The study was carried out on a commercial dairy farm located in the Waikato. Samples collected monthly from May until October 2012 included faeces (cow, bird and rat), feed (pasture and supplements), soil and trough water. *C. jejuni* was identified by culture and species-specific PCR. Isolates were compared and grouped according to ERIC fingerprint analysis (>90% similarity); a representative of each group was characterised by MLST. *Campylobacter jejuni* was present in 150 of 380 samples. Thirty-one ERIC groups and 16 MLST types were identified from the 264 *C. jejuni* isolates recovered. ERIC types that were identified as ST42 and ST2345 were each recovered from cows, birds, pasture, supplementary feed and soil. ERIC types that were identified as MLST ST45 were recovered from cows, birds, rats, pasture and supplementary feed. Cows, birds and rats were all identified as sources of *C. jejuni* and feed, pasture and supplements, together with trough water were potential vectors of cycling within the farm environment. These findings suggest that farm mitigation practices developed to control the faecal shedding of *Campylobacter jejuni* by cows should consider the broad ecology of this bacterium in the farm environment.

P029

Implementation of whole genome sequencing for routine public health surveillance of Campylobacter species

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Background: Seven-locus MLST as a method for strains discrimination amongst isolates of *C. jejuni* and *C. coli* was introduced in April 2014 at the UK national laboratory to replace the phenotypic typing

Methods: which included serotyping and phage typing. However, MLST based on Sanger Sequencing data from PCR amplicons has proved costly and labour intensive. Objective: Derive MLST and speciation from Whole Genome Sequencing (WGS) from cultures as a routine method, thereby improving speed and accuracy of results. Method: WGS is performed using Illumina technology. FASTQ reads are quality trimmed and a K-mer identification step identifies strains to species level. Bioinformatics software (BIGSbd and in-house mapping and SNP calling tools) uses short read Illumina FASTQ files to determine MLST profiles.

Results: From 122 isolates, WGS determined full STs in 96% of cases using an in-house bioinformatics pipeline, 93% using PubMLST and, 81% by the classical method of PCR and Sanger Sequencing. Identification of only MLST clonal-complex was possible for 17% by Sanger sequencing and 7% by WGS. One isolate of *C. coli* gave a discrepant result at ST level from WGS data analysed by the in-house and PubMLST methods, due to a mixture of two different strains (ST7346 and ST1764).

Conclusion: WGS is an accurate, rapid, cost effective method for determining both the MLST and species identification for Campylobacter in a single assay. Sequence data can easily be shared and potentially used in further analysis for detecting antimicrobial resistance mechanisms and in whole genome MLST schemes to provide greater strain discrimination for outbreak investigations.

P030

The role of lipid asymmetry in maintaining the integrity of the outer membrane of *Campylobacter jejuni*

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Background and objectives: The outer membrane (OM) of Gram-negative pathogens plays crucial roles in adhesion, interaction with the immune system, cell-signalling and in nutrient uptake. The OM has asymmetrical lipid distribution, with the outer-leaflet containing lipid A conjugated to oligosaccharide (LOS) and the inner leaflet primarily comprised of phospholipids (PLs). Under stress conditions, PLs can migrate from the inner leaflet and accumulate in the outer leaflet, forming aberrant regions of phospholipid bilayer, causing destabilisation and loss of barrier function of the OM. *Methods and results*: In *E. coli*, lipid asymmetry is maintained either by the destruction of outer leaflet PLs by the phospholipase PldA, or their translocation to the inner membrane by the Mla system (maintenance of lipid asymmetry), comprising an outer membrane protein, MlaA, a periplasmic lipid-binding protein, MlaC, and an ABC transporter-like inner membrane complex, MlaBDEF. Homology searches identified a homologous Mla system in *C. jejuni*, as well as PldA. We have constructed a complete set of *C. jejuni mla* mutants and complemented strains, and characterised growth, antimicrobial sensitivity, *in vivo* infection ability in the *Galleria* model, and the ability of PldA to collude with the Mla system in maintaining OM integrity. We show that *mla* mutants have significant growth defects, increased sensitivity to osmotic, oxidative and antimicrobial stressors, and have attenuated colonisation *in vivo*. *Conclusions:* We suggest that the Mla system plays a crucial role in *C. jejuni* in maintaining lipid asymmetry, and therefore integrity, of the OM, which directly impacts on colonisation ability and pathogenicity.

P031

Shedding patterns of C. jejuni by New Zealand dairy cows

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Dairy cows have been identified as common carriers of *Campylobacter jejuni*, the cause of many human gastroenteritis cases reported in New Zealand. The first step in developing on-farm mitigation that controls human infection linked to dairy activities is to determine *C. jejuni* excretion patterns in naturally infected cows. In this study, the same 35 cows were monitored fortnightly over one year to determine the temporal fluctuations in *C. jejuni* faecal concentration and the underlying changes in the excreted genotypes. Studied cows were from two farms in the Waikato region (New Zealand). A total of 20 to 25 samples per cow were enumerated for *C. jejuni* by a Most Probable Number technique (detection limit: 0.3 *C. jejuni*.g^{-1} faeces), and two isolates per positive sample were genotyped by Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR. The individual cows exhibited unique *C. jejuni* excretion patterns, and on both farm excretion patterns ranged from sporadic to chronic. Most cows excreted *C. jejuni* intermittently with excretion periods ranging from less than 2 to 33 weeks interrupted by periods of no excretion that lasted between 4 to 45 weeks. Chronic excretion (>90% of the collected faeces positive for *C. jejuni*) was observed for 13 cows. The *C. jejuni* concentration in consecutive samples was variable but changes in concentration were not linked to changes in the observed ERIC genotypes. The variable individual pattern of excretion suggests that more than one mechanism of infection may occur within dairy cows. Further work is required to understand the drivers for this variability.

P032

Molecular epidemiology of human clinical cases of campylobacteriosis in Nova Scotia, Canada

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The incidence rate of campylobacteriosis in Nova Scotia (18/100,000 population) is similar to that of salmonellosis. While molecular subtyping of isolates from human clinical cases is routinely conducted for Salmonella, Campylobacter isolates are not subtyped, limiting the ability to understand case etiology, monitor trends and detect outbreaks. This study investigated the use of high resolution subtyping with Comparative Genomic Fingerprinting (CGF40) to examine the epidemiology of campylobacteriosis in Nova Scotia. Campylobacter isolates from human stool specimens collected from January 1, 2012 to March 31, 2015 (n=288) were subtyped using CGF40 in order to identify genotypic clusters. Information from case report forms identified geographical exposure locations and epidemiologically-linked case clusters. Fingerprints were also compared to those in a national CGF database (n=19,049) to assess source associations. The 288 isolates (51% of reported campylobacteriosis cases) comprised 139 distinct subtypes, with 72% of isolates sharing fingerprints with one or more isolates. Some subtypes were detected in multiple years with exposures in local and overseas locations, while others were rare. All four *Campylobacter jejuni* case clusters identified by public health were confirmed by CGF40 and CGF40 identified additional case associations. The majority of isolates (86%) matched existing subtypes in the CGF database, of which chicken represented the sole or dominant non-human source (69%). CGF identified isolates from patients with known epidemiologic links. Routine analysis of human clinical isolates using CGF40 may play a useful role in public health surveillance and outbreak investigations of campylobacteriosis.

P033

Effects of 'end of life stressors' on Campylobacter jejuni biology in a chicken model

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Background andd **Objectives:** The understanding of *Campylobacter* population responses in the chicken to stresses in the farm and processing environments is limited. Broiler chickens will experience acute stress during de-population and transport. Consequently, we have assessed how *C. jejuni* behaves in standard commercial broiler chickens following stress by handling, feed withdrawal, and simulated transport.

Methods: We infected 40 Ross 308 birds by oral gavage with *C. jejuni* at 21 days of age. Twenty birds were infected with M1 strain of *C. jejuni*, and twenty were infected with the 13126 strain of *C. jejuni*. At 35 days of age, 10 birds from each strain group had their feed withdrawn for 4 hours and were placed into transport crates for 3 hours to stimulate transport to the slaughterhouse, prior to being killed for post mortem analysis (along with the unstressed birds). The contents of the caeca and ileum, and the liver were enumerated for *C. jejuni*. Blood was collected to assess heterophil:lymphocyte ratios as a marker of stress.

Results: There was a significant difference between the numbers of 13126 in the ileum between stressed and unstressed birds (Mann-Whitney U test; $p < 0.001$). In contrast, there was a significant difference between the numbers of M1, between stressed and unstressed birds, in the caeca (Mann-Whitney U test; $p < 0.05$).

Conclusions: This data suggests that host stress can have an impact on the *Campylobacter* populations within the chicken gut. A greater understanding and control of *Campylobacter* population responses in the chicken gut may reduce the infection of edible tissues in broilers.

P034

Survival of Campylobacter in the poultry farm environment

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Background and Objectives: Mechanisms by which *Campylobacter* survives in the farm environment, transmits to broilers and colonises them, are not well understood. Currently, we are determining the population structure of *Campylobacter* circulating in the external broiler farm environment and determining how this correlates with those in the broiler house environment infecting broilers on a standard commercial farm.

Methods: Boot socks are used to sample the different environmental compartments (for example, around each broiler house, the paths to the broiler houses, entrance to the farm and neighbouring fields) and water samples collected from transient and permanent water sources on the farm, to capture the presence and strain diversity of *Campylobacter* over space and time.

Results and Conclusions: Out of 734 boot socks collected, a higher proportion of culture-positive ones were obtained over the winter period (often preceding and following flock-positivity), compared to the summer months. Out of 516 water samples collected, only 24% were culture-positive for *Campylobacter*. Furthermore, no one area of the external farm environment was consistently culture-positive for *Campylobacter*. Most broiler flocks became positive with *C. jejuni* or *C. coli* following partial depopulation; however during the summer months, flocks became positive prior to this process. A greater understanding of *Campylobacter* survival in the environment could improve the control mechanisms implemented by farmers to reduce *Campylobacter* colonisation of broilers.

P035

Molecular typing of Campylobacter isolated from the poultry farm environment

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Background and Objectives: *Campylobacter* survival in the external poultry farm environment and onward transmission to the broilers are not well understood. We are investigating the population structure of campylobacters circulating in the external broiler farm environment and establishing how this compares with those inside the poultry houses colonising broilers on a standard commercial farm.

Methods, results, and Conclusions: *Campylobacter* spp. were isolated from inside the poultry houses, the external farm environment (including around the poultry houses, paths, and neighbouring fields) and from transient bodies of water. Molecular typing of the *porA* gene of 282 *C. jejuni* isolates and 90 *C. coli* isolates has demonstrated a high turnover of different *porA* alleles over time, in both the broiler flocks and the external farm environment. There also appeared to be more diversity in the *porA* alleles in the external environment compared to those isolated from the poultry houses. Furthermore, *Campylobacter* spp. with the same *porA* alleles were isolated from both the external environment and inside the poultry houses, suggesting that the external environment might represent a source of *Campylobacter* colonising the broilers. Further work will involve whole genome sequencing of 147 *C. jejuni* isolates obtained from the external and internal poultry farm environment during two flock cycles to determine population structure circulating in these environments.

P036

Prevalence of prophage zot-like genes CCC13826_0191 and CCC13826_1210 in oral *Campylobacter concisus* strains isolated from patients with inflammatory bowel disease and healthy controls

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Campylobacter concisus is an oral Gram-negative bacterium that has been associated with inflammatory bowel disease (IBD). *C. concisus* holds multiple prophages in its genome, one of which is CON_phi2 and contains the zonula occludens toxin (*zot*) gene. Two prophages, CON_phi3 and CON_phi4 contain *zot*-like genes CCC13826_0191 and CCC13826_1210 respectively. It was previously reported that 30% of all oral *C. concisus* strains possess the *zot* gene. The prevalence of CCC13826_0191 and CCC13826_1210 genes in *C. concisus* strains was investigated in this study. A total of 56 oral *C. concisus* strains were examined. Of these, 32 strains were isolated from patients with IBD and 24 strains were isolated from healthy controls. Polymerase chain reaction (PCR) was used to detect the *zot*-like genes CCC13826_0191 and CCC13826_1210. All PCR products were sequenced. The CCC13826_0191 gene was detected in 8.9% strains (5/56); the CCC13826_1210 gene was detected in 23.2% strains (13/56) and 5.4% (3/56) of the strains carried both genes. The prevalence of the CCC13826_0191 gene in *C. concisus* strains isolated from patients with IBD was 9.4% (3/32), which was not significantly different from healthy controls (8.3%, 2/24). The prevalence of the CCC13826_1210 gene in *C. concisus* strains isolated from patients with IBD was 15.6% (5/32), which was not statistically different as compared to that in healthy controls (33.3%, 8/24). These data indicate that 8.9% and 23.2% of *C. concisus* strains have acquired prophages CON_phi3 and CON_phi4 respectively. Whether proteins encoded by these prophages have biological effects on human intestinal epithelial cells remains to be investigated.

P037

Identification of Helicobacter species in the biliary tract of Mexican patients

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Background: The presence of Enterohepatic Helicobacter species (EHS) has been reported in the human bile duct, and it has been suggested that *H. bilis* and *H. hepaticus* infection may be a risk factor for cholangiocarcinoma (CCA), which represent the sixth cause of malignant lesions in the gastrointestinal tract in Western countries. Objectives. We investigated the presence of *H. bilis*, *H. hepaticus* and *H. pylori* in patients diagnosed with extrahepatic cholangiocarcinoma (ECCA) or with benign pathology of the common bile duct in a Mexican population.

Methods: A total of 192 samples from the bile ducts were obtained during endoscopic retrograde endoscopic cholangiopancreatography (ERCP), and diagnosed as ECCA (102) or with benign pathology (90). DNA was extracted and used to amplify the 16S rRNA gene from *H. bilis* and *H. hepaticus* and the *vacA* and *cagA* genes from *H. pylori* by PCR.

Results: We identified the presence of DNA from the three Helicobacter species. EHS were detected in 84/192 (43.8%) samples, whereas *H. pylori* was detected in 141/192 (73.4%) samples. From all three Helicobacter species, only the presence of DNA from *H. bilis* was significantly associated with cases of cancer in the common bile duct (p=0.0005; OR 3.56, 95% CI 1.77-7.17). Conclusions. Infection with *H. bilis* was significantly associated with tumors located in the common bile duct. The high frequency of *H. pylori* DNA was interesting but unexpected since it is considered that the natural niche for this pathogen is the human gastric epithelium.

P038

Transcriptome and proteome analysis of *Helicobacter pylori* in co culture with *Acanthamoeba castellanii.*

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Helicobacter pylori is a causative agent for acute and chronic gastritis. Transmission route of *H. pylori* is not clearly known, and it is implied that *H. pylori* are transmitted from various environments because their DNA are detected in water. Protozoa including free-living amoeba are universally developed in water and soil. They generally eat bacteria, however, some species of bacteria have resistance against digestion system of protozoa. Thus, some protozoa are regarded as reservoir of bacteria in environments.

Objective: In this study, it was analyzed if *H. pylori* survive in co-culture with *Acanthamoeba castellanii* and expression of mRNA and proteins in *H. pylori* are different in between co-culture with the protozoa and single culture.

Method: *A. castellanii* Neff strain and *H. pylori* TK1402 strain were co-cultured in PBS. Microplates were incubated and sampled for fluorescence microscopy, CFU assay, electron microscopy, SDS PAGE, and RNA-seq.

Results: 24 hours after incubation, the numbers of *H. pylori* were $1.6x10^6$ 0.3CFU/ml and $1.8x10^4$ 0.1CFU/ml in co-culture and bacterial single culture, respectively. SEM showed that coccoid formation of *H. pylori* was mainly observed in bacterial single culture but spiral form was kept in co-culture with *A. castellanii*. By SDS PAGE analysis, produced proteins of *H. pylori* in *A. castellanii* were different from those in bacterial single culture. Transcriptome analysis showed that proteins related with outer membrane protein and cell motility were up-regulated in co-culture with *A. castellanii*.

Conclusions: The results obtained suggest a possibility that *H. pylori* might survive in co-culture with protozoa.

P039

Structural identification and antibacterial mechanism analysis of a novel anti-microbial peptide against *Helicobacter pylori*

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Helicobacter pylori(Hp) infection is the major cause of chronic gastritis and peptic ulcer diseases. Routinely, combination of antibiotics with other interventions is used to treat Hp infection. However, this therapy results in side effects e.g. reemergence of Hp infection after initial clearing. Thus, new biological therapies, to completely or partially replace antibiotic therapy is warranted. We previously isolated and purified a novel peptide from the supernatant of *B .subtilis* strains with anti-Hp activity. The purity of this peptide is 99.01%, with high thermal stability and a wide range of pH adaptability. We preliminarily evaluated its molecular weight and amino acid composition. The peptide structure will further be identified and characterized by combining use of amino acid sequence analysis, C Nuclear magnetic resonance (CNMR), H-nuclear magnetic resonance (HNMR) and Distortionless Enhancement by Polarization Transfer (DEPT) methods. Electron microscope analysis showed that the cell membrane of *H. pylori* was still intact when treated with this peptide, indicating a non-lytic mode of antimicrobial action that goes beyond membrane disruption. RNAseq and iTRAQ

Methods: will further be used to find differential expressed genes in *H. pylori* treated with and without this peptide. The downstream effect of these differential expressed genes will be identified by comet assay and DNA binding assay to reveal the antimicrobial mechanisms of this peptide to *H. pylori*. This study reveals a non-lytic mode of antimicrobial action of the peptide and provides a theoretical basis for its development as the drug candidate for the prevention of *H. pylori* infection.

P040

Interactions between broth type and incubation atmosphere influence dissolved oxygen content of media and biofilm formation by *Campylobacter jejuni*

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Campylobacter jejuni biofilms have been suggested to play a role in their survival in the environment. Reports indicate that biofilm formation is enhanced under aerobic conditions. Different growth media used to study this feature may confound results because of the presence or absence of reducing agents. Biofilm formation by eight *C. jejuni* strains under both aerobic and microaerobic conditions in different broths (Mueller-Hinton (MH), Bolton and Brucella) was quantified. The dissolved oxygen (DO) content of the broths under both incubation atmospheres was determined. The results showed that biofilm formation for all strains was highest in MH broth under both incubation atmospheres. Four strains had lower biofilm formation in MH under aerobic as compared to microaerobic incubation, while biofilm formation by the other four strains did not differ under the two atmospheres. Two strains had higher biofilm formation under aerobic as compared to microaerobic atmospheres in Bolton broth, while biofilm formation by all other strains in Bolton, and all strains in Brucella, broth did not differ under the two atmospheres. Under aerobic incubation DO levels in MH > Brucella > Bolton broth, while under microaerobic conditions levels in MH = Brucella > Bolton broth. Levels of DO in MH and Brucella broth were lower under microaerobic conditions but those of Bolton did not differ under the two atmospheres. Results indicate that interaction of broth type with incubation atmosphere influence DO content. These findings impact on interpretation of reports that aerobic atmospheres enhance biofilm formation by *C. jejuni*.

P041

Exploring genetic diversity of the porA gene in *Campylobacter jejuni* and *Campylobacter coli* from poultry in Kenya.

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Background: Campylobacter are the most commonly reported bacterial food-borne pathogens in the world. A number of different molecular typing schemes exist for Campylobacter and have revealed considerable diversity amongst the genus. More recently, diversity of the major outer-membrane protein (MOMP) of *C. jejuni* and *C. coli*, encoded by the porA gene has been exploited in genotyping studies. Although a few studies have examined genetic diversity of Campylobacter in Africa, very little is known about the Campylobacter population in poultry in Kenya.

Objectives and Methods: The aims of this study were to examine diversity in the porA gene of 162 *C. jejuni* and 56 *C. coli* isolates collected from poultry in Nairobi, Kenya between January 2012 and August 2013. Results In total, 76 different porA alleles were found amongst the 162 *C. jejuni* and 56 *C. coli* isolates; of which 26 were new porA alleles. The most common alleles were 11, 57, 173, 1806 and 1807, representing 4.6%, 6.4%, 4.1%, 5.0% and 5.5% of the strains, respectively. Phylogenetic analysis identified three porA allele clusters; the first cluster contained predominantly *C. jejuni* (n=95) and one *C. coli* isolate, the second contained solely *C. jejuni* isolates (n=65), and the third predominantly *C. coli* (n=55) and two *C. jejuni* isolates. There was no geographical association between the different porA alleles found.

Conclusions: Diversity in the porA gene is useful for assessing diversity in the Campylobacter population prior to using the more time consuming and labour intensive molecular typing schemes or whole genome sequencing.

P042

Assessing the effect of sub-MIC antibiotic treatment at multiple molecular levels in *Campylobacter jejuni*

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Rising antibiotic resistance and the propensity of campylobacters for carrying antibiotic resistance markers, make these bacteria an increasing public health threat. Campylobacters are likely to be exposed to sub-lethal or sub-MIC levels of antibiotics in their natural avian host which has previously been linked to tolerance & persistence in poultry. The aim of this work was to examine the effect of sub-MIC concentrations of antibiotics (tetracycline, erythromycin and ciprofloxacin) on *Campylobacter jejuni* NCTC11168 & RM1221 on multiple molecular levels; DNA, RNA, and DNA methylation. A step wise training method was used to produce tolerant or resistant isolates. Cells exhibiting a developed tolerance were assessed for stability via repeated sub-culturing onto antibiotic-free medium. The nature of either the tolerance or resistance of each 'trained' isolate was established by PCR & Sanger sequencing of known regions of mutation, such as cmeB & gyrA. Isolates were then examined at a methylome and transcriptome level using an Oxford Nanopore technologies MinION™ in combination with other techniques such as restriction digests and bisulphite modification. Exposure to sub-inhibitory levels of antibiotics can lead to changes in the bacterium that have not yet been investigated and although the molecular mechanisms of campylobacteriosis are not completely defined, from studying the enhanced survival of C. jejuni following response to antibiotics, may allow for insight to be gained, with regards to virulence.

P043

The effect of temperature and rainfall on human Campylobacter infections in England and Wales

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The direct linkage of Campylobacter cases in England and Wales to temperature and rainfall parameters was achieved by using an algorithm developed in the Met Office and using specimen date and laboratory postcode. Weather variables in the previous 30 days were tested using regression analyses. Patient postcode data was used to assign cases to Lower Super Output Areas (LSOA) and the cases and populations of LSOAs was then used to compile a database of weekly records over five years 2005 to 2009. Data on 227,398 cases and 258 weeks within LSOAs were then ordered by temperature into deciles. The Campylobacter incidence showed a polynomial regression relationship to average temperature per week ($R^2 = 0.9916$), maximum temperature per week ($R^2 = 0.9982$) and minimum temperature per week ($R^2 = 0.9974$) two weeks before the specimen date. The approach allows an examination of relationships to weather at different times of the year and with different lag periods. A limitation is that the analyses only include data on the incidence in LSOA populations with one or more cases within a week. The results were compared to a generalized structural time-series model (GEST), generalised additive model of location, scale and shape (GAMLSS) and wavelet analysis. This approach demonstrates the value of using direct data linkage between Campylobacter cases and local weather parameters prior to diagnosis, along with cumulative incidence. The method provides evidence that can contribute to a better understanding of the main drivers for the annual exponential increase in Campylobacter cases in the late spring, and may also prove useful in estimating the likely impact of climate change on Campylobacter infections if there are no public health interventions.

P044

Arcobacter butzleri induce inflammatory responses in IL-10 deficient gnotobiotic mice

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Background: Acute gastroenteritis with abdominal pain and acute or prolonged watery diarrhoea has been described for humans infected with *Arcobacter* (*A.*) *butzleri*. Adhesive, invasive and cytotoxic capacities have been described for *A. butzleri in vitro*. So far, only limited information is available about the immune-pathogenic mechanisms of infection *in vivo*. Objective: The aim of this study was to investigate the immune-pathological properties of *A. butzleri* in a well-established murine infection model, reflecting major clinical aspects of human campylobacteriosis.

Methods: Gnotobiotic IL-10-/- mice, achieved by broad-spectrum antibiotic treatment of conventionally raised mice, were orally infected with two different *A. butzleri* strains and clinical signs as well as fecal shedding were determined over time. At day 6 and day 16 post-infection apoptotic and proliferating cells, intestinal infiltration with immune cells and cytokine expression patterns were determined.

Results: Despite no overt macroscopic signs of disease, stable infection of IL-10-/- gnotobiotic mice with *A. butzleri* led to increased numbers of apoptotic cells, influx of immune cells and higher expression of pro-inflammatory cytokines in the intestine, depending on the respective *A. butzleri* strain. For instance host responses were delayed after infection with strain C1 as compared to the H1 strain.

Conclusion: Even though no overt clinical signs have been observed we could clearly show that *A. butzleri* is able to stably colonize and induce apoptosis paralleled by induction of pro-inflammatory immune responses in the intestine of infected IL-10-/- gnotobiotic mice, pointing towards an immune-pathogenic potential of *A. butzleri in vivo*.

P045

Different Campylobacter species show variances in their heat stress response

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Background: Compared to other zoonotic bacteria, *Campylobacter* species are quite susceptible to environmental or technological stressors. This might be due to the lack of many stress response mechanisms described in other bacteria. So far, only for *Campylobacter* (*C.*) *jejuni* details of the heat stress response are known.
Objectives: The heat stress response of *C. coli* and *C. lari* was compared to the response of *C. jejuni*.

Methods: Survival rates and whole transcriptome analyses at 46°C were investigated for the strain *C. jejuni* NCTC11168, *C. coli* RM2228 and *C. lari* RM2100.

Results: Under heat stress (46°C) *C. jejuni* showed the highest survival rates, followed by *C. lari* and *C. coli*. Transcriptomic analyses revealed that only 3 % of the genes in *C. jejuni* and approx. 20 % of the genes of *C. coli* and *C. lari* were differentially expressed after heat stress, respectively. The transcriptomic profiles showed enhanced gene expression of several chaperones in all strains, but differences in the gene expression of transcriptional regulators as well as for genes involved in metabolic pathways, translation processes and membrane components. However, the function of many of the differentially expressed gene is unknown so far.

Conclusion: Our data suggest that the heat stress response of *C. coli* and *C. lari* are more similar to each other compared to *C. jejuni*. This indicates that stress response mechanisms described for *C. jejuni* might be unique for this species and not necessarily transferable to other *Campylobacter* species.

P046

Farming environment and litter practices adopted in Australia have little impact on Campylobacter levels in caeca and carcasses

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The study was carried over two years (2012 - 2013) and resulted in 24 different farm samplings from randomly selected sheds on the farms adopting four different litter practices. The practices were the (a) conventional practice of cleaning out litter after each cycle, (b) the Australian practice of re-using litter and free-range with the adoption of both practices (a) and (b). Caeca, litter, soil samples were collected from the various farms in a random manner and carcasses from the processing plant. This study assessed *Campylobacter* levels and *Campylobacter* species diversity. This study using a quantitative approach has provided a better understanding of *Campylobacter* dynamics relevant to the different farming practices studied. One of the key outcomes of the study is there were no statistical differences in the high *Campylobacter* levels in caeca, and the levels present in litter, soil or carcasses irrespective of vastly different litter practices adopted by the various farms. The litter practices also had no impact on *Campylobacter* key species diversity (*C. jejuni* and *C. coli*) and their distribution across farm. Thus although major differences exist within the four litter practices in terms of how bedding is used, the litter practices adopted had little to do with what dictated *Campylobacter* levels in the bird (caeca and carcass) and the farming environment (i.e. litter and soil).

P047

Community profiling of Campylobacter isolates across various farms adopting a range of litter practices in Australia

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Campylobacter was isolated from 24 sheds (17 farms) during a survey (2012 -2013) carried across farms adopting a range of farming practices in Queensland, Australia. The practices were (a) conventional practice of cleaning out litter after each cycle, (b) the Australian practice of re-using litter and (c) free-range with the adoption of both practices (a) and (b). The isolates were sourced from the bird (caeca), the environment (litter and soil) and the processing plant (carcasses). Initially PCR screening of the *flaA* small variable region (SVR) and DGGE were carried out, following which the profiles for caeca, litter, soil and carcasses for the various farms, locations and litter practices were compared using bionumerics and ecological analyses. The outcome provided an understanding of the diversity of *Campylobacter* isolates and their relationships with *Campylobacter* species, farms (or their geographic locations), sample type and the litter practices adopted. One of the key outcomes was that the *flaA*-SVR groups between 2012 and 2013 changed indicating a changing pattern of isolates and thus diversity. The overall outcomes of the study provided a better understanding of *Campylobacter* diversity across the farms.

P048

Accounting for Campylobacter biology and epidemiology in source attribution modelling

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Campylobacter is the leading foodborne bacterial pathogen in Europe. Various sources of these bacteria have been identified, but their relative contribution to human disease is not clear. Though, broiler chickens are considered to be the largest single source. Source attribution for *Campylobacter* has been performed by several countries using the microbial subtyping approach (based on multi locus sequence typing (MLST)), apportioning human illness cases to different animal sources based on subtype distribution. Microbial subtyping-based source attribution has been performed with success for *Salmonella*; instigating the implementation of targeted control measures resulting in the reduction of the human incidence in Denmark. However, *Campylobacter* genotypes are widely distributed among reservoirs, with a large variety of transmission routes, resulting in wider uncertainty intervals for source estimates. We propose a modelling framework which combines the microbial subtyping approach with quantitative exposure assessment, to provide estimates of the proportion of human cases attributable to different transmission routes from each *Campylobacter* reservoir. As a first step, the existing Asymmetric Island attribution model will be used to apportion human campylobacteriosis cases to their primary reservoirs, based on subtype distribution. Cases assigned to each reservoir will then be subdivided among relevant transmission routes, based on quantitative exposure estimates and the overlap of subtypes between each route and reservoir, expressed by the proportional similarity index (PSI). Although this approach is greatly data intensive, it is expected to provide more detailed results with narrower uncertainty ranges, enabling risk managers to target control strategies more precisely.

P049

Danish approaches to control Campylobacter

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Close collaboration between researchers, authorities and industry combined with political will and persistence have kept *Campylobacter* in Danish conventional broiler flocks and broiler meat relatively low compared with other European countries. Voluntary control initiatives started in late 1990ies and action plans continue to aim for a reduced burden of this organism. Monitoring, risk factor studies, intervention studies and risk assessments have guided the Danish management strategies. Monitoring has identified high risk slaughter processes and foods, quantified effects of action plans and informed risk assessments and source attribution. Risk factor studies have identified farm management operations leading to an increased risk of *Campylobacter*, and intervention studies have looked into biosecurity and physical and chemical decontamination of broiler meat. Furthermore, risk assessments have estimated the most effective control measures for Danish conditions and emphasized the importance of reducing the *Campylobacter* concentration. Risk models have been developed to work on a day-to-day basis in the case-by-case control of poultry meat batches and as a plant tool estimating individual plant performances. The Danish approach includes initiatives in all steps of the broiler production chain; strict biosecurity, fly screens (experimental only), good slaughter hygiene, an industrial code of practice, audits, freezing to the extent possible (stopped), case-by-case control of meat batches, consumer information campaigns and education of school children. That strict biosecurity works, also in countries with a warmer climate, was shown in the EU project CamCon. An overview of possible control options identified in CamCon and Danish research will be presented.

P050

C-di-GMP undergoes a transient increase during active dispersal- new insight from real time, in situ ratiometric imaging and quantification of c-di-GMP

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Bis-(3'-5 ')-cyclic dimeric guanosine monophosphate (c-di-GMP) is a dynamic intracellular signaling molecule that plays a central role in the biofilm life cycle. Current methodologies for the quantification of c-di-GMP are typically based on chemical extraction, representing end-point measurements. Chemical methodologies also fail to take into consideration of the physiological heterogeneity of biofilm and thus represent an average c-di-GMP concentration across the entire biofilm. To address these problems, a ratiometric, image-based quantification method has been developed based on expression of green fluorescence protein under the control of c-di-GMP responsive cdrA promoter (Rybtke., et al. 2012). The approach has been successfully applied to biofilms at different developmental stages and as well as during dispersal. Using this dynamic, real-time monitor, a transient state of increased c-di-GMP before the expected decrease in c-di-GMP was observed for biofilms under starvation conditions. The observation has been validated by comparison with chemical measurements of c-di-GMP with increased temporal resolution. Transcriptomic analysis of starved biofilms and planktonic cultures indicated that 50 out of 52 signal transduction genes were positively induced upon starvation, including three cyclases and three phosphodiesterase genes. Additionally, it was observed that c-di-GMP was localized to the outer boundary of mature colonies rather than showing a uniform distribution in young, small colonies. These data advocates the application of in situ, real time quantification methodology for dynamic signaling molecules.

P051

Genomic attribution of human clinical campylobacteriosis to putative host sources using STRUCTURE Bayesian attribution model.

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Background: Reducing the campylobacteriosis burden requires targeted public health interventions and an understanding of the reservoirs of *Campylobacter jejuni* infection in humans. The link between livestock and Campylobacter has been demonstrated by a number of methods including Bayesian attribution modelling, using MLST data. MLST-based attribution lacks accuracy, particularly in the case of multi-host STs. The availability of whole genome sequence data has enabled differentiation between closely related isolates.

Objectives: To examine the potential to use genomic data to construct an alternative typing scheme for more accurate attribution of human campylobacteriosis cases to host sources.

Methods: A test set of 656 human clinical *Campylobacter jejuni* isolates from the John Radcliffe Hospital was compared with a reference set of 454 *C. jejuni* isolates of animal origin, for which whole genome sequences were available. Variable loci were identified using the BIGSDB Genome Comparator, and population genetic analyses were carried out in Arlequin. Self-Attribution of isolates from known source and attribution of clinical isolates to putative source was carried out using the STRUCTURE Bayesian attribution model. Results Chicken was identified as the major source of campylobacteriosis, consistent with previous studies. Population genetic analyses and self-attribution using genomic data reveal low differentiation between populations of Campylobacter isolated from livestock sources.

Conclusions: The farm environment has created an artificial ecological niche in which different livestock species represent different samples from a common pool of *Campylobacter jejuni* genotypes, therefore attribution is inherently prone to some inaccuracy.

P052

Involvement of AlpB as a key role in in biofilm formation of *Helicobacter pylori*

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Bacterial biofilms are communities of microorganisms attached to a surface. *Helicobacter pylori* is one of the most common causes of bacterial infection in humans and this microorganism has biofilm forming ability on human gastric mucosal epithelium as well as on in vitro abiotic surfaces. We previously demonstrated that strain TK1402, which was isolated from a Japanese patient with duodenal and gastric ulcers, showed significantly higher levels of biofilm formation relative to the other strains. In addition, outer membrane vesicles (OMV) play an important role in biofilm formation. The aim of this study was to analyze what protein in OMV contributes to the biofilm formation in strain TK1402. We obtained a spontaneous mutant derived from strain TK1402 without biofilm forming ability. The protein profile of OMV was compared between parental and the mutant strain. It was found that three proteins, VacA, HpaA and AlpB, of the mutant were decreased compared to those of parental strain. Since HpaA and AlpB proteins are outer membrane proteins, we constructed an *hpaA* or *alpB* deficient mutant and the ability to form biofilm was assessed. Both mutants strains showed a decreased biofilm forming activity, especially *alpB* deficient strain did not form biofilm conspicuously. Complementation of *alpB* to the mutant strain recovered the ability to form biofilm. These results indicated that *alpB* of strain TK1402 plays a central role for biofilm formation.

P053

Antibodies against recombinant *Campylobacter jejuni* flagellar proteins enhanced colonization of this microorganism in broiler gastrointestinal tract

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Background: Poultry products are a major source of *Campylobacter jejuni* for human infection. Risk assessments have demonstrated that reduction of *C. jejuni* in chicken gut or carcasses reduces human health risk. Vaccination is regarded as the effective means to control this microorganism in poultry production. Because flagella of *C. jejuni* play an important role in colonization in gastrointestinal mucosa and major targets for inducing immune response, we selected the flagellar proteins as targets for potential vaccine development. Objective: The objective of this study was to determine whether the flagellar proteins could induce immune response in broilers and protect them from *C. jejuni* colonization.

Methods: Four flagellar proteins were produced by the recombinant technique, and were purified by the nickel-chelated affinity chromatography. Broilers were administered with the proteins emulsified in a Freund incomplete adjuvant. After 10 days of post immunization, the broilers were challenged with *C. jejuni*. The ELISA and immunoblot were performed. Cecal contents were collected for colonization assay. Appropriate controls were also included.

Results: The ELISA and immunoblot analyses showed all broilers produced strong specific humoral immune response to the flagellar proteins in the immunized groups. However, the broilers with immunization resulted in a statistically significant increase in the number of *C. jejuni* in the broiler ceca compared to those were not immunized but challenged.

Conclusion: These results provide insight of the specificity of host antibodies to the *C. jejuni* flagellar proteins, and provide a rationale for further evaluating the roles of antibodies in *C. jejuni* colonization in broiler gut.

P054

Genome and epigenome evolution of *H. pylori*

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Here I review genome/epigenome dynamics in the short- and long-term evolution of *H. pylori* as revealed by comparison of complete genome/methylomes. *H. pylori* genomes rapidly evolve through high mutation as well as high mutual homologous recombination. They show wide phylogeographic divergence. The evolutionary pathway suggested for cagA oncogene is Western type – Amerind type – East Asian type. Chromosome painting *in silico*, which detects transfer of sequence chunks through homologous recombination, revealed their fine population structure and admixture. *H. pylori* have a large number of sequence-specific DNA methyltransferase genes, with different strains carrying unique repertoires. Using single-molecule real-time (SMRT) sequencing technology in a Pac Bio machine, we studied methylated DNA bases throughout H. pylori genomes. The results demonstrated that these methyltransferases often change DNA sequence specificity through allelic recombination as well as domain movement – the movement of coding sequences of target recognition domains between genes and within a gene. Knocking out these specificity determinant genes led to unique changes in transcriptome and phenotype. These results are consistent with the concept of adaptive evolution driven by changes in the methylome. Most of these DNA methyltransferases are paired with a restriction endonuclease to form a restriction-modification system. One family of restriction enzymes present in *Helicobacter* and *Campylobacter* excises a base from their recognition sequence by DNA glycosylase activity and then cleaves the strand by AP lyase activity. This surprising finding reminds us of the demethylation by base excision in plant and animal epigenetics.

P055

Minding the gap on Campylobacter epidemiology in broiler meat and the public health risk in developing countries: The model of the Swedish Research Links Collaborative Project in Egypt

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Background: In Egypt, *Campylobacter* is a leading cause of pediatric diarrhea with infants and one year olds experiencing 1.2 and 0.4 episodes per year, respectively. In such hyper-endemic settings, the burden of *Campylobacter* diarrhea could be substantial. The gap of knowledge about the epidemiology of *Campylobacter* in food sources hinders accurate assessment of the human health burden.

Objectives: The goal of this collaborative research is to enhance better risk assessment of *Campylobacter* in the Egyptian setting. The project will provide the first baseline data on *Campylobacter* in broiler meat. Such data will be used for a quantitative risk assessment for such important zoonotic pathogen.

Methods: Funded by the Swedish Research Council, we describe here a model for research that could be extended to other resembling developing countries. The research is founded on a model of exchange of senior researchers, capacity building of junior researchers, and organizing technical and laboratory capacity building trainings.

Results: Partners from Sweden (the Swedish University of Agricultural Sciences and the Swedish National Veterinary Institute), Egypt (High Institute of Public Health, Alexandria University), and Australia (School of Veterinary and Life Sciences, Murdoch University) will be collaborating in order to generate baseline data on prevalence, counts, genotypic diversity, antimicrobial resistance, and virulence of *Campylobacter* in retail chicken meat in Egypt; and to develop a quantitative model for human campylobacteriosis infection risk from consumption of broiler meat in Egypt.

Conclusions: We present this research project as a model for providing a science based contribution toward reducing the risk of foodborne pathogens and enhancing safe food resources the developing world.

P056

Antimicrobial resistance of Campylobacter in Swedish chicken

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Campylobacter jejuni isolated from intestinal contents from broilers sampled within the Swedish Campylobacter programme have been routinely tested for susceptibility to ciprofloxacin, erythromycin, gentamicin, nalidixic acid, streptomycin and tetracycline since 2001. Until 2010 quinolone resistance was less than 5%. However, in 2010, the proportion of quinolone resistant isolated increased notably to around 20%. In 2012 the prevalence was 17 %. However, a lower proportion (8%) was detected in 2014. Notably, resistance to erythromycin, the drug of choice for treatment of human campylobacteriosis, was not found in Campylobacter from animals in Sweden. In 2013, testing of antimicrobial susceptibility was performed on isolates from Swedish broiler products from conventional, organic and other small scale production. All the isolates (n=17) from organic and small scale producers were susceptible to all tested antimicrobials, whereas 30% of the 47 isolates from conventional produced broiler products were resistant to ciprofloxacin. The reasons for the variation and increased quinolone resistance are not known but selection through use of antimicrobials is unlikely as an explanation since fluoroquinolones are not used in the Swedish broiler production. Quinolone resistance in *C. jejuni* is usually caused by a mutation in the *gyrA* gene. The mutation usually leads to high MICs for both ciprofloxacin and nalidixic. PFGE analyses are performed of the resistant *Campylobacter jejuni* strains to find out the variation and similarity. When analyzing the origin of the quinolone resistant isolates there is a tendency that resistant strains are more frequently found in certain regions and producers in Sweden.

P057

Comparison of MLST types of *C. coli* from animals and raw water with human cases in Sweden

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Campylobacter jejuni is the most commonly isolated species from humans with campylobacteriosis. In a study of Swedish domestic cases in 2012, 95-96% of isolates consisted of *C. jejuni* and the rest of *C. coli*. Several possible sources of *Campylobacter* infection were sampled: domestic animals, wild birds, raw water and imported chicken meat. The majority of non-human *C. coli* isolates came from pigs and raw water. The objectives were to investigate the genetic diversity among the *C. coli* isolates using MLST typing and to find overlapping genotypes in the isolates from humans and different sources that could clarify the epidemiology of human disease with *C. coli*. A total of 174 *C. coli* isolates from humans (37), pigs (100), chicken (7), cattle (2), sheep (2), imported chicken meat (1) and raw water (25), were genotyped by MLST (http://pubmlst.org/campylobacter/). A high genetic diversity was seen in the *C. coli* isolates as 87 STs were identified among the 174 isolates. The majority (54 STs) were assigned to the ST-828 clonal complex (CC) and the remainder (33 STs) did not belong to any defined CC. Six of 37 human isolates had STs that were shared with pigs and 4 with poultry; the remainder were from uniquely human STs. The raw water isolates were of 19 different STs unique to water. Phylogenetic analysis indicated that animal and human isolates belong largely to a single large cluster and water to two other clusters. This indicates that domestic animals, rather than raw water constitute sources for human *C. coli* infection.

P058

Isolation and characterization of a new species of the genus Helicobacter recovered from wild birds faecal samples

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Background: The genus Helicobacter is currently comprised of 35 species, being Helicobacter pylori the most studied species for its pathogenic role in humans. However, recently other helicobacters have gained importance as emerging pathogens and potential zoonotic agents associated with intestinal and hepatobiliary diseases. During a study on diversity of campylobacters in fecal samples from wild birds, held in Valdivia (Southern Chile) between 2013 and 2015, has been isolated a strain (WBE - 107) initially identified as a Campylobacter-like organisms. However, this isolate showed a PCR -RFLP pattern unknown so far, so it was selected for a polyphasic taxonomic characterization.

Objectives: The aim of this study was to determine the taxonomic position of strain WBE - 107.

Methods: A taxonomic study, using phenotyping (morphological, physiological and biochemical differentiation) and genotypic (PCR based methods and phylogenetic analysis of DNA sequences) techniques was performed.

Results: Using specific-PCR of Campylobacter, Arcobacter and Helicobacter it was found that WBE-107 belonged to the latter genus. This was confirmed by analysis of 16S rRNA, gyrB and atpA genes where clearly this strain forms a new phylogenetic lineage. WBE-107 also presents phenotypic characteristics different from other helicobacters by API Campy (bioMérieux).

Conclusions: The results obtained confirm that the strain WBE - 107 represents a new species of the genus Helicobacter. The epidemiological significance of this new taxon is unknown and further studies are needed to define its natural reservoirs, possible zoonotic potential and clinical relevance.

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P059

How variability varies: do different Campylobacter strains have different phase variable genes?

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Multiple genes of *Campylobacter jejuni* exhibit high frequency, stochastic, reversible switching between ON/OFF expression states. Genetically, the main mechanism of this "phase variation" is instability in homonucleotide (poly-G/C) repeat tracts producing frame shift mutations. An observation from the rapidly-increasing number of *Campylobacter* genome sequences is that the number of phase variable genes differs between strains. We present a bioinformatic analysis of the number and conservation of phase variable genes in *Campylobacter* genome sequences. We search for poly-G/C homonucleotide tracts of seven nucleotides or longer in each genome and identify the nearest gene. A BLAST analysis is performed for each repeat-containing gene sequence against whole genome sequences of all the other strains. These comparisons indicate the conservation of both the gene and the mechanism of phase variation (i.e. the repeat region). Finally, the identified genes are grouped together according to links established by the BLAST search. Based on 16 complete genome sequences, we found >3-fold variation in the number of repeat tracts per genome between strains of *C. jejuni* subsp. *jejuni* (from 11 to 36 tracts) and as many as 76 tracts in *C. jejuni* subsp. *doylei*. We also identified patterns of presence or absence that indicate a mixture of conserved phase variation and of genes that are phase variable in some strains and non-phase variable or absent in others. We are extending this analysis to ~200 sequences of strains of known provenance in order to identify trends in phase-variable gene conservation with the epidemiological source of *C. jejuni*.

P060

Helicobacter pylori outer membrane vesicles modulate human immune responses

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The long-term persistence of *Helicobacter pylori* bacteria in the human stomach is attributed to the co-evolution of complex immunosuppressive mechanisms that modulate the activity of a range of effector and regulatory host immune cell populations. *H. pylori*, in common with other gram-negative bacteria, constitutively shed outer membrane vesicles (OMV) and there is increasing interest in the concept that OMV provide a mechanism by which bacteria can deliver immunomodulatory signals. To date however only limited studies on the immunomodulatory effects of *H. pylori*-derived OMV on circulating human immune populations have been performed. We investigated the immunomodulatory effects of OMV prepared from H. pylori strain 60190 with (OMV60190) and without (OMV60190:v1) the vacuolating cytotoxin VacA, which is reported to inhibit T cell activity. Addition of both OMV60190 and OMV660190:v1 to peripheral blood mononuclear cells (PBMC) strongly suppressed subsequent T cell proliferation. This suppression was associated with the up regulation of COX-2 expression by monocytes, was inhibited by the presence of COX-2 inhibitors and did not involve induction of T cell apoptosis. This data suggests OMV can indirectly modulate T cell responses by inducing suppressive activity in monocyte populations.

P061

Comparison of *Helicobacter pylori* eradication rate among concomitant, tailored, and sequential therapy

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Background: Currently, the *Helicobacter pylori* eradication rate of clarithromycin-based triple therapy has decreased to an unacceptably low level, and novel therapeutic strategies are necessary.

Aim: To compare the eradication rates of concomitant, tailored, and sequential therapy.

Methods: A total 712 patients infected with *Helicobacter pylori* were divided into 3 groups, and each group was treated with a different eradication therapy. The first group was simultaneously treated with rabeprazole, amoxicillin clarithromycin, and metronidazole for 7 days (concomitant therapy group). A test for point mutations in the 23S rRNA gene was conducted in the second group (tailored therapy group) which was treated with amoxicillin, rabeprazole, and clarithromycin in the absence of resistence for 7 days, whereas clarithromycin was replaced by metronidazole if the resistence was detected. The final group was treated with rabeprazole and amoxicillin for 5 days, followed by rabeprazole, clarithromycin, and metronidazole for 5 days (sequential group).

Results: The eradication rates were 92.4% (194/210) in the concomitant group, 90.6% (193/213) in the tailored group (p=0.514), and 84.6% (176/208) in the sequential group (p=0.012).

Conclusion: The eradication rates for concomitant and tailored therapy were similar. And both were higher than sequential therapy.

P062

Evaluation of cephamycins as supplements (Cefotetan and Cefoxitin) to selective agar for detecting *Campylobacter spp.* in chicken carcass rinses

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Although cefoperazone is the most commonly used antibiotic in Campylobacter-selective media, the distribution of cefoperazone-resistant bacteria such as extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* is increasing. Here we evaluated the potential of cephamycins for use as supplements to improve modified charcoal-cefoperazone-deoxycholate agar (mCCDA) by replacing cefoperazone with the same concentrations (32 mg/L) of cefotetan and cefoxitin, which are resistant to ESBLs. Modified charcoal-cefotetan-deoxycholate agar (mCCtDA) and modified charcoal-cefoxitin-deoxycholate agar (mCCxDA) were significantly more sensitive and selective ($p < 0.05$) than mCCDA. The number of mCCDA plates positive for Campylobacter (18/70, 26%) was significantly lower ($p < 0.05$) than that of mCCtDA (42/70, 60%) or mCCxDA plates (40/70, 57%). The number of mCCDA plates (70/70, 100%) that were contaminated with non-Campylobacter species was significantly higher than that of mCCtDA (20/70, 29%) or mCCxDA plates (21/70, 30%). The most common competing species identified using mCCDA were ESBL-producing *E. coli*, while Pseudomonas species frequently appeared on mCCtDA and mCCxDA. The emergence and spread of ESBL-producing *E. coli* requires changing the standard antibiotics in Campylobacter-selective agar, and cefotetan and cefoxitin show promise as useful options.

P063

A novel immunoproteomics method for identifying in vivo-induced *Campylobacter jejuni* antigens using pre-adsorbed sera from infected patients

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Campylobacter jejuni is an important food-borne and zoonotic pathogen with a worldwide distribution. Humans and chickens are hosts of this pathogen. At present, there is no ideal vaccine for controlling human campylobacteriosis or the carriage of *C. jejuni* by chickens. Bacterial in vivo-induced antigens are useful as potential vaccine candidates and biomarkers of virulence. In this study, we developed a novel systematic immunoproteomics approach to identify in vivo-induced antigens among the total cell proteins of *C. jejuni* using pre-adsorbed sera from patients infected with *C. jejuni*. Overall, 14 immunoreactive spots were probed on a PVDF membrane using pre-adsorbed human sera against *C. jejuni*. Then, we excised these protein spots from a duplicate gel and identified using MALDI-TOF MS. In total, 14 in vivo-induced antigens were identified using PMF and BLAST analysis. The identified proteins include CadF (CadF-1 and CadF-2), CheW, TufB, DnaK, MetK, LpxB, HslU, DmsA, PorA, ProS, CJBH_0976, CSU_0396 and hypothetical protein cje135_05017. Real-time RT-PCR was performed on 9 genes to compare their expression levels in vivo and in vitro. The data showed that 8 of the 9 analyzed genes were significantly upregulated in vivo relative to in vitro. We successfully developed a novel immunoproteomics method for identifying in vivo-induced Campylobacter jejuni antigens by using pre-adsorbed sera from infected patients. This new analysis method may prove to be useful for identifying in vivo-induced antigens within any host infected by bacteria and will contribute to the development of new subunit vaccines

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P064

Campylobacter in children under the age of 5 years old

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Background: Campylobacter is a common cause of diarrhoeal disease worldwide. Children suffer disproportionately but risk factors in this group tend not to be investigated as intensively as they are in adults.

Objectives: To describe the prevalence of and risk factors for campylobacteriosis in children under the age of five years old. To calculate population attributable risks (PAR) for statistically significant risk factors.

Methods: We performed a literature review using search terms including "children", "Campylobacter", "case control", "Campylobacteriosis" and "risk factors". Databases searched included Google Scholar, PubMed and Web of Science restricted to years 1979 and June 2014. Results Fifty-six papers were found to be relevant and used in the review. The prevalence of Campylobacter was lowest in children in the Middle East. Population attributable risks were calculated from nine papers. The PAR for chicken, in the small number of studies where it was identified as a risk factor, was higher than for other factors although the confidence intervals were very large. Not breast feeding was the other risk factor with a PAR greater than 10%. The most effective ways of reducing the likelihood of infection were hand-washing, using a toilet and preparing meat products. Few factors were found to be protective or attributable to 10% or more symptomatic infections.

Conclusions: Wide variations in prevalence were observed but may reflect differences in surveillance systems, healthcare seeking behaviours, or differences in exposure e.g. different diets, animal husbandry or sanitation. Good hygiene measures and sanitation are essential for reducing the burden of Campylobacteriosis.

P065

The efficacy of broiler farm boot-dip disinfectants against *Campylobacter jejuni*

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Campylobacteriosis is a common cause of bacterial enteritis and scientific opinion suggests that 50% to 80% of cases are attributed to the chicken reservoir. Reducing *Campylobacter* colonisation in chicken flocks could help lower the burden of disease to society. Disinfectant boot-dips at access points to broiler flocks are associated with a reduced risk of flock colonisation. The type of disinfectant and how it is used varies and it is known that, after dilution, disinfectants may lose efficacy over time, and may also become inactivated by organic matter. This study aimed to assess the suitability of disinfectants for use in boot-dips as on-farm *Campylobacter* controls. Twelve products that covered the main disinfectant classes used on farms were selected for testing. A disinfectant boot-dip model was created to simulate use through daily loading with poultry litter and to assess the effect of contact time. A test method for disinfectant efficacy against *Campylobacter* (based on BS6734:1986) was developed for this study. All products were effective (>4 log10 *Campylobacter* reduction) after a 30 minute contact time in clean boot-dips; however a shortened contact time or increasing contamination of boot-dips with poultry litter resulted in some products becoming ineffective. After seven days of simulated boot-dip usage (with litter loading), only chlorocresol products remained effective at the one minute contact time. In conclusion, different disinfectant groups vary in the time needed to inactivate *Campylobacter* and resistance to interference by organic matter. These properties should be considered when selecting and managing boot-dips for optimal *Campylobacter* control.

P066

Analysis of results from a monitoring programme for Campylobacter in broiler carcases at slaughter in the UK

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In December 2010, the UK Food Standards Agency announced a joint Government and Industry target to reduce the percentage of chickens produced in UK poultry slaughterhouses that have the highest level of *Campylobacter* contamination, i.e. those with more than 1,000 colony forming units per gram of carcase neck skin sample, from a baseline of 27% in 2008 to 10% by April 2015. A UK-wide, stratified and randomised survey of broiler chicken flocks at slaughter began in March 2012 to monitor progress against this target. The survey design and sampling protocols were based on the EU technical specifications for EC decision 2007/516. One carcase per slaughter batch was sampled after chilling and before further processing. The samples were then transported to the laboratory for detection, quantification and speciation of *Campylobacter* spp. based on the methods described in ISO 10272:2006. Overall, 30.7% of the carcases were found to be highly contaminated with *Campylobacter* (>1,000 cfu/g), on average, in the first two years of the monitoring programme. Increasing bird age, certain abattoirs, line speed, presence of skin lesions, presence of processing damage, mortality at 14 days and increasing proportion of birds that were dead-on-arrival were all identified as significant independent risk factors for the most heavily *Campylobacter*-contaminated carcases (>1,000 cfu/g). The findings reported here provide a robust estimate of the percentage of chicken carcases that are highly contaminated in the UK and indicate some important factors associated with this.

P067

Investigations into the association of Campylobacter contamination in chicken carcase and caeca samples in the UK

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Campylobacter is the leading zoonotic enteric disease in Europe. The main source of infection is believed to be the handling, preparation and consumption of chicken meat. *Campylobacter*-colonised chickens entering the slaughterhouses are considered to be the main source of carcase contamination and it has been suggested that low numbers of *Campylobacter* in the caeca results in substantially reduced carcase contamination. This study aimed to investigate the concentration of *Campylobacter* contamination in caeca and carcase samples from 732 slaughter batches sampled at 19 abattoirs over a three year period in the UK (2012-2015). One caeca and one carcase sample was collected per batch and tested to detect, quantify and speciate *Campylobacter* following

Methods: described in ISO 10272: 2006. The prevalence of *Campylobacter* on carcases (76.0%) was very similar to the prevalence obtained in caeca samples (75.3%) and results from both samples showed a good level of agreement (k=0.69). The level of *Campylobacter* contamination was much higher in caeca than in carcase samples and most (95.8%) of the highly contaminated carcases (>1,000 cfu/g) were obtained from batches with high loads of *Campylobacter* in the caeca (>10^6 cfu/g). Although the number of *Campylobacter* on the carcase samples was correlated with the concentration of contamination in the caeca, the strength of this association varied within and between abattoirs, suggesting differences in hygiene control. The results from this study show that a reduction of the level of carcase contamination could be obtained by interventions aimed at reducing the concentration of *Campylobacter* in the colonised birds.

P068

Characterisation of a YceI-like protein in *Campylobacter jejuni*

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Background: YceI-like proteins are ubiquitous in Proteobacteria but their function remains largely unknown. Structural data from studies in other bacteria suggest that YceI-like proteins may act as lipid/polyisoprenoid binding proteins and might be involved in isoprenoid quinone synthesis or turnover. Isoprenoid quinones are essential components of the bacterial membrane and are implicated in electron transport and cell-wall metabolism. *Objectives*: We have recently identified a YceI-like protein in *C. jejuni* which shares 37.2% protein identity to the corresponding homologue in *Helicobacter pylori*, which is thought to be involved in acid stress responses. We aimed to functionally characterise the *C. jejuni* YceI protein in order to determine its role in virulence and cell envelope maintenance.

Methods and Results: We identified that YceI accumulates in the periplasm of strains which have mutations in genes that are putatively involved in the maintenance of the *C. jejuni* cell envelope. Mutation of the *C. jejuni ycel* gene resulted in increased sensitivity to polymyxin B of the mutant compared to wild-type which indicated a role for YceI in maintaining cell membrane integrity. Furthermore, the *yceI* mutant also showed significant attenuation during *in vivo* colonisation of the *Galleria mellonella* insect model.

Conclusions: This study suggests that YceI could be involved in cell envelope maintenance, in conjunction with another family of proteins which are currently under investigation. YceI is also a potential virulence factor and further colonisation studies in the chicken host could provide a useful insight into the role of YceI in colonisation and infection.

P069

flgA is essential for flagellar biosynthesis and important for the biofilm formation of *Campylobacter jejuni* NCTC11168

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Campylobacter jejuni is a major pathogenic bacterium causing foodborne illness worldwide. A transposon-mediated insertional mutation of *flgA* gene (cj0769c) was identified to have significantly decreased the biofilm formation (<10%) on polystyrene surface in *C. jejuni* NCTC11168 (P<0.05). The mutation of *flgA* also decreased the biofilm formation on other food contact surfaces such as stainless steel by 1.6-log CFU and borosilicate glass by 2.8-log CFU, respectively, compared to 11168 wild-type. *flgA* mutant was completely devoid of flagella and non-motile while 11168 wild-type had the full-length flagella and was motile. It supports that flagellar-mediated motility play a significant role in the biofilm formation of *C. jejuni* NCTC11168. It was further supported by that the biofilm formation of 11168 wild-type was affected by the bacterial swimming motility modulated by the viscosity of glycerol in media. SEM analysis revealed that 11168 wild-type formed biofilm on glass surface with a net-like structure of extracellular fiber-like material, but *flgA* mutant formed biofilm lacking such a structure. It suggests that the extracellular fiber-like material may play a significant role in the biofilm formation of *C. jejuni*. This study demonstrates that *flgA* is essential for flagellar biosynthesis and plays a significant role in the biofilm formation of *C. jejuni* NCTC11168.

P070

Correlation between helicobacter eradication and body mass index

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Background: *Helicobacter pylori* plays an important role in gastric ulcer and lymphoma. Eradication of such causative agent thus is crucial when dealing with upper gastric diseases. Numbers of studies are actively in progress with subjects such as standardized first line therapy with decreased amount of dosage, dosage-related therapy effectiveness.

Objectives: To determine clinical dosage effectiveness in *H. pylori* therapy, we authors here tried to find correlation between eradication result and difference in body mass index.

Method: Total of 166 patients were analyzed who were diagnosed with *Helicobacter pylori* infection between 2011 and 2014. Standard triple therapy were used in all cases. Eradication result after each therapy was checked with parameters that could affect the outcome such as age, sex, weight, height, body surface area, BMI, underlying disease and eradication indication. Correlation between BMI and eradication effectiveness were measured based on category of low weight (below 18.5), overweight (over 23), obesity (over 25), and morbid obesity (over 30). Results Among 166 patients, 117 people showed successful eradication outcome and 49 did not. 108 were men and 58 were women. Average BMI between successful and failed group were 23.5 and 24.7 (P value=0.028), showing higher index in failed group. Obese subjects with BMI over 25 were 50 (30.1%). Those who were not were 116 (69.9%). Eradication success rate were 56% in obese group and 76.7% in non-obese group.

Conclusions: Between successful and failed group, BMI did not differ prominently. However, the index was statistically higher in failed group. This result shows that obese patients have significantly lower success rate for *H. pylori* eradication, thus dosage modification in obese person should be considered henceforth.

P071

The Campylobacter Risk Management Strategy in New Zealand

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A very high rate of foodborne campylobacteriosis was evident in New Zealand in 2006 and attribution studies estimated that more than 50% of human cases were due to the preparation and consumption of poultry meat. This led to the implementation of a national risk management strategy for *Campylobacter* to significantly reduce what was judged to be an unacceptably high human illness burden. The primary focus was on improving the food safety control systems for slaughter and dressing of broiler chickens in order to get a reduction in *Campylobacter* levels on carcasses at the end of primary processing.Improvements in broiler chicken processing, together with improvements in hygienic practice at other steps in the farm-to-plate food chain has resulted in the following reductions in relation to Campylobacter: 49.7% (2007-8) to 27.3% (2014-15) positive carcass rinsates, and 22.4% (2007-8) to 3.9% (2014-15) carcass rinsates greater than 3.78 log10 cfu. Human campylobacteriosis cases in New Zealand have reduced by more than 50% since 2006 (15,873) compared to 2014 (6,776). Source attribution studies from 2005 – 2014 show that the proportion of notified cases of human campylobacteriosis in New Zealand that is attributable to consumption of poultry is changing and a dynamic risk management programme needs to take into account other exposure pathways when developing mitigation measures. These include the increasing proportion of overall notified cases that are attributable to Campylobacter strains carried by ruminants, as well as potential changes in exposure due to the increased consumption of raw milk in New Zealand.

P072

A co-transformation strategy identified Cj1501c as an important player involved in conjugation in *Campylobacter jejuni*

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Campylobacter jejuni displays significant strain diversity due to horizontal gene transfer. Conjugation is an important horizontal gene transfer mechanism contributing to the evolution of bacterial pathogenesis and antimicrobial resistance. *C. jejuni* strains display great variation in conjugation efficiency; however, the underlying mechanisms are still unknown. In this study, a co-transformation strategy was developed to obtain high frequency conjugation (HFC) derivatives of *C. jejuni* NCTC 11168, a standard strain with extremely low conjugation efficiency (6.3 × 10E-8 CFU/recipient). Specifically, erythromycin resistance marker (*erm*) was first introduced into a HFC *C. jejuni* strain (2.2 x 10E-4CFU/recipient) and genomic DNA from this strain was introduced into *C. jejuni* NCTC 11168 via natural transformation. Given that multiple recombination loci could occur within one recipient cell independently during natural transformation, the genetic components involved in HFC may be co-transformed and enriched with the erythromycin selection marker. All transformants were then pooled and harvested for conjugation. Nine transformants were identified and demonstrated to display HFC phenotype. The genome of six HFC derivatives and two low frequency derivatives were subjected to whole genome sequencing using MiSeq. Comparative genomics analysis and genetic manipulation indicated that the Cj1051c, which encodes a putative restriction-modification enzyme, plays a significant role in conjugation in *C. jejuni* NCTC 11168. Together, this study successfully developed and utilized a unique co-transformation strategy to identify Cj1051c as an important component involved in conjugation in *C. jejuni*.

P073

A refined method for assessing the efficacy of live vaccines to reduce *Campylobacter jejuni* colonisation levels in chickens in vivo

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Campylobacter is a leading cause of human bacterial gastroenteritis in developed nations and is a serious public health concern worldwide. The annual cost of food-borne illness in Australia is ~ $1.2 -2.6 billion and *Campylobacter* was isolated in 67% of gastroenteritis cases in 2003 in Australia. *Campylobacter* infection can also trigger rare, but severe complications. Poultry are an important reservoir for transmission to humans. Reducing the levels of *Campylobacter* in chicken meat would reduce the risk of human infection and improve safety for consumers. Therefore, intervention to reduce *Campylobacter* levels in chickens is a worthwhile goal for the poultry industry. A cost benefit analysis predicted a gain of > $57.4 annually in New Zealand in 2014 resulting from such interventions. Vaccines are likely to be the risk reduction strategy with the most promise. They have the potential advantages of being very specific and offering life-long protection. The added benefit of live vaccines is that they are relatively inexpensive and simple to produce, store and administer. This work describes a colonisation trial protocol that compares a range of challenge dose titres with a view to more accurately reflect the natural infection process. The Australian isolate used for this work, *C. jejuni* strain 354, was originally a human enteritis isolate that also reliably colonises the caeca of chickens to high levels. This model compares direct dosing with in-contact dosing and the shedding levels of the challenge strain are monitored in the faeces to determine the timing of necropsy to evaluate colonisation levels.

P074

Increased isolation rates of *Campylobacter concisus* from mucosal biopsies by cultivation in both micro-aerobic and anaerobic atmospheres

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Background: Some *Campylobacter concisus* isolates have been shown to possess several pathogenic capacities that can facilitate increased intestinal permeability, a pathognomonic finding in inflammatory bowel disease (IBD). Previous studies have confirmed a high prevalence of *C. concisus* DNA in mucosal biopsies from IBD patients using PCR techniques, but cultivation of isolates from biopsies has been sparse.

Objectives: To investigate if cultivation in both micro-aerobic and anaerobic atmospheres, can improve *C. concisus* isolation rates from intestinal mucosal biopsies.

Methods: Thirty-three patients with IBD and 14 healthy subjects were examined. Biopsies from different anatomic locations of the intestine were immediately smeared onto two 5% blood agar plates with added yeast extract. These were incubated for 48 hours at 37°C in micro-aerobic and anaerobic conditions, respectively. Smears were transferred to a polycarbonate filter, incubated for one hour at 37°C after which the filters were removed, and the agar plates subsequently incubated for four days. Colonies resembling *C. concisus* were assessed and validated by MALDI-TOF analysis.

Results: *C. concisus* was isolated from 17/33 (51%) IBD patients, and from 8/12 (67%) healthy subjects. The total number of isolates was 60, of which 23 were derived from micro-aerobic and 13 from anaerobic incubation exclusively. From 12 sites, isolates grew in both atmospheres (p=0.0001).

Conclusions: Cultivation of *C. concisus* from mucosal biopsies is tedious, but the prevalence of *C. concisus* is high in both IBD and healthy subjects. Incubation in both micro-aerobic and anaerobic atmospheres facilitates a higher isolation rate than by micro-aerobic incubation exclusively.

P075

In vitro properties of the point mutated forms of *Helicobacter pylori* HP0231 protein

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Background: The mechanism of disulfide bond formation in microorganisms is extremely diverse. The *H. pylori* Dsb system seems to be novel and different from that functioning the model microorganism - *E. coli*.

Objectives: HP0231 was characterized as a protein functioning parallel as a molecular chaperone and in an oxidizing pathway in H. pylori cells. HP0231 acts as periplasmic oxidase, as EcDsbA, despite its structural resemblance to EcDsbG. However, the characteristic motifs of HP0231 are identical to that of EcDsbA (CPHC/VcP) but different from that of EcDsbG (CPYC/TcP). To assess relations between HP0231 structure and function we tested *in vitro* properties of mutated forms of HP0231 containing motifs characteristic for EcDsbG.

Methods: Mutated forms of HP0231 were constructed by site-directed mutagenesis and overexpressed in *E. coli* Rosetta strain. Proteins were purified using NGC™Medium-Pressure Chromatography and used to evaluate their redox potential, ability to reduce insulin and their chaperone activity. Results All tested mutated forms of HP0231 possess chaperone activity slightly higher than that of native protein. At the same time the ability to reduce insulin revealed by HP0231 CPYC/TcP and CPHC/TcP decreased compared to HP0231 native form what correlates with their higher redox potential.

Conclusions: The redox potential and oxidizing properties of the HP0231 are dependent on both the XX dipeptde within the active site CXXC motif and a residue located upstream of the *cis*-Proline loop, whereas the high chaperone activity is rather conditioned by dimeric HP0231 stucture. The work was supported by the National Science Centre (grant no. 2012/05/B/NZ1/00039).

P076

Diversity of the Campylobacter Dsb (disulfide bond) oxidative protein folding

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Background: The bacterial proteins of the Dsb family catalyze the formation of disulfide bridges. While the *Escherichia coli* disulfide bond-forming system is quite well understood, the mechanisms of action of Dsb systems in other bacteria, including *Campylobacter* genus are poorly characterized.

Objectives: The aim of the presented work was to gain knowledge about oxidative protein folding within various species of *Campylobacter* genus.

Methods: The BLASTp search, versus a manually curated database including reference proteins, was applied to identify *Campylobacter dsb* genes. Transmembrane topology predictions of envelope located Dsb (DsbB and DsbI) were determined using Protter, TOPCONS, PSIPRED and PredictProtein programs. Results *In silico* analysis of the proteomes of 31 members of *Campylobacter* genus, with respect to the presence of Dsb oxidoreductases was performed. The significant differences among the *Campylobacter* genomes with respect to *dsb* gene numbers and their genetic organization were noticed between thermotolerant and non-thermotolerant bacteria. Predictions of the transmembrane domain topologies of the Dsb envelope proteins revealed differences between *Campylobacter* species. We found that *C. jejuni* DsbB and also *C. coli* DsbB, both contain five membrane-spanning domains compared with four for EcDsbB; whereas the DsbBs of *C. fetus*, *C. concisus*, *C. curvus* and *C. hominis* revealed the same topology as EcDsbB.

Conclusions: The Dsb oxidative pathway of *Campylobacter* is more complex than that of *E. coli*. The significant differences were noticed not only between various species of *Campylobacter* genus but also between strains of the same species. The work was supported by the National Science Centre (grant no. 2012/05/B/NZ1/00039).

P077

Comparative methylome analysis of *Campylobacter jejuni* strains of human and animal origin reveals unique methylation motifs

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Background and Objectives: Previous studies showed evidence of syntenic genomes between a *Campylobacter jejuni* sheep abortion clone (clone SA) with commonly studied human gastroenteric *C. jejuni* strains 11168 and 81-176. However, the highly virulent abortion phenotype of clone SA has launched investigations to try and understand what other factors could contribute to clone SA's unique ecological preference and pathobiology. We hypothesized that the methylome profile of IA3902, a clinical isolate of clone SA, would differ from the syntenic but phenotypically distinct gastrointestinal-specific strains 11168 and 81-176.

Methods: Utilizing Pacific Biosciences' SMRT sequencing technology we determined the methylome profile of IA3902 and compared that with methylation data of 11168 and 81-176. We also compared the distribution of methylation motifs across the genome and within functional gene categories to identify any distinguishing features in their methylomes.

Results and conclusion: Unique to the IA3902 methylome is a GAAGAA motif that has been tentatively assigned to the CjeIAORF32P restriction-modification system. The predicted methyltransferase for this system is associated with a previously described phase variable methyltransferase in other *C. jejuni* strains. In IA3902 the gene encoding this methyltransferase appears to have undergone a horizontal gene transfer event resulting in a constitutive "phase ON" methyltransferase. These changes result in unique motif specific patterns of hyper- and hypomethylation that are associated with unique changes in the methylation of functional gene categories. Additional assays are underway to further characterize this gene and its role in the ecology of *C. jejuni*.

P078

The endoscopic future of non-*Helicobacter pylori helicobacter* gastritis.

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Helicobacter pylori (*H. pylori*) infection is specific to the human stomach and has been shown to be associated with chronic gastritis, gastroduodenal ulcers, gastric MALT lymphoma, and gastric cancer. Besides *H. pylori*, non-*Helicobacter pylori* helicobacter (NHPH) have been observed in the animal and human stomach. NHPH has been shown to be associated with chronic gastritis, peptic ulcers and MALT lymphoma, but details of NHPH infection have not been clarified because endoscopic characteristic findings of NHPH-infected gastritis are unknown and differential diagnosis from *H. pylori* is difficult. We found that the white marbled appearance limited to the gastric angle and antrum is characteristic finding of NHPH-infected gastritis. Combination therapies with a proton pump inhibitor, amoxicillin, and clarithromycin or metronidazole as well as *H. pylori* treatment have been shown to be effective for NHPH. Both endoscopic finding such as white marbled appearance and histological findings of activity and inflammation were improved after successful eradication.

P079

Campylobacter spp. from a season-long "FARM-TO-FORK" study of all natural, anitbiotic- free, pasture-raised broiler flocks in the Southeastern United States

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Background: The transmission of *Campylobacter* spp. and baseline level of antimicrobial resistance associated with this organisms has significant implications for environmental, animal, and human health. One focus is the use of antibiotics in animal agriculture and its effects on antibiotic resistant bacterial populations within those systems; however, before this causal effect can be elucidated, a greater understanding of the background/ reference levels of antibiotic resistance devoid of antibiotic use is needed.

Methods: An all-natural, multi-species, pasture-raised broiler flocks was sampled along the entire "farm-to-fork" continuum, including fecal and soil samples during grow-out, cecal content and carcass rinses during processing, and carcass rinses of the final products delivered to the consumer. Traditional culture methods, as well as the Campycheck method, were used to isolate *Campylobacter* spp. Isolates were subtyped using *flaA* SVR while antimicrobial sensitivity esistance profiles were determined using the CDC's NARMS protocol.

Conclusions: Resitances to a variety of antibiotics were found points along the "farm-to-fork" continuum. While not surprising, these results show that background levels for resistance in these production systems need to be considered when determining the causal effect of antibiotic use within the production animals to the proliferation of antibiotic resistance organisms.

P080

Antimicrobial sensitivity patterns of major zoonotic pathogens from a season long "farm-to-fork" study of all natural, antibiotic-fress, pasture-raised broiler flocks in the Southeastern United States

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Background: The prevalence of antibiotic resistance microorganisms has significant implications for environmental, animal, and human health. One focus is the use of antibiotics in animal agriculture and its effects on antibiotic resistant bacterial populations within those systems, but before this causal effect can be elucidated, a greater understanding of the background/reference levels of antibiotic resistance devoid of antibiotic use is needed.

Objectives: What are the antibiotic resistance profiles of bacteria in agricultural production environments when antibiotics are not used for production purposes? All-natural, pasture-raised production systems where antibiotics are not used nor have been used historically should allow us to better determine background levels of antibiotic resistance in relevant bacteria associated with broiler chicken production.

Methods: 15 all-natural, pasture-raised broiler flocks were sampled along the entire "farm-to-fork" continuum, including fecal and soil samples during grow-out, cecal content and carcass rinses during processing, and carcass rinses of the final products delivered to the consumer. Traditional culture methods were used to isolate 3 zoonotic bacterial pathogens (Salmonella, Campylobacter, and Listeria) and generic Escherichia coli and their resistance profiles were determined using the CDC's NARMS protocol.

Conclusions:S ensitivities to a variety of antibiotics were found for not only generic *E. coli* isolates, but also for the three zoonotic bacterial pathogens, from various points along the "farm-to-fork" continuum. While not surprising, these results show that background levels for resistance in these production systems need to be considered when determining the causal effect of antibiotic use within the production animals to the proliferation of antibiotic resistance organisms.

P081

The effect of embryonic age and breeder flock age on the gastrointestinal microbiome of developing broiler chicken: potential implications for food safety

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Background: There are several food-safety issues related to broiler egg production, including the introduction/proliferation of zoonotic pathogens during embryonic gastrointestinal (GI) tract development. Little is known about the overall GI bacterial communities, how they change over time, or how their composition could influence zoonotic pathogen survival/transmission.

Objectives: The objectives of this study were: (1) use 16S microbiomic sequencing to determine the structure of the overall developing GI bacterial community; (2) determine the effects of embryonic age and broiler breeder flock age on developing GI bacterial communities; and, (3) focus on the abundance of Salmonella and Campylobacter within these GI communities and how these temporal changes affect their abundance.

Methods: GI tracts were aseptically removed from commercial broiler eggs at 4 times (7-, 15-, 20-days post fertilization, and 1-day post-hatch) from broiler breeder flocks of three different ages (20, 35, and 60 weeks). As part of a second study, eggs from the 20-week-old flock were re-sampled at 35 and 60 weeks of age. DNA was extracted and 16S microbiomic sequencing analysis (using QIIME) was performed using the Illumina MiSeq platform.

Conclusions: Both embryonic and breeder flock age had significant effects on the developing GI microbiome of broiler eggs. While Salmonella sequences were present in low abundances, fluctuations in the abundance of Campylobacter sequences were more pronounced, especially in the second study. Considering Campylobacter has never been recovered culturally from broiler eggs, these results show the presence of a significant Campylobacter population during embryonic development and how these abundances are potentially related to breeder flock age.

P082

Determining risk factors of a non-point source outbreak of Campylobacter using case-case and case-control studies

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Introduction: Investigating foodborne outbreaks is a resource and time-intensive process using traditional case-control methodology. The use of case-case studies in outbreak investigations is not well studied, although they require fewer resources to conduct and limit selection and recall bias.

Objectives: In this study we investigated a cluster of Campylobacter infections using almost simultaneous case-control and case-case studies to compare results from the two methodologies.

Methods: In 2011 a significant increase in Campylobacter cases was detected in Pima County, AZ through routine surveillance. To determine potential sources of the outbreak we conducted two studies. The case-control study used randomly selected non-ill controls. The case-case study used historical surveillance data. Logistic regression analysis was used to determine risk factors for infection.

Results: Statistically significant risk factors associated with disease differed by design with travel (OR= 4.1), Hispanic (OR =4.5), and youth (OR=3.6) in the case-control and untreated water (OR=3.4) and fresh eggs (OR=2.5) in the case-case. Effect modification by travel was found for untreated water (OR=14.0 for travelers vs. OR=undefined for non-travelers) and eggs (OR=11.5 for travelers vs. OR=1.5 for non-travelers).

Conclusions: Travel history, a commonly reported risk factor, is a distal part of the exposure pathway. These studies exposed the more proximal cause to be largely attributed to travelers who had exposure to untreated water and fresh eggs. Case-case methods were found to be useful in outbreak investigations of a foodborne illness. This outbreak is also an example where a student response team response with a local public health department.

P083

Diagnosis of campylobacteriosis in humans from the Manawatu, New Zealand

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Campylobacteriosis is among the most frequently reported gastrointestinal diseases in the developed world. In New Zealand most diagnostic laboratories use culture methods optimised for detection of *C. jejuni/coli*, thus other species may be underdiagnosed. This study aimed to evaluate diagnostic methods for detection of *Campylobacter* species. A total of 594 faecal samples from people with clinical gastroenteritis were tested by four diagnostic methods during 2014-2015. A direct PCR targeting five *Campylobacter* species (Lund_PCR), 2 culture methods (CAT agar at 37°C in a hydrogen-enriched microaerobic atmosphere and mCCDA at 42°C in a microaerobic atmosphere) and the ProSpecT™ *Campylobacter* assay (ELISA) were performed on each sample. From all samples, 109 (18%) tested positive for *Campylobacter* spp. by at least one method. Individually, Lund_PCR detected 95 (16%), CAT 64 (11%), mCCDA 61 (10%) and ELISA 38 (6%) positive samples. Samples positive by only one method and negative by all other were 29, 11, 1 and 1 for Lund_PCR, CAT, mCCDA and EIA respectively. Only 28 samples were positive by all methods. *C. jejuni* was confirmed in 59 and 51 samples by mCCDA and CAT respectively. The CAT method detected 2 *C. coli* and 2 *C. hyointestinalis* samples undetected by other methods. Comparison of positive rates showed significant differences for each pair-wise combination of methods ($p<0.01$) except between CAT and mCCDA methods ($p=0.85$). The proportion of cultured campylobacters confirmed as *C. jejuni* was higher than that seen in studies in other countries. ELISA was the least sensitive method and not comparable to culture.

P084

Enumeration of campylobacter by using Potassium-Clavulanate-Supplemented Modified Charcoal-Cefoperazone-Deoxycholate agar from chicken

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Potassium-clavulanate-supplemented modified charcoal-cefoperazone-deoxycholate agar (C-mCCDA) that was described in our previous study was compared with original mCCDA for the enumeration of Campylobacter in pure culture and chicken carcass rinse. The quantitative detection of viable Campylobacter cells from a pure culture, plated onC-mCCDA, is statistically similar ($P > 0.05$) to mCCDA. In total, 120 chickens were rinsed using 400 mL bufferedpeptone water. The rinses were inoculated onto C-mCCDA and mCCDA followed by incubation at 42°C for 48 h.There was no statistical difference between C-mCCDA (45 of 120 plates; mean count, 145.5 CFU/mL) and normal mCCDA (46 of 120 plates; mean count, 160.8 CFU/mL) in the isolation rate and recovery of Campylobacter ($P > 0.05$)from chicken carcass rinse. The Pearson correlation coefficient value for the number of Campylobacter cells recovered in the 2 media was 0.942. However, the selectivity was much better on C-mCCDA than on mCCDA plates ($P < 0.05$). Significantly fewer C-mCCDA plates (33 out of 120 plates; mean count, 1.9 CFU/mL) were contaminated with non-Campylobacter cells than the normal mCCDA plates (67 out of 120 plates; mean count, 27.1 CFU/mL). The C-mCCDA may provide improved results for enumeration of Campylobacter in chicken meat alternative to mCCDA with its increased selectivity the modified agar possess.

P085

Evaluation of Cephamycins as antibacterial supplementation of selective agar for detecting Campylobacter spp. in chicken carcass rinse

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Although cefoperazone has been the most commonly used antibacterial supplement in Campylobacter selective media, cefoperazone-resistance bacteria such as extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* (*E. coli*) has recently become more widespread. In this study, we improved modified charcoal-cefoperazone-deoxycholate agar (mCCDA) by replacing cefoperazone with the same concentration (32 mg/L) of cefotetan and cefoxitin, which suppress ESBL-producing bacteria. Both improved agar plates [modified charcoal-cefotetan-deoxycholate agar (mCCtDA) and modified charcoal-cefoxitin-deoxycholate agar (mCCxDA)] provided much better ($p < 0.05$) sensitivity and selectivity compared to original mCCDA. The number of positive mCCDA plates for Campylobacter (18 out of 70, 26%) was significantly lower ($p < 0.05$) than that of mCCtDA (42 out of 70, 60%) or mCCxDA plates (40 out of 70, 57%). In terms of selectivity, significantly higher ($p < 0.05$) number of mCCDA plates (70 out of 70, 100%) were contaminated with non-Campylobacter than that of mCCtDA (20 out of 70, 29%) or mCCxDA plates (21 out of 70, 30%). The most common competing flora on original mCCDA was ESBL-producing *E. coli*, while Pseudomonas was frequently appeared on mCCtDA and mCCxDA. The emergence and spread of ESBL-producing *E. coli* strongly required the change of traditionally used antibacterial agent in Campylobacter agars, and cefotetan and cefoxitin would be a useful option as a selective supplementation.

P086

Performances of the LUMINEX xTAG molecular screening approach for detection of *Campylobacter* Spp. in stool samples

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Background: Early reliable detection of pathogens responsible of gastroenteritis is important. The xTAG(®) Gastrointestinal Pathogen Panel (GPP; Luminex Corporation, Austin, USA) is a molecular qualitative assay for the simultaneous detection of *Campylobacter spp. (jejuni, coli* and *lari)* plus 14 other gastroenteritis-causing pathogens from stool specimens.

Objectives: The aim of this study was to evaluate the performance of the xTAG® GPP for the detection of *Campylobacter spp.* compared to conventional culture methods.

Methods: Five hundred and fourty-two stool samples sent for culture to our laboratory were prospectively enrolled (one specimen/patient). Samples were from both pediatric (n=181; 89% outpatients) and from adult patients (n=361; 66.2% outpatients). Conventional method (Butzler agar plate and filtration method followed by identification by mass spectrometry) and xTAG® GPP were simultaneously performed.

Results: Five hundred and one samples gave concordant negative results (373 negative by both methods, 38 positive by culture for organisms not targeted by the xTAG, 90 positive for other organisms targeted by the xTAG) and 19 were concordantly positive (all *C. jejuni*). Twenty-two samples yielded discordant results (7 xTAG "false-negative" and 15 xTAG "false-positive"). Sensitivity, specificity, PPV and NPV were 73.08%, 97.09%, 55.88% and 98.62%, respectively.

Conclusion: Compared to culture, xTAG® GPP assay was able to accurately detect *Campylobacter jejuni* with a low turnaround time. In our setting, the low positivity rate contributed to the low PPV observed for the xTAG® GPP. Nevertheless, the high NPV value makes the xTAG a suitable first line screening tool.

P087

Use of molecular techniques for identification of unknown Campylobacter species

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Background: *Campylobacter* spp. has been isolated from humans and animals and is associated with foodborne and waterborne illnesses. *C. jejuni* and *C. coli* are the two most common species responsible for acute bacterial gastroenteritis in humans. Our current phenotypic method can differentiate these two species, however, there are other *Campylobacter* species frequently isolated from human stool samples of which species cannot be determined.

Objectives: To apply molecular techniques for identification and subtyping of human *Campylobacter* isolates of which species were unable to be determined by phenotypic and biochemical testing.

Methods: PCRs for 16S rRNA, 14 species specific *Campylobacter* and Chaperonin gene (cpn60) typing and sequencing were applied. 107 *Campylobacter* isolates from diarrhea cases and controls from diarrhea surveillance studies were included.

Results: Of the 107 isolates, 4 (4%) were excluded by 16S rRNA as not *Campylobacter*, 20 (19%) were *Campylobacter*-related genus (14 *A. buzleri*, 3 *A. cryaerophilus* and 3 *Helicobacter* spp.) and 83 (77%) were confirmed as *Campylobacter* spp. 80/83 isolates tested by *Campylobacter* species specific PCR and cpn60 typing PCR and sequencing were identified as *C. jejuni* (21), *C. coli* (11), *C. upsaliensis* (29), *C. hyointestinalis* (8), *C. concisus* (4), *C. lari* (4) and *C. fetus* (3). Three isolates were identified as *C. jejuni*, *C. coli* and *C. cuniculorum* by cpn60 typing and sequencing only.

Conclusions: PCRs for species specific *Campylobacter* and cpn60 typing and sequencing are useful for speciation of unknown *Campylobacter* isolates from our diarrhea surveillance studies. This application will enable us to understand an association of other *Campylobacter* species with diarrheal disease and an emergence of new *Campylobacter* spp. as potential diarrhea pathogens.

P088

Control of Campylobacter within pig farms: a realistic project?

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Pigs are known to be an important reservoir of *Campylobacter*. For the implementation of control measures in farms, it is essential to understand the epidemiology of *Campylobacter* in livestock animals. This study aimed at (i) investigating the carriage and the excretion of *Campylobacter* by pigs, (ii) describing the contamination of the environment, and (iii) assessing the efficiency of control measures. Faecal and environmental samples were monitored by quantitative real-time PCRs for quantification and species identification in a longitudinal study in two farrow-to-finish farms. No *Campylobacter* have been detected after the down period showing that good cleaning and disinfecting processes allow the elimination of *Campylobacter* in the rooms. There was no significant correlation between the presence and the level of *Campylobacter* excretion by pigs and the presence and the level of *Campylobacter* in the environment, whatever the samples. The early contamination of the piglets seems linked to their contact with their mothers. The environment including feed and water play a role as source of indirect contamination for pigs. Constant exposure of the environment to animal faeces during the pig life can explain this dynamic of infection and the pattern of *Campylobacter* excretion by pigs. Limiting this contamination could reduce the excretion of *Campylobacter* by pigs. Our study underlined the efficiency of good measures of hygiene to reduce even eliminate *Campylobacter* from the environment. However, animals participate to that contamination. Reducing the excretion of *Campylobacter* by pigs is the best option for limiting the presence of the bacteria in the farm.

P089

Inhibition of growth of Campylobacter as affected by bile salts in broth or agar

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The concentration of bile salts in the human small intestine ranges from approximately 0.2 to 2% (wt/vol), depending upon the individual and the type and amount of food ingested. Resistance of *Campylobacter* to bile salts is important for colonization and in the presence of bile *Campylobacter* may upregulate some virulence factors. The effect of various deoxycholate concentrations on the growth of 32 *Campylobacter* strains (21 C. jejuni and 11 C. coli) was measured and compared to growth at 0%, using two methods. The bioassay method assessed the inhibition of growth of each of the strains in broth against twelve deoxycholate concentrations (0.00195 to 4%). The bile salt dilution method utilised agar plates supplemented with four levels of deoxycholate (0 to 1%). In the bioassay most strains (22) demonstrated ≥ 95% inhibition of growth at 1%. A total of seven strains promoted growth at 0.125% compared to the no bile salts control. A single strain from these seven also demonstrated positive growth at 0.25%. In the dilution method only three strains demonstrated positive growth at any concentration. A single strain demonstrated positive growth at 0.75 and 1% and was significantly ($P<0.05$) different from 22 other strains. The concentration at which significant ($P<0.05$) differences in inhibition of growth were noted varied between strains and methods. These eight strains demonstrate an increase in growth when subjected to low levels of deoxycholate and may have the potential to resist bile salt concentrations that can be found within the human gut.

P090

Optimization of the filtration method for Campylobacter and related-organism isolation

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Background: It has been well demonstrated that using filtration through a membrane filter onto an antibiotic-free blood agar and subsequent incubation at 37°C in a microaerobic atmosphere dramatically increases the number of campylobacters isolated in clinical laboratories by allowing the growth of strains inhibited by the conventional 42°C incubation temperature or by the selective medium used.

Objectives: This work aims to optimize the filtration method by determining which blood agar -between Columbia agar (Becton Dickinson, Erembodegem, Belgium) and Mueller Hinton agar with 5% sheep blood (MHB; ThermoFisher Scientific, Erembodegem, Belgium)- is the best for *Campylobacter* isolation using such a method.

Methods: During a 3 month-period, all stool samples transmitted to our laboratory for *Campylobacter* isolation were inoculated onto both Columbia agar and MHB using the filtration method and onto a conventional Butlzer selective agar (ThermoFisher Scientific) in parallel.

Results: A total of 1146 stool specimens were collected from July to September 2014, 72 of which led to a *Campylobacter*-positive culture (6.3%). Amongst these, about a third were revealed by using the filtration method only (22/72): 11 on Columbia agar only, 1 on MHB only and 10 on both media. The Columbia agar appeared to be statistically better than the MHB (McNemar statistical test, $p<0.05$).

Conclusion: When performing the filtration method, the Columbia agar allows the isolation of more campylobacters that are missed by conventional selective media than the MHB. The Columbia agar is now used in our daily practice for the isolation of *Campylobacter* by this method.

P091

Recognition of two genomovars within *Arcobacter cryaerophilus* after a polyphasic taxonomic re-evaluation

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Background: *Arcobacter cryaerophilus* is an emerging human and animal pathogen, for which originally two hybridization groups (named either 1A and 1B or 1 and 2) were defined. They showed different restriction fragment length polymorphisms (RFLP), whole-cell protein and fatty acid contents suggesting that they could belong to different taxa, but this was unclear using amplified fragment length polymorphisms (AFLP) and the sequences of the *cpn60* gene.

Objectives: To re-evaluate the taxonomic status of *A. cryaerophilus* by mean of a polyphasic taxonomic study, using a strain collection representative of the heterogeneous population of this pathogen.

Methods: Strains (n=42) obtained from clinical and environmental origin, 9 different countries and isolated in a broad time frame (1985 -2013) were characterized by phenotypic tests and Multilocus Phylogenetic Analysis (MLPA).The data set also included previously published data (16S rDNA sequences and DDH values) from reference strains.

Results: Individual and concatenated analyses of the *gyrB*, *gyrA*, *rpoB*, *atpA* and *cpn60* genes showed the strains phylogenetically grouped into two main clusters. The 16S rDNA sequences similarity between representative strains of both clusters (LMG 9904T and LMG 10829), was 98.56%, while DDH value was 55%. However, none of the phenotypic tests used was able to differentiate them.

Conclusions: The two main phylogenetic clusters found here represent two genomovars, which did not completely correlate with the former 1A and 1B groups. The only requirement to formally differentiate the genomovars into two different species requires finding a phenotypic test able to differentiate them.
Acknowledgements: Projects DID S-2013-06 and AGL2011-30461-C02-02

P092

Identifying reservoir hosts of emergent Campylobacter and Arcobacter species in Chilean wildlife rehabilitation centers

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Background: *Campylobacter jejuni* and *C. coli* are the leading cause of bacterial gastroenteritis worldwide. However, other emergent campylobacteria has become increasingly important in recent years. In contrast with common campylobacters, the natural reservoir of most emergent species are yet unknown.

Objectives: Given that some emergent campylobacters have been originally isolated from wild birds and mammals, we hypothesized that wildlife could be playing a role as reservoir of these emergent pathogens. Therefore, our objective was to identify natural hosts of campylobacteria in wildlife rehabilitation centers from Southern Chile.

Methods: Ninety three faecal samples obtained from 21 different kind of wild bird species and from three species of mammals were analyzed. The specimens were cultured in Bolton broth and, after incubation at 37°C and 42°C for 48h in microaerobic condition, an aliquot of broth was plated in mCCDA and re-incubated in the same mentioned conditions. The isolates were identified by a polyphasic taxonomic approach including phenotypic and molecular methods (PCR-RFLP, m-PCR and 16S rRNA gene sequencing).

Results: The global campylobacteria prevalence found in birds was 33.3%. The most prevalent species was *C. jejuni* (58%) followed by *C. lari* (12.9%), *A. cryaerophilus* (6.5%), *A. butzleri* (3.2%) and *C. coli* (3.2%). The rest of bird´s isolates corresponded to a potential new species belonging to the *Campylobacter lari*-group. In mammals, *C. lanienae* was the only species recovered.

Conclusions: Chilean wildlife harbours a number of emergent and novel campylobacteria, and therefore would contribute to the spread and transmission of these zoonotic pathogens. Acknowledgements: Project Fondecyt No. 11130402

P093

Resistance and virulence profile of Helicobacter pylori strains isolated in Brussels, Belgium

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Background: Management of patients infected by *Helicobacter pylori (Hp)* depends of local epidemiology as current treatment strategy guidelines are based on local resistance patterns. Additionally, several *Hp* virulence genes are known to be associated with severe outcomes. These include *cag*A and some allelic variants of *vac*A.
Objectives: Our goal was to determine the resistance and virulence profile of *Hp* strains isolated in Brussels, a city mixing multiple populations.

Methods: One hundred and three strains collected in Brussels during 2012-2013 from gastric biopsy of non-treated patients were included. Demographics (age, gender, ethnicity) and endoscopic results were retrieved. *vac*A genotype and *cag*A detection were assessed by PCR while amoxicillin, metronidazole, levofloxacin and clarithromycin MIC were assessed by E-test.

Results: Patients originated predominantly from North- (45) and Sub-Saharian Africa (14), Western (14) and Eastern Europe (13); 20 presented ulcers. Twenty-seven strains were resistant to metronidazole, 18 to levofloxacin and 15 to clarithromycin. Fifty-two strains were *cag*A positive. The most frequent *vac*A genotypes were s2m2 (41.7%) and s1m1 (37.9%). Ninety-eight percent of *vac*As2m2 strains were *cag*A negative while 89% of s1m1 and s1m2 strains were *cag*A positive ($p<0.001$). While ulcer was significantly more frequent in patients infected with *vac*As1m2 strains, a significant association between virulence gene profile and ethnicity was observed as *vac*As2m2-*cag*A-negative strains were not found among patients from Eastern Europe.

Conclusions: These results highlight significant associations between certain virulence gene profiles, ethnicity and occurrence of ulcer. Further investigations are needed to assess their usefulness regarding the clinical management of *Hp* infected patients.

P094

Comparative genomics of *Campylobacter fetus* from reptiles and mammals reveals divergent evolution in host-associated lineages

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Background: Ectothermic reptiles display an intestinal Epsilonproteobacteria composition distinct from endothermic mammals and birds, which includes several *Campylobacter* and *Helicobacter* taxa exclusively isolated from reptiles. The novel taxa *C. fetus* subsp. *testudinum* (*Cft*) and *C. iguaniorum* show highest Epsilonproteobacteria prevalence in reptiles. Interestingly, *C. fetus* shows a distinct intraspecies host dichotomy; both *C. fetus* subspecies *fetus* and *venerealis* are associated with mammals, primarily ruminants, whereas *Cft* is primarily associated with reptiles. Both mammal- and reptile-associated *C. fetus* have been associated with severe infections, often with a systemic component, in humans.

Objectives: To study the genetic determinants associated with the distinct host dichotomy in *C. fetus* and to study the genetic determinants associated with pathogenicity of *Cft* in humans.

Methods: Whole-genome comparison of 60 strains of mammal- and reptile-associated *C. fetus* and most closely related *Campylobacter* species was performed using BLAST and orthology clustering. Recombination events were detected using Gubbins.

Results: Genomic comparisons showed a clear distinction between mammal- and reptile-associated *C. fetus*. Several genomic regions were subspecies specific, including a tricarballylate catabolism locus, exclusively present in reptile-associated taxa *Cft* and *C. iguaniorum*. Within *Cft*, *sapA*-, *sapB*-, and *sapAB*-type strains were observed. A recombinant *iamABC* locus, derived from mammal-associated *C. fetus*, was exclusively associated with invasive *Cft* strains isolated from humans. A phylogenomic reconstruction was consistent with divergent evolution in niche-associated strains and the existence of a barrier to lateral gene transfer in the speciation of *C. fetus*.

Conclusion: *C. fetus* shows signs of divergent evolution based on host-associated allopatric speciation.

P095

Isomerase activity of HP0377 mutated forms

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Background: *Helicobacter pylori* HP0377 protein is a Dsb thiol oxidoreductase which acts as CcmG protein, involved in the maturation of apocytochrome c. However, in contrast to other so far characterized CcmGs, it possesses disulfide isomerase activity, as it catalyzes the refolding of scrambled RNase.

Objectives: The biochemical properties of Dsb oxidoreductases are conditioned by the presence of two highly conserved motifs: the CXXC active site within the TRX fold and the *cis*-proline loop (distant in the linear sequence but close in the three-dimensional structure to the CXXC).The aim of this study was to establish the link between the amino acid residues of HP0377 active motifs (the cysteine-flanked dipeptide sequence of the CXXC motif as well as the *cis*-Pro motif) and its isomerization activity.

Methods: Site-directed mutagenesis of *hp0377* were carried out using a QuickChange mutagenesis kit. DNA fragments were then cloned into *E.coli* expression vector. Genes encoded mutated forms of HP0377 were overexpressed by autoinduction. Subsequently, wild type HP0377 and its variants were purified by affinity chromatography and used for in vitro assay (scrambled RNase refolding). Results We found that HP0377 variants with cysteine of the CXXC motif changed to serine (HP0377_CSYS and HP0377_SSYC) does not reveal isomerase activity. HP0377 variant with active site of ResA (*Bacillus subtilis* CcmG which does not possess isomerase activity) showed significant decrease in isomerase activity.

Conclusions: Isomerase activity of HP0377 is, at least partially, determined by active site CXXC-dipeptide.

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P096

Genetic characteristic and source attribution analysis of Campylobacter isolated in China

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Background: Campylobacteriosis is mainly caused by *C. jejuni* and *C. coli*. Production animals can function as the mainly reservoir in China. Identifying the most important source of human disease is essential for prioritizing food safety interventions and setting public health goals. Molecular sub-typing play an important role for source attribution of human cases and MLST is the most widely applied one. Objective: To assess the mainly source of the human cases of *Campylobacter* infection.

Methods: A total of 624 (278 *C. jejuni* and 346 *C. coli*) isolates collected between 2011 and 2013 were included in the present analysis. Among these isolates, 272 isolates were collected from the stool samples of the diarrheal patients and 352 isolates were collected from the samples of the food producing animals. MLST was performed by sequencing seven housekeeping gene loci. The fixation indices (Fst) between populations were determined by ARLEQUIN software and the genotypic assignment with STRUCTURE was applied to estimate the proportion of human cases attributable to each source using the concatenated sequences of the MLST alleles.

Results: 226 STs were identified among 624 isolates. The Fst between the chicken isolates and human isolates (Fst=0.0124, p=0.027) is lower than the Fst between the swine isolates and human isolates (Fst=0.31701, P<0.001). 53.2% of the human isolates were assigned to the chicken reservoir while 46.8% were assigned to the swine reservoir.

Conclusion: The source attribution study indicated that both the chicken and the swine were the principal sources of *Campylobacter* infection in humans in China.

P097

MALDI-TOF and rpoB sequencing reveals previously undetected case of *Arcobacter butzleri* from human diarrhoea.

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Background: The genus *Arcobacter* includes aerotolerant gram-negative bacteria and was the fourth most common pathogenic bacteria genus isolated from stool specimens of patients with acute enteritis in Belgium when using a specific culture media. However, this is not the case in many other studies where Arcobacter is identified by chance from media used for Campylobacter or other enterobacteria.

Objectives: To characterize the sequence types and virulence genes present in Arcobacter butzleri strains associated to cases of human diarrhea.

Methods: Four *A. butzleri* strains, three recovered from CIN agar (BD) and identified by MALDI-TOF and one isolated from Campylosel agar (bioMerieux) and identified by sequencing the *rpoB* gene were investigated. Sequence types were determined after sequencing the *aspA*, *atpA*, *glnA*, *gltA*, *glyA*, *pgm* and *tkt* genes by comparison with the MLST database. The presence by PCR of 5 virulence genes (*ciaB*, *cadF*, *cj1349*, *hecA* and *irgA*) was determined using primers described previously.

Results: The 4 *A. butzleri* strains were all confirmed as this species with the sequences of housekeeping genes. All strains showed to belong to new sequence types on the basis of the new alleles found for the genes *glyA* and *tkt* despite alleles found for *atpA* and *glnA* were already present at the database. At least 3 virulence genes were present in all the strains.

Conclusions : Isolation of new sequence types of *A.butzleri* form CIN agar seems to be common and MALDI-TOF enabled their fast and reliable identification. Acknowledgments: Projects AGL2011-30461-C02-02 and FP7/2007-2013 (no. 311846).

P098

Implementation of key biosecurity measures in Spanish broiler houses to reduce Campylobacter prevalence: hygienic barrier and training of farm personnel.

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The main tool available at present to control *Campylobacter* in the primary broiler production is implementation of biosecurity measures at house level. Key interventions undertaken at the Spanish farms were to create a hygienic barrier in the anteroom and to train farmers adequately in entry and exit procedures. The hygienic barrier consisted of firstly dividing the anteroom in two zones: the dirty zone (the part of anteroom closest to the outer door), and the clean zone (the part closest to the door leading to the broiler room). In the dirty zone, farm personnel should remove outerwear and footwear and wash and disinfect their hands. Once in the clean zone, they put on working clothes and footwear dedicated for each specific broiler house. Secondly, for adequate training of farm personnel, weekly farm visits were carried out, where around 50 control points were checked at each visit and deviations noted and discussed with the farmer. Supportive training material was provided, such as instructive posters for the anteroom. The implementation of these key biosecurity measures faced several difficulties; the main one was the fact that anterooms in the Spanish houses were not built appropriately for installing hygienic barriers. An e-learning program for access at the internet has been developed currently, including the experience gained at the farm visits. The whole process in upgrading the biosecurity together with practical issues will be presented and discussed.

P099

Diversity and distribution of multi-locus sequence typing (MLST) subtypes of *Campylobacter jejuni* and *Campylobacter coli* from broiler chickens in Spain.

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The distribution of subtypes of *Campylobacter jejuni* and *Campylobacter coli* isolates from broiler chickens in Spain was investigated by Multi-locus Sequence Typing. A total of 129 isolates (70 *C. jejuni*, 59 *C. coli*) from 20 farms covering the period 2011-2012 were analyzed. All isolates were cultured from the caeca of intensively reared broiler chickens and were evenly spread across the seasons to avoid seasonal bias. *C. coli* strains were genetically more conserved than *C. jejuni*, with a single clonal complex (CC828) that included 21 sequence types (ST). *C. jejuni* isolates generated 41 ST and most of them clustered within the overall 11 different CC. Most of the farms showed a high diversity of strains, which clustered in several CC, but in four of them the isolates clustered in only one CC (CC828). The number of different ST per farm ranged from 2 to 11. Widespread ST21, 257 and 572 that have been reported from a range of animal species and humans, have also been isolated in the studied farms. This study highlights the diversity of *C. jejuni* and *C. coli* isolates on Spanish broiler farms.

P100

Detection of antigenic *Helicobacter hepaticus* protein recognized by child serum antibodies

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Background: *H. hepaticus* causes chronic hepatitis and colitis in mice. However, the association of the microorganism in the liver diseases is obscure in human yet. *H. hepaticus* has not been isolated from human patients. The diagnostic PCR methods of *H. hepaticus* infection using human samples were reported, but other methods need to be developed for confirmation of the infection or epidemiological study.

Objectives: The purpose of this study was to detect the antigenic *H. hepaticus* protein recognized by child serum antibodies.

Materials and Methods: Subjects were sera from eight children (3-10 years old, average 5.9). The serum samples were assayed by ELISA using *H. hepaticus* monoclonal antibody (HRII 51) or total lysate of *H. hepaticus* as antigens, and divided into low or high reactivity groups by the OD. Furthermore, diluted serum samples were used on PVDF membranes blotted by the total *H. hepaticus* lysate after two-dimensional electrophoresis (2-DE), followed by anti-human IgG secondary antibody to detect antigenic protein spots. The same spots were isolated from the 2-DE gels and the proteins were identified by mass spectrometry (LC-MS/MS). **R**

esults: *H. hepaticus* ELISA reactivity of five sera were high, but those of remaining three sera were low. Total 25 proteins were identified as candidate antigens by LS-MS/MS after the 2-DE immunoblot analysis. Among the proteins, flagelin A was the most reactive protein, particularly against highly reactive sera, although low reactive sera also detected the spot slightly.

Conclusions: Flagelin A is one of the main antigenic protein in *H. hepaticus* infection in children.

P101

The importance of CiaB and cellular invasion to *Campylobacter rectus pathogenesis*

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Background: *Campylobacter rectus* is a Gram-negative anaerobe and a putative pathogen of periodontitis. Similarly to *Campylobacter jejuni, C. rectus* encodes a protein known as Campylobacter invasion antigen B (CiaB).

Objectives: Objectives of this research include: 1) to test whether CiaB in *C. rectus* functions similarly to *C. jejuni* CiaB in pathogenesis (related to invasion); 2) to determine the conditions of CiaB secretion (e.g. signals and pathways).

Methods: A complete gene deletion of the *ciaB* gene in *C. rectus* ATCC 33238 was generated by electroporation with a targeting plasmid created using Invitrogen's Gateway cloning system. To assess cellular invasion, microscopy methods will be used to observe invasion rates for wild-type *C. rectus* strains as well as *ciaB* null mutants using HGF-1 and BeWo cells. RT-PCR-based analysis of inflammation-related genes in both HGF-1 and BeWo cells will be used to assess host response to wild-type and *ciaB* mutants. The secretion of *C. rectus* CiaB protein will be assessed in response to human pregnancy hormones as well as other potential effectors.

Results: The successful generation of a complete gene deletion of the *ciaB* gene in *C. rectus* allows the investigation of invasion related phenotypes. While preliminary phenotypes of the *C. rectus ciaB* mutant are consistent with CiaB function in *C. jejuni*, studies are continuing to verify mode of and signals for CiaB secretion, and importance to pathogenesis.

Conclusion: Based on the results of these studies, the molecular involvement of the CiaB protein in the pathogenesis of *C. rectus* will be clarified.

P102

A novel regulatory system controlling genes for cell envelope functions in *C. jejuni.*

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Background and Objectives: Elucidating how *Campylobacter jejuni* regulates gene expression in response to extracytoplasmic signals is crucial to understanding its infection biology. We have identified a previously uncharacterized regulatory system, which controls expression of a small set of genes encoding novel cell envelope proteins.

Methods and Results: The *mceS* and *mceR* genes in *C. jejuni* NCTC 11168 encode a putative membrane bound sensor protein and a putative DNA binding protein. Microarray analysis of a *mceR* null mutant showed up-regulation of a divergently transcribed 3-gene operon encoding cell envelope proteins, indicating that MceR is a repressor of these genes. qRT-PCR analysis of a *mceS* null mutant showed that it affected the expression of the *mceR* regulator gene. MceR was purified as a his-tagged recombinant protein and shown by gel-shift assays to bind to the divergent promoter region and to the promoters of other genes identified by the microarray. To identify signals for the de-repression of the controlled genes, a *lacZ* fusion has been constructed and inducers are being screened using Phenotype Microarray plates in high-throughput assays. Bioinformatic analysis has shown that while the two regulatory genes are highly conserved between *C. jejuni* strains, the divergent structural genes within the same regulon are more variable. Null mutants have been made in each of the genes regulated by MceR and their phenotypes are being characterized.

Conclusions: This system controls expression of periplasmic and membrane proteins in *C. jejuni* which may be involved in maintenance of cell envelope functions.

P103

Comparative whole genome sequence (WGS) analysis of *Campylobacter fetus* subspecies; a Canadian perspective.

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Background: Traditional phenotypic and genotypic tests to differentiate *C. fetus* subspecies demonstrate variable reliability. Existing comparative genomic studies of *C. fetus* subspecies are notably absent of a North American perspective.

Objectives: Comparative WGS of *C. fetus* isolates from clinical and animal sources was performed to provide a phylogenetic representation of the subspecies through core and accessory genome analysis and to enable the identification and characterization of subspecies-defining genomic elements.

Methods: Twenty-six clinical and reference isolates representing *fetus, venerealis,* and *testudinum* were sequenced on an Illumina MiSeq. Sequencing reads were de novo assembled and annotated using SPAdes and Prokka. Core genome SNP phylogenies and a 7-gene MLST scheme were employed to inform the phylogenetic relatedness between subspecies. Pangenomes built using GView revealed variable genetic regions within the accessory genome. MALDI-TOF, parA/cstA multiplex PCR and 1% glycine tolerance (gold standard) were performed.

Results: Core genome SNP phylogenies delineated subspecies *venerealis* and *testudinum* as homogenous clusters distinct from subspecies *fetus,* while *fetus* isolates grouped into several clades, indicative of increased genetic diversity. Accessory genome analysis revealed genetic heterogeneity between subspecies including the presence of indels and a CRISPR-Cas system within a subset of testudinum and fetus isolates. Pangenome analysis identified a previously uncharacterized 46-kb plasmid harbouring genes for a Type IVSS and AMR genes.

Conclusions: Core genome analysis revealed that subspecies fetus is more genetically diverse than other subspecies. Significant intra-and inter-subspecies diversity in accessory genome was discovered, a finding potentially leading to the development of new genetic tools for subspecies identification and differentiation.

P104

Cytopathic effects of toxogenic strains of *Helicobacter pylori* on different cell lines

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Background: Many virulence factors are involved in the pathomechanism of infection caused by *Helicobacter pylori*. Toxins such as vacuolating cytotoxin, encoded by the vacA gene and the immunogenic protein cagA, encoded by the cagA gene (cytotoxin-associated gene) are major factors conferring the property of virulence. The current study is aimed at isolation of *H. pylori* and separation of its toxin from antral biopsies of patients.

Materials and Methods: The following cell lines were used to demonstrate the cytopathic effect (CPE) of the separated toxin: African green monkey kidney (Vero), baby hamster kidney, human lung carcinoma (LLC-MK2), and human epithelial.

Results: *H. pylori* was isolated from 27 out of 45 patients (60%) selected for the study. CPE of *H. pylori* toxin was highly significant on Vero cells than other cell lines used as it reached a high dilution titer of toxin (1/16) in 13 isolated strains (48.15%). No significant difference in CPE of toxin in different dilutions was detected among other cell lines used in different groups. *H. pylori* toxin could be detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis as a distinct band with a molecular weight ranging between 66 and 97 kDa and closely related to 87 kDa.

Conclusion: *H. pylori* vacuolating cytotoxin plays a vital role in the pathogenesis of gastroduodenal diseases (gastritis, gastric ulcer, duodenal ulcer, and gastric cancer). The Vero cell lines were found to be the most suitable form of tissue culture when compared with other cell lines used in our study for demonstrating the activity of *H. pylori* toxin.

P105

Patterns of *Helicobacter pylori* Resistance to Metronidazole, Clarithormycin and Amoxicillin in Saudi Arabia

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There are no generally accepted regimens for the treatment of *H. pylori* infection in patients with gastritis or duodenalulcers. However, metronidazole based regimens have been reported to be among the most successful. Resistance tometronidazole, clarithromycin, and amoxicillin was determined for 46 clinical isolates of *Helicobacter pylori* in SaudiArabia and tested by E test. Of these isolates, 69.5% was resistant to metronidazole (MIC > 8 mg/l), 21% to clarithromycin(MIC > 1 mg/l) and 11% were multiresistant. No resistance to amoxicillin was observed. Resistance to metronidazolewas more common in isolates from females than in those form males. In conclusion, the present study demonstrateshigh metronidazole resistance rate of *H. pylori* isolates in Saudi Arabia. Regimens containing metronidazole are bestavoided. Trials to test other antimicrobial combinations are recommended.

P106

Is the inhibitory effect of honey against Campylobacter cost related?

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Honey is the only beneficial food with high popularity, consumption and availability. It has inhibition effect against most food-borne pathogens including *Campylobacter*. The effective activity without any side effects reported increases the use of this traditional medicine in treating all kinds of infection. This study was examined the activity of commercially available honey against *Campylobacter*. Different types of honey were used and a comparison between the activity of low cost and high cost honey was observed. Honey samples were obtained from local supermarket or imported. Honey was introduced to Mueller Hinton media by filtration. *Campylobacter jejuni* NCTC 11168 was sensitive to all honey samples used in this study. The minimum inhibitory concentrations were in the range between 2 and 10% honey. The result showed that all types were effective against *Campylobacter* regardless the price of honey. The recommendation from this study is to use honey to treat *Campylobacter* infection and food poisoning

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P107

Development of an effective method for decontamination of Campylobacter species on chicken carcasses using physical treatment with a direct-drive-pulse cleaner

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Although many risk factors for transmission of *Campylobacter* to humans have been identified, the most frequent mode of infection is considered to be consumption of chicken meat that has been contaminated during processing. While the most desirable approach would be a suitable physical treatment that carries no risk of chemical residues in the product, a fully satisfactory system for decontamination is not yet available. In the present study, we evaluated an alternative strategy to reduce the numbers of contaminating bacteria using a direct-drive-pulse cleaner (DDPC), which rapidly washes out any bacteria attached to a carcass using an intermittent high-impact pressure spray and ultrasonic energy. Broiler carcasses after evisceration were inoculated artificially with *C. jejuni* and then spray-washed against the breast skin or back skin with tap water (20 or 43°C) or sodium hypochlorite (100 ppm) for 5 minutes each. The numbers of *Campylobacter* attached to the skin before and after spray-washing were determined by the most probable number method. In comparison with prewashed skin, the mean reductions obtained by the new decontamination treatment exceeded the one to two log CFU per 10 g breast or back skin achieved with chemical antimicrobials. Moreover, the decontamination effect of water at 43°C was higher than that for water at 20°C. The decontamination effect achieved using a DDPC was observed as the reduction in both the standard count and coliforms. The present study has shown that treatment of carcasses by spray-washing using a DDPC can remove microorganisms attached to chicken skin surfaces effectively.

P108

Helicobacter pylori infection is a consistent protective factor against inflammatory bowel diseases

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Background and Objectives: Previous studies suggest that *Helicobacter pylori* infection is a protective factor against several diseases including inflammatory bowel diseases (IBD). Our aim was to conduct a comprehensive meta-analysis on *H. pylori* infection and risk of IBD to estimate true pooled effect sizes and identify potential causes for previous conflicting results.

Methods: Electronic databases were searched up to July 2015 for all case-control studies on *H. pylori* infection/ enterohepatic *Helicobacter* spp. (EHS)/*Campylobacter* spp. and IBD. Pooled odds ratios (P-OR) and 95% confidence intervals were obtained using the random effects model. Heterogeneity, sensitivity and stratified analyses were performed.

Results: The total study sample included 6,130 IBD patients and 74,659 controls. Analyses comprising all IBD patients showed a consistent negative association between gastric *H. pylori* infection and IBD (P-OR: 0.43, *P*-value: <1e-10). This association appears to be stronger in Crohn's disease and IBD unclassified patients than ulcerative colitis patients. Stratification by age, ethnicity and medications showed significant results. In contrast to gastric *H. pylori*, non *H. pylori*-EHS (P-OR: 2.62, *P*-value: 0.001) and *Campylobacter* spp., in particular *C. concisus* (P-OR: 3.76, *P*-value: 0.006) and *C. showae* (P-OR: 2.39, *P*-value: 0.027), increase IBD risk.

Conclusion: *H. pylori* infection is negatively associated with IBD regardless of ethnicity, age, *H. pylori* detection methods and previous use of common medications. Closely related bacteria including EHS and *Campylobacter* spp. increase the risk of IBD. These results support the notion that *H. pylori* might be an immune modulator playing a role in the pathogenesis of IBD.

P109

Assessing the survival of *Campylobacter jejuni* and *C. coli* under different temperature and pH regimes.

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Background: New predictive models are urgently needed that can inform the food industry about treatments to eliminate *Campylobacter* from chicken products. Data used in current models do not recognise the population biology of the pathogen, or its prior "environmental experience".

Objectives: The aims of this project were to determine the susceptibility of strains of *C. jejuni* (ST21, ST45, ST51, ST257, ST74) and *C. coli* (ST825, ST829, CC828) to instant heat challenge in media and also to altered pH.

Methods: Broth was pre-heated prior to inoculation with the test strain. The full panel were subjected to heat challenge at 56, 60°C and 64°C. A subset of isolates was subjected to challenge at 56, 60 and 64°C and at neutral pH 7.2-4, and at pH 4.5, 5.5, 6.5 and 8.5. Results The response of *Campylobacter* populations to challenge at 56 and 60°C were similar and hence highly reproducible across replicates for individual strains. Some differences however were observed between different strains of *Campylobacter* in terms of their response to and viability following heat treatments. When challenged at 56°C, D values were significantly higher for pH 5.5 - 8.5 in comparison to D values recorded at 60°C and 64°C.

Conclusions: Results indicate that *Campylobacter* is susceptible to stress at pH 4.5 and 8.5, but was found to be more resilient between pH 5.5- 6.5 at 56°C. Significant differences in D values were found between moderate and higher temperatures. Such findings have implications for the food industry and treatments used to eradicate *Campylobacter*.

P110

Investigating the interaction of Campylobacter with chicken meat and its influence of survival at high temperatures.

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Background; Campylobacter can be present on the surface or interior of broiler meat and it is important to understand the impact of attachment or internalisation in the meat tissue on survival following heat treatment. Objectives; The aims of this project were to determine the susceptibility of strains of C. jejuni (ST-21, ST-45, ST-257) and C. coli (ST-825) to heat challenge following inoculation of meat. Methods; Chicken meat fragments (0.1g) were surface inoculated with Campylobacter and subjected to direct heat or gradual heating (56-70°C) to examine the effects of attachment, whilst pieces of chicken meat (2g) were inoculated internally and subjected to gradual heating from 25°C to 66 and 70°C. Results Attachment to meat tissue was shown to enhance survival and heat resistance at low temperatures, but at higher temperatures attachment to meat had no impact on survival. For internally contaminated meat it took ~20 minutes for the interior to reach 68°C (70°C in the water bath) and whilst there was a large reduction in counts, Campylobacter could still be detected for all four strains. Conclusions; Large inoculums (~10^7 CFU/ml) were used in these experiments to allow modelling of survival following challenge, however given the small size of these pieces used, extrapolating to large meat portions and also internal contamination reported in poultry meat, these results are worrying and may suggest the low level persistence of Campylobacter in meat following heating.

P111

Investigating survival of Campylobacter in whole chicken fillets during sous vide cooking.

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Background: Sous vide cooking of meat has been growing in popularity over recent years and involves low temperature cooking for long periods. There are however few data on the survival of *Campylobacter* in poultry meat cooked by this method.

Objectives: To determine the response of *C. jejuni* (ST257, ST45) and *C. coli* (ST828) strains to cooking within whole chicken fillets under vacuum conditions at low temperature using a commercially available sous vide water bath. Methods; Each fillet (100-120g) was injected with *Campylobacter* (10^6 CFU/g) and placed individually in a vacuum sealed bag and then placed into a sous vide machine. Experimental simulations were undertaken covering a range of potentially inadequate heating temperatures (50-56°C), with samples taken at 0, 20, 40 and 60 minutes for enumeration/*Campylobacter* culture. At the lower temperatures, extended heating times were also examined up to 2 h at 52°C and 3h at 50°C.

Results: At 56°C, *Campylobacter* was mostly eliminated after 1 hour following sous vide cooking. Additionally, simulations were extended and samples taken following 2 hours at 52°C and 3 hours at 50°C, which indicated that a population of *Campylobacter* was able to survive at these lower temperatures for long periods of time.

Conclusions: These results indicate that the temperature used for sous vide cooking is important with temperatures of 52°C and below are inadequate in killing all *Campylobacter* even after 3 hours. Our work indicates that temperatures of 56°C or above should be used to cook meat which may potentially be contaminated with *Campylobacter*.

P112

CDT variants produced by *Campylobacter hyointestinalis* show different biological activity

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Cytolethal distending toxin (CDT) consisting of three subunits, CdtA, CdtB and CdtC, is a potential virulence factor produced by *Campylobacter* species. CdtA and CdtC are responsible for receptor binding while CdtB exerts DNaseI-like activity causing cell cycle arrest at G2/M phase and eventual cell death. We have found that *C. hyointestinalis* produced at least three CDT variants named as ChCDT-I, ChCDT-II and ChCDT-III. In this study, recombinant ChCDT-II and ChCDT-III were prepared to compare the biological activity between rChCDT-II and rChCDT-III by cell assay. rChCDT-II and rChCDT-III demonstrated different biological activities. In Vero cells, rChCDT-II caused both cell distention and G2/M arrest, but rChCDT-III could cause only cell distention. While in CHO cells, rChCDT-III could cause cell distention and G2/M arrest, but rChCDT-II could not demonstrate any biological activity. To examine which subunit is responsible for the different biological activity between rChCDT-II and rChCDT-III, various chimeric rChCDTs were generated and analyzed for their cytotoxicity activities. Three chimeric rChCDTs, rChCdt-IIA/IIIB/IIC, rChCdt-IIA/IIIB/IIIC and rChCdt-IIIA/IIIB/IIC caused cell distention in Vero cells. Interestingly, only rChCdt-IIA/IIIB/IIIC showed cytotoxicity against CHO cells as demonstrated by cell distention and G2/M arrest. The result indicates that 'CdtC' subunit may be important for cytotoxic activity of rChCDT-III against CHO cells. When Vero cells were co-cultured with rChCdt-IIA/IIIB/IIIC, both G2/M arrest and γH2AX could be observed. However, γH2AX was not observed by co-culture of Vero cells with rChCdt-IIA/IIIB/IIC or rChCdt-IIIA/IIIB/IIC. These findings suggest that ChCDT variants may have different receptors and intracellular pathways to cause cell distention and G2/M arrest.

P113

A longitudinal study of interventions and Campylobacter genotypes in British broiler farms

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Background: Colonisation of broiler flocks on farm is a major challenge for the poultry industry.

Objectives: To determine the efficacy of interventions (biosecurity barrier, organic acid treatment of drinking water and fly-screen). To utilise genotyping data to detect carry over between crops and identify potential sources of flock colonisation.

Methods: Broiler farms (n=24) were sampled prior to thinning and at clearance for 8 crops. *Campylobacter* was isolated, whole genome sequenced and MLST genotypes obtained.

Results: Prevalence was higher (P0.05). At clear 6 farms were always positive (P=0.005). There were 64 out of 168 flocks that were positive at clear and also positive pre-thin in the following crop. Of these 64 flocks 6 had the same genotype at clear and at the following pre-thin (higher than by chance, P=0.04). Of the 6 positive flocks, two farms have two carry overs each (P=0.009). However, carry-over of the same genotype only explains 4% of overall flock positivity prevalence. STRUCTURE inferred putative source pre-thin as cattle (0.315), sheep (0.217), wild birds (0.381) and pigs (0.086). Two sequence types (ST814 and ST257) were more common at clear and flocks were more likely to change from C. jejuni to C. coli (P=0.03).

Conclusions: Interventions did not reduce flock prevalence, carry over between flocks explains only a small amount of positivity, some farms are continually positive and a number of external source reservoirs contribute to flock prevalence.

P114

Prevalence of capsule types and hemolysin co-regulated protein (hcp) gene among *Campylobacter jejuni* isolates isolated from adult travelers and children with diarrhea in Thailand

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Background: *Campylobacter jejuni* is one of the most common bacteria responsible for human gastroenteritis worldwide. The prevalence among children, travelers and military personnel deployed to developing countries is high with limited understanding of virulence and pathogenesis. Recent findings have shown that the presence of Type 6 secretion systems (T6SS) in *C. jejuni* can result in association with virulence mechanisms.

Objectives: Our objective was to characterize and determine prevalence of capsule types and hemolysin co-regulated protein (hcp) gene marker in T6SS among *C. jejuni* isolated from travelers and pediatric cases of diarrhea in Thailand.

Methods: A total of 524 *C. jejuni* isolates from travelers and children with diarrhea were included in this study. All isolates were characterized by PCR assays to determine capsule types/Penner serotypes and hcp gene.
Results: A total of 20 capsule types were detected from 524 *C. jejuni* isolates by capsular PCR assays. The six most common capsule types among these *C. jejuni* isolates were as follows: HS4 complex (17.6%), HS2 (12.4%), HS23/36 complex (10.1%), HS53 (9.5%), HS8/17 complex (9.2%), and HS5/31complex (9.2%) which accounted for nearly 70% of all isolates. However, variations in capsule types were observed among isolates from travelers and children. Hcp was detected in 79% of all *C. jejuni* isolates that belonged to 10/20 capsule types detected.

Conclusions: High prevalence of hcp has been observed among clinical *C. jejuni* isolates from Thailand and this prevalence was concerned in this study. This finding highlights necessity for further characterization of the role of T6SS in Campylobacter pathogenesis.

P115

Key role of capsular polysaccharide in the induction of systemic infection and abortion by *Campylobacter jejuni*

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Campylobacter jejuni clone SA has emerged as the predominant cause of ovine abortion in the U.S., and is highly pathogenic in pregnant sheep. To induce abortion, orally ingested *Campylobacter* must translocate across the intestinal epithelium, spread systemically in the circulation, and reach fetoplacental tissue; however, bacterial factors involved in these steps are not well understood. *C. jejuni* possesses a capsular polysaccharide (CPS), which is presumed to be crucial in the overall pathobiology of this organism; however, the specific role CPS plays in systemic infection and in particular abortion in animals remains to be determined. Here, contribution of capsule production to hypervirulence of clone SA was evaluated using a mice model for bacteremia and pregnant guinea pig model for abortion following oral challenge. Compared with *C. jejuni* strains NCTC11168 and 81-176, a clone SA isolate (IA3902) resulted in significantly higher magnitude and duration of bacteremia in mice. Loss of capsule production via gene mutagenesis in IA3902 led to complete abolishment of bacteremia in mice and induction of abortion in pregnant guinea pigs; in-trans complementation of capsule expression almost fully restored these phenotypes to the wild-type level. The capsule was found important for resistance of IA3902 to guinea pig sera. Sequence-based analyses suggested possession of a distinct and stable CPS structure by clone SA isolates derived from various hosts and times. These findings identify a unique capsular locus of a highly pathogenic *C. jejuni* clone as a key virulence factor for induction of systemic infection and abortion in pregnant animals.

P116

Rising fluoroquinolone resistance in Campylobacter isolated from feedlot cattle in the United States

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Campylobacter is a major foodborne pathogen causing gastroenteritis in humans. Antibiotic resistance, particularly to fluoroquinolones and macrolides, in Campylobacter is considered a serious threat to public health. Despite ruminants serve as a significant reservoir for this zoonotic organism, limited information is available on antimicrobial resistance of ruminant Campylobacter. Here, we analyzed the antimicrobial susceptibilities of *C. jejuni* and *C. coli* obtained from 65 different feedlot cattle in Iowa, Texas, Colorado, Missouri, and Kansas. In total, 320 *C. jejuni* and 115 *C. coli* isolates were randomly chosen for antimicrobial susceptibility tests using the microbroth dilution method. Among the *C. jejuni* isolates, 281(88.1%) were resistant to tetracycline, 113 (35.4%) were resistant to ciprofloxacin, and 109 (34.2%) were resistant to nalidixic acid. Of the *C. coli* isolates, 86 (74.4%) were resistant to tetracycline, 89 (77.3%) were resistant to ciprofloxacin, 95 (82.6%) were resistant to nalidixic acid, and 4 (3.5%) were resistant to florfenicol. The isolates were generally susceptible to azithromycin, clindamycin, erythromycin, florfenicol, gentamicin and telithromycin. The antibiotic resistance patterns did not differ significantly among the 5 different states. The tested fluoroquinolone resistant isolates harbored the Thr-86-Ile mutation in GyrA. Interestingly, the fluoroquinolone-resistant *C. coli* isolates from different states had very similar PFGE patterns, suggesting that they are genetically related. These findings reveal the drastic increase in the prevalence of fluoroquinolone-resistant Campylobacter in feedlot cattle in the United States and highlight the need for enhanced effort to understand the ecology of antibiotic resistant Campylobacter in the ruminant reservoir.

P117

Construction of CjaA protein presenting CjaD epitopes using structure-based approach.

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Background: Two highly immunogenic *Campylobacter* antigens, CjaA and CjaD, were originally identified by immunological screening of a *Campylobacter* genomic DNA library. Selected proteins satisfy several criteria used for vaccine candidate evaluation.

Objectives: The aim of the work presented here was to generate CjaA protein presenting CjaD epitopes using structure-based approach.

Methods: CjaA and CjaD epitopes predictions and hybrid protein design were done using bioinformatics tools. DNA fragment encoding hybrid (rCjaAD) protein was synthetized by Genecust. rCjaAD was produced using *Escherichia coli* expression system and purified by affinity chromatography. Western blot analysis with specific rabbit anti-CjaA and anti-CjaD antibodies was used to confirm protein specificity. Purified rCjaAD was used for rabbit immunization.

Results: The structure-based approach combine with the identification of the CjaA and CjaD epitopes allowed us to engineer CjaA antigen and construct CjaA antigen that presents CjaD epitopes on its surface. The protein was named rCjaAD. As shown by Western blot experiments, the rCjaAD protein reacts with specific rabbit anti-CjaA, as well as with specific rabbit anti-CjaD sera. Additionally, and more importantly from a vaccine standpoint, the specific serum obtained by rabbit immunization with rCjaAD recognized both the native CjaA and the native CjaD produced by a wild type *C. jejuni* strain.

Conclusions: The presented work might facilitate construction of a recombinant immunogenic proteins presenting epitopes of several antigens for clinical applications and vaccine development. Acknowledgements: The work was supported by grant from the National Science Center, Poland (grant No 2011/03/B/NZ1/00592).

P118

Cell wall anchoring of the Campylobacter antigens to *Lactococcus lactis*

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Background: There is increasing interest in using lactic acid bacteria (LAB) as mucosal delivery vehicles. Studies investigating the use of LAB as vaccine delivery vehicles suggested that the cell-wall-anchored (CWA) protein form may possess superior ability to induce a strong immune response. The most exploited anchoring regions are those with the LPXTG box that ensure covalent protein binding to the bacterial peptidoglycan.

Objectives: The aim of the work presented here was to generate LAB surface display system for *C. jejuni* antigens.

Methods: All genetic manipulations was performed using standard molecular biology procedures. The correctness of generated constructs was verified by sequencing. Protein localization was determined by Western blot analysis with specific rabbit sera and by immunofluorescence assay.

Results: Two model *C. jejuni* antigens (strongly immunogenic CjaA protein) and hybrid protein rCjaAD (CjaA protein presenting CjaD epitopes) were fused with C-terminal domain of *Lactococcus lactis* YndF or YhgE proteins, which contain LPXTG motif. All genetic manipulation were performed in *E. coli*. Subsequently recombinant plasmids encoding hybrid proteins were introduced into *L. lactis* and protein localization was confirmed by two strategies. Recombinant plasmids encoding the same *Campylobacter* antigens in cytoplasmic location were also constructed. They will be used as a control for animal experiments in the future.

Conclusions: The study demonstrated the possibility of using LAB strains as a platform for immunogenic proteins presentation. Acknowledgements: The work was supported by grant from the National Science Center, Poland (grant No 2011/03/B/NZ1/00592).

P119

Heterogeneity in the infection biology of *Campylobacter jejuni* isolates in three infection models reveals an invasive and virulent phenotype in a ST21 isolate from poultry.

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Background: Our understanding of the infection biology of *Campylobacter jejuni* is based on relatively few isolates. Our recent work has shown that there can be considerable variation in the infection ecology between isolates in the chicken. Understanding phenotypic variation in infection is important in understanding risk and developing controls.

Objectives: To understand the degree of heterogeneity of infection biology of *C. jejuni* in relevant models.

Methods: A panel of 5 *C. jejuni* isolates (M1, 13126, 12662, DBM1 and NCTC 11168H) was tested in three infection models to determine their infection phenotype: 1. Oral infection of broiler chickens, 2. Invasion of human intestinal epithelial cells, and 3, Virulence in the *Galleria melonella* insect model.

Results: All isolates tested colonized the caeca of broiler chickens to similar levels. Extra-intestinal spread to the liver varied; ranging from 9% of birds infected with DBM1 to 60% infected with 13126. All isolates invaded CaCo2 intestinal epithelial cells at a level of <0.01% of the inoculum, except 13126 that invaded to levels around 0.2% of the inoculum. *Galleria* mortality rates at 48 h ranged from 3% for 12262, 17% M1, 33% for NCTC 11168H to 47% for 13126.47%.

Conclusions: The infection biology of *C. jejuni* varies and assumptions based on 'commonly used isolates may not accurately reflect the biology of field isolates such as 13126 that show increased invasion and pathogenicity across a range of infection models. Such isolate may pose an increased risk of spread to the edible tissues of poultry and in causing disease.

P120

Effect of oat hulls addition and whole wheat addition on cecal morphology and *Campylobacter jejuni* colonization of broilers orally infected

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An experiment was conducted within the EU-FP7 project CAMPYBRO for evaluating the effect of whole wheat (WW) and oat hulls (OH) addition on cecal morphology and *Campylobacter jejuni* colonization in orally infected broilers. There were three treatments: a mash diet (T1), T1+ WW (7.5/15% from 0-21/21-42d, respectively), and T3: T2+5%OH. A total of 108 one-day-old Ross 308 broilers were divided into floor pens (36 birds/pen) and experimental treatments (36 birds/treatment). At 14 days of age, 3 broilers per pen were orally gavaged with 100 µl of a solution containing 1 x 10^5 cfu/ml of ST-45 *C. jejuni* strain. On days 21 and 42, caeca from 12 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). There were not significant differences between treatments at 21d. However, the diet with WW at 7.5% from 0-21d and 15% from 21 to 42d, and 5% OH, showed a 1.4 $\log_{10}$ cfu/g reduction in cecal *C. jejuni* counts with respect to Control diet (9.48 *vs* 8.10 $\log_{10}$ cfu/g for Control diet *vs* Control+whole wheat+oat hulls, respectively; P=0.001). The WW alone decreased 0.48 $\log_{10}$ cfu/g with respect to Control diet, but differences were not significant. It is concluded that WW at 7.5/15% plus 5% OH showed less *C. jejuni* population than control diet at 42d.

P121

Worldwide *Helicobacter pylori CagL* diversity and its association with gastric cancer development.

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Background: Overrepresentation of particular polymorphisms within the CagL protein of *Helicobacter pylori* isolates recovered from gastric cancer patients has been reported. These differences occur within the CagL hypervariable motif (CagLHM) at amino acid residues 58 to 62. However these correlations vary geographically such that gastric cancer appears to correlate with Y58E59 in Taiwanese patients, and with D58K59 in Indian patients.

Objectives: To examine the geographical diversity of CagLHM and the association of its sequence promiscuity with *H. pylori*-mediated disease outcomes.

Methods: We compared 500 publically available CagL nucleotide and amino acid sequences of isolates with known geographical origin for CagLHM diversity. We also examined disease correlation by comparing >300 sequences for which the host health state was known.

Results: We identified 33 *H. pylori* CagLHM sequence combinations with disparate geographical distribution, suggesting substantial worldwide CagLHM diversity, particularly within Asian countries. We found that polymorphisms E59 and I60 were significantly overrepresented in isolates from *H. pylori* patients with gastric cancer compared to isolates from non-cancer patients, whereas polymorphisms D58 and E62 were underrepresented in isolates from gastric cancer patients. These associations were consistent across isolates from both Asian and non-Asian countries. Other CagLHM sequence polymorphisms showed only regional associations with disease outcome.

Conclusions: Our findings suggest that there is substantial CagLHM diversity worldwide with regional characteristics. We propose that host tolerance of strains carrying diverse CagLHM alleles may vary geographically, thus contributing to variation in the severity of *H. pylori*-related disease outcomes observed in different populations worldwide.

P122

Effect of temperature and media on *Campylobacter concisus* growth

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Campylobacter concisus is an oral bacterium which has been implicated in inflammatory bowel disease. Previous studies suggest that *C. concisus* colonising lower intestinal tract originate from oral sources. To better understand transmission and provide useful information for clinical isolation of *C. concisus*, we investigated the growth of *C. concisus* following storage in different temperature and media. A total of 17 previously isolated oral *C. concisus* strains were examined. Same amounts of bacteria of each *C. concisus* strain were suspended in two tubes of heart infusion broth (HIB) and sterile water. One tube of *C. concisus* suspended in HIB and water was stored at room temperature (RT) and the other in 4ÂºC. Each week, the *C. concisus* suspension was streaked onto horse blood agar plates and incubated anaerobically supplemented with 5% hydrogen. Of *C. concisus* strains kept at RT, one strain (6%) suspended in HIB and three strains (18%) suspended in water were able to grow after one week. After 2 weeks storage at RT, none of the strains grew. When stored in 4ºC, all 17 *C. concisus* strains suspended in water were still able to grow after 4 weeks. Of *C. concisus* strains suspended in HIB, 15 strains (88%) grew at week 3 and 13 strains (76%) grew at week 4. These data suggest that clinical samples used for *C. concisus* isolation should be kept at 4ºC prior to processing. Furthermore, cold storage of food contaminated with *C. concisus* may be a means of *C. concisus* transmission.

P123

Analysis of the potential use of *Campylobacter jejuni* outer membrane vesicles (OMVs) for the immunization of chickens.

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Background: *Campylobacter jejuni* is the most prevalent cause of food-borne gastroenteritis in the developed world. Like other Gram-negative bacteria it constitutively release outer membrane vesicles (OMVs) during cell growth. With their immunogenic properties, self-adjuvanticity, ability to be taken up by mammalian cells, and capacity for enhancement by recombinant engineering, OMVs are attractive candidates for vaccine delivery platforms.

Objectives: The aim of the presented work was analysis of the potential use of *Campylobacter jejuni* outer membrane vesicles (OMVs) for in ovo immunization.

Methods: Eighteen day-old embryos were orally immunized by injection of different types of *C. jejuni* OMVs, and isotonic saline (control) into the amniotic fluid. Both the percentage of colonized chickens and the estimated numbers of organisms shed in feces (CFU/gram of feces) in colonized birds were calculated and used to determine the degree of protection. Fourteen days post-hatch chicks were orally challenged with live *C. jejuni* strain isolated from chicken carcasses. Every two weeks (at 0, 2, 4 and 6 weeks post-hatch) birds were sacrificed and their cecal contents were aseptically removed for enumeration of *C. jejuni* colonies.

Results: Seven days after challenge all the birds' ceca became colonized with *C. jejuni*, reaching mean concentrations of about 1.9E+9 per g of cecal contents of nonimmunized birds and 4.9E+7 per g of cecal contents of immunized bird.

Conclusions: OMVs are promising candidate for anti-Campylobacter vaccine. Vaccination in ovo induce protective effect against *C. jejuni* colonization of chicken. Acknowledgements: The project was carried-out within the PARENT BRIDGE programme of the Foundation for Polish Science, co-financed from the European Union under the European Regional Development Fund, POMOST/2012-6/4

P124

Recovery of thermophilic Campylobacter by three sampling methods from classified river sites in Northeast Georgia, USA.

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It is not clear how best to sample streams for the detection of *Campylobacter* which may be introduced from agricultural or community land use. Fifteen sites in the watershed of the South Fork of the Broad River (SFBR) in Northeastern Georgia, USA, were sampled in three seasons. Seven sites were classified as mostly influenced by forest, six sites mostly pasture, and two sites were downstream from waste water pollution control plants (WPCP). Collections were made at or near base-flow rate. Sampling was repeated twice in the fall of 2012 and three times in the spring and fall of 2013 at two or more week intervals for a total of 120 samplings. Free-catch water and sediment grab samples were taken at each sampling; Moore's swabs were placed for up to three days at most sites. A total of 58 isolates of thermophilic *Campylobacter* were recovered at least once from all the sites except for one of the forest. Fourteen samplings were positive by two or three methods and 29 samplings were positive by only one method; twice by Moore's swab and 27 times by free-catch water. *Campylobacter* was detected at 58% of cattle grazed pastures sites, 30% of forested sites and 81% of WPCP sites. Water grab samples were more efficient than Moore's swabs or sediment samples for recovery of *Campylobacter*, which is more likely to be detected in streams near cattle pastures and human communities than in forested land.

P125

Campylobacter is present as a contaminant in chicken meat and products but absent in traditional chicken curries in Sri Lanka

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Campylobacter is a frequently reported causative agent of food-borne disease and often associated with poultry and related products. Though there is no published evidences on importance of *Campylobacter* as a cause of food poisoning in Sri Lanka, previous studies have shown high prevalence (60-70% of 125 flocks tested) of this pathogen in broiler flocks. Hence, the objective of this study was to identify the exposure of humans to *Campylobacter* through chicken meat and related products, and ready to eat, chicken curries prepared according to traditional recipes which are very popular among Sri Lankans. A cross sectional study was conducted from April to October 2014 analysing 127 samples of chicken meat or products (chilled meat=28, frozen meat=23, sausages=26, meat balls=25, chicken curries =25) purchased from retail outlets in and around Kandy city, the second largest city of Sri Lanka. Standardized analytical method elaborated under ISO 10272E was adopted with certain modifications to identify *Campylobacter* in all types of meat samples. As expected higher percentage of contamination was seen with fresh chilled meat (21%) followed by frozen meat (8%) compared to meat products. Out of the tested products 4% of sausage samples and 7% of meat ball samples were contaminated with *Campylobacter*. The absence of *Campylobacter* in chicken curries was an important finding. It is suggestive that despite of significant level of *Campylobacter* contamination in chicken meat and products available at retail, traditional cooking methods and spices may reduce the risk of acquiring campylobacteriosis by Sri Lankans.

P126

Genomic comparisons of multiple *Campylobacter rectus* strains

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Background: Our interest in comparative genomics led us to focus on the oral *Campylobacter* species, in particular *Campylobacter rectus*. *C. rectus* strain ATCC 33238 was the first strain sequenced and submitted to NCBI by JCVI in 2008. This strain is considered the *C. rectus* reference genome. Four other *C. rectus* strains were obtained directly from CCUG in Sweden and sequenced at North Carolina State University in 2011.

Objectives: There are three major objectives for this research endeavor: 1) to provide genome sequences that will be publishable and useful to other research endeavors; 2) to compare the five genomes to one another and identify regions of variation; and 3) to identify and compare potential pathogenesis-associated genomic regions and determine which strains retain these genes/regions.

Methods: The four genomes were sequenced using Illumina sequencing and the raw data assembled into scaffolds and contigs. The RAST server was used to initially compare the five sequenced genomes to one another with *C. rectus* 33238 used as the reference. Additional genomic analysis was done (and is continuing) to look at similarities and differences between pairs of genomes.

Results: Genomic comparisons thus far demonstrate conserved regions, but also some regions displaying potentially missing or additional genes in several strains. In particular several conserved secretion systems (including flagellar/type III) have been noted and will be further examined.

Conclusions: This research endeavor has the potential to shine new light on the *C. rectus* species at a genetic level using whole genome analysis.

P127

Eradication of *Helicobacter pylori* in Mongolian gerbils using colostral antibody obtained from dairy cows immunized with H. pylori and complement.

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The effectiveness of immune colostral antibody and its antibody with bovine complement to eradicate *Helicobacter pylori* infection was investigated in Mongolian gerbils. A pregnant dairy cow, 3 months prior to delivery, was used for preparation of immune colostral antibody against *H. pylori*. One hundred and one Mongolian gerbils aged 5-10 weeks were used. Mongolian gerbils were orally inoculated with 1 ml of BHI culture fluid suspension of 5.0 x107. Mongolian gerbils were orally administered with 0.5 ml of colostral antibody against *H. pylori*. Colostral antibody against *H. pylori* or whey was administered to Mongolian gerbils twice a day for one month or two months. Mongolian gerbils were orally administered 0.5 ml of colostral antibody and complement twice a day for 2-3 days. One month after finishing administration experiments, stomachs from euthanized gerbils were extracted and homogenized to be cultured. Eradication rates in the groups administered colostral antibody for one month and two months were 83% and 92%, respectively. On the other hand, the eradication rate was 0% in the group administered whey without colostral antibody. The eradication rates were 100% in Mongolian gerbils administered complement and colostral antibody twice a day or for three days. On the other hand, in groups administered medication twice a day for two days, the eradication rate was 83% and, the rate was 17% in the control group given inactivated complement. Bovine immune colostral antibody or complement with immune colostral antibody is useful because it can eradicate *H. pylori* in a very short period of time.

P128

No association between *Helicobacter pylori* infection and homocysteine levels in Korean healthy volunteers

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Background: Homocysteine levels may be an indicator of risk for the development of cardiovascular disease. Recent studies have suggested that *Helicobacter pylori* (*H. pylori*) infection might be a risk factor for atherosclerosis by increasing levels of plasma homocysteine. *H. pylor*i may cause malabsorption of vitamin B_{12}, leading to an increase in circulating homocysteine levels.

Objectives: We investigate whether the infection of *H. pylori* affect the level of homocysteine in healthy individuals.

Methods: Between January and December 2014, 286 healthy volunteer were enrolled. Plasma samples were tested for the presence of IgG antibody to *H. pylori* using enzyme linked immunosorbent assay method. Homocysteine levels were measured enzymatically.

Results: One hundred sixty eight were men (58.7%), and mean age was 50.1 ± 8.7 years. One hundred eleven (38.8%) were *H. pylori* positive. The proportions of Body Mass Index ≥ 25, alcohol drinking, ever smokers, and hypertension were not different significantly between *H. pylori* positive and negative groups. Although, the concentration of vitamin B12 was slightly higher in *H. pylori* negative group (735 ± 121 vs 647 ± 128 pg/mL, p = 0.37), both groups were within reference levels. There was no significant difference in homocysteine levels between *H. pylori* positive and negative groups (9.0 ± 2.9 vs 8.2 ± 3.1 mmol/L; p = 0.48).

Conclusions: Although there is homocysteine level increase in *H. pylori* positive subjects than controls, the difference was not significant. Therefore, even if *H. pylori* infection influences the risk of atherosclerosis, the influence may not be through the elevation of homocysteine.

P129

Systematic analysis of phosphotyrosine antibodies recognizing single phosphorylated EPIYA-motifs in CagA of Western and East Asian type *Helicobacter pylori* strains

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Background: Highly virulent *Helicobacter pylori* strains encode a type IV secretion system (T4SS) that delivers the effector protein CagA into gastric epithelial cells. Translocated CagA undergoes tyrosine phosphorylation by members of the oncogenic c-Src and c-Abl host kinases at EPIYA-sequence motifs A, B and C in Western-type strains or EPIYA-motifs A, B and D in East Asian-type strains. Phosphorylated EPIYA-motifs can then mediate interactions of CagA with host signaling factors – in particular various SH2-domain containing human proteins – thereby hijacking multiple downstream signaling cascades. Research question: Detection of tyrosine-phosphorylated CagA is mainly based on the use of commercial pan-phosphotyrosine antibodies, which originally were selected to detect phosphotyrosines in mammalian proteins. Specific anti-phospho-EPIYA antibodies for each of the four sites are not forthcoming. This study was therefore designed to systematically analyze the detection preferences of each phosphorylated EPIYA-motif by seven different pan-phosphotyrosine antibodies and to determine a minimal recognition sequence.

Methods: We first synthesized a series of phospho- and non-phosphopeptides derived from the EPIYA-A motif exhibiting the phosphotyrosine residue in the middle +/- five, four, three or two flanking amino acids, including the STEPIYAKVNK (11-mer), TEPIYAKVN (9-mer), EPIYAKV (7-mer) and PIYAK (5-mer) sequences, and determined the recognition patterns by pan-phosphotyrosine antibodies in Western blots. We compared these results with those from phospho- and non-phosphopeptides representing each predominant Western and East Asian CagA EPIYA-motif B, C and D and also performed infection studies with diverse representative worldwide *H. pylori* strains.

Results: Our results show that a total of 9-11 amino acids containing the phosphorylated EPIYA-motifs are necessary and sufficient for specific detection by these antibodies, but revealed great variability in sequence recognition. Three of the antibodies recognized phosphorylated EPIYA-motifs A, B, C and D similarly well; whereas preferential binding to phosphorylated motif A was found with two antibodies. In addition, phosphorylated motifs A and C or A and D was found were found to be recognized by a sixth anti-phosphotyrosine antibody, and the seventh antibody did not recognize any phosphorylated EPIYA-motif. Controls showed that none of the antibodies recognized the corresponding non-phospho CagA peptides or non-phospho CagA, and that all of them recognized phosphotyrosines in mammalian proteins.

Conclusions: We unraveled the recognition preferences by seven different anti-phosphotyrosine antibodies in all four described phospho-EPIYA-motifs of CagA. These data are valuable in judicious application of commercial anti-phosphotyrosine antibodies in general and in particular for the characterization of CagA phosphorylation events during infection and disease development. A model for successive CagA phosphorylation steps at the EPIYA-motifs is also presented.

P130

Genetic complementation of Campylobacter jejuni serine protease HtrA confirms its important role in heat tolerance, oxygen stress resistance, host cell adhesion, invasion and transmigration

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Background: *Campylobacter jejuni* is a highly important bacterial pathogen involved in foodborne illness. Transmigration across the host intestinal epithelial barrier and cellular invasion are primary reasons for tissue damage triggered by *C. jejuni*, but the involved molecular mechanisms are widely unknown. The serine protease HtrA (high temperature resistant protein A) of *C. jejuni* is important for stress tolerance and physiology, but is also secreted in the extracellular space, where it can cleave host cell proteins such as E-cadherin.

Objectives: Aim of the present study was to develop a genetic complementation system in two *C. jejuni* strains in order to introduce the wild-type *htrA* gene in trans, test all known *htrA* phenotypes and perform mutagenesis across the *htrA* gene.

Methods: We complemented the Δ*htrA* mutant with wild-type *htrA* gene by introduction in the *C. jejuni* pseudogene downstream of Cj0208. Growth of *C. jejuni* under stress conditions was tested on MH agar plates. For HtrA secretion assays *C. jejuni* was grown in BHI broth medium followed by fractionation. Casein zymography was done with bacterial lysates, culture supernatants or recombinant HtrA as separated under non-reducing conditions in gels containing casein. To study bacterial transmigration across polarised MKN-28 epithelial cells, infection experiments were done in a transwell filter system (pore size 3.0 µm) and determination of CFU. Tight polarized cell monolayers were confirmed by measuring the transepithelial resistance (TER) and by immunofluorescence against E-cadherin. Cell-attached and intracellular bacteria were determined with conventional gentamycin protection assays.

Results: We confirmed that re-expression of the *htrA* wild-type gene in Δ*htrA* mutants restored the following phenotypes: (i) *C. jejuni* growth at high temperature (44°C), (ii) growth under high oxygen stress conditions, (iii) expression of proteolytically active HtrA multimers, (iv) secretion of HtrA into the supernatant, (v) cell attachment and invasion as well as (vi) transmigration across MKN-28 cells.

Conclusions: These results establish a genetic complementation system in *C. jejuni*, exclude polar effects in the Δ*htrA* mutants, confirm important *htrA* functions and permit further dissection of HtrA functions *in vitro* and *in vivo*.

P131

Comparative proteomic analysis of two *Campylobacter jejuni* strains infers significant differences in cold-shock response pathways

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Improved understanding of the stress survival mechanisms of *Campylobacter jejuni* may help develop new mitigation strategies. Using a high-resolution, gel-free approach, we examined and compared the proteomes of two unrelated but well studied *C. jejuni* strains (NCTC 11168 and SVS 5001, both from human diarrhoeal cases) subjected to cold shock. Strains were cultured at 42°C in BHI broth under microarobic conditions overnight before subjecting aliquots to refrigeration (4°C) in microaerobic conditions. Cell viabilities were examined after 6 h, 1 day, 2 days, 6 days and 8 days and whole-cell protein extracts prepared at T0 and at these intervals. Proteins were resolved and identified using iTraq labelling coupled to UPLC-MS/MS. The viability profiles of the strains were similar, with the major decrease in cell counts seen in the first 24 h period. The protein responses however appeared to be quite different. In NCTC 11168, the number of proteins that were up-regulated, down-regulated, or associated with the cessation of protein synthesis or cold induction were 56, 24, 22 and zero. In contrast, the corresponding results for SVS 5001 were 85, 24, 21 and 10. Within these categories, there were also qualitative differences in the individual protein expressions. Our study suggests that different *C. jejuni* strains possess different systems for adaptation to cold shock, which may have implications for their control.

P132

Campys in Kiwis: a preliminary study

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The genus *Apteryx* contains five species of small flightless birds, collectively commonly referred to as "kiwi", that are related to ostriches, emus and cassowaries. Kiwi birds are unique to New Zealand and frequently used as a national iconic symbol. Their diminutive size, inability to fly and defenceless physiology render them highly susceptible to predation and thus kiwi are protected by law. Specialist reserves such as Willowbank in Christchurch protect kiwi through breeding programmes and emergency care. Willowbank routinely screen kiwi for the presence of pathogens but not for *Campylobacter* or related species. We examined fresh (~12h) faecal samples from 13 kiwi (12 captive and one recovering wild bird) using enrichment methods combined with subculture onto Exeter medium; and a filter-based approach; to determine the presence of CHRO. An established PCR method was used to identify suspect isolates. We recovered one strain of an as-yet unclassified thermophilic *Campylobacter* species using an initial enrichment step performed at 42°C; and a *C. jejuni* strain using an initial enrichment step performed at 37°C. Isolates were from different kiwi housed in distinct areas of the sanctuary and are undergoing further characterisation that will help determine their origin (i.e. native flora or zooanthroponotic). We believe this is the first investigation, and first report, of CHRO in New Zealand's native iconic bird.

P133

Protein-protein interactions among the *Campylobacter jejuni* post-transcriptional regulator CsrA and components of the flagellar system

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Background: We have shown that the CsrA protein is a central post-transcriptional regulator of *Campylobacter jejuni* metabolic and pathogenesis-related characteristics, including motility, biofilm formation, and host cell invasion, each of which require functional flagella. We have also demonstrated that *C. jejuni* CsrA regulatory activity is modulated by protein-protein interactions with the putative flagellar chaperone FliW. Objectives. In this study, we analyzed the biochemical interactions between FliW, CsrA, and the major flagellin FlaA. Methods. We assessed protein-protein interactions of CsrA-FliW and FliW-FlaA using pull-down assays and protein cross-linking. We also performed mutational analysis on the C-terminus of *C. jejuni* CsrA, and assessed the ability of the CsrA mutants to bind FliW.

Results: We demonstrated specific interactions of FlaA with FliW, and of FliW with CsrA. We hypothesized that FliW binds to the C-terminus of CsrA, as this region is significantly divergent from the C-terminus of E. coli CsrA; PHYRE analysis predicted that this region would be on the outside of CsrA homodimers and available for protein-protein interactions. Deletion analysis showed that the FliW binding site on CsrA is in the region of -15 to -28 amino acids relative to the CsrA C-terminus.

Conclusions: These results suggest a model in which FliW association destabilizes the RNA binding properties of CsrA, thereby affecting its regulatory activity. Binding of FliW to FlaA supports the proposed role of FliW as a flagellar chaperone. Together, these data provide further evidence for the coordinate regulation of flagellar synthesis and pathogenesis-related characteristics by *C. jejuni* CsrA.

P134

Investigating virulence capabilities of selected *C. concisus* oral strains

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Background: *Campylobacter concisus* is a genetically diverse species that normally colonises the human oral cavity. It was reported to be more frequently isolated from permanent than deciduous teeth in young children. Our previous studies found that children can be colonised with this bacterium in their first year of age. Yet, *C. concisus* has been considered as an opportunistic pathogen due to its isolation from periodontitis, gastroenteritis, and other infections.

Objectives: It is not known if a particular genomospecies colonises the oral cavity of healthy individuals and if strains belonging to this genomospecies possess virulence characteristics.

Methods: We investigated the motility of five *C. concisus* oral strains isolated from healthy volunteers. Bacterial motility was measured using a modified method from Differential Dynamic Microscopy. Expression of *flaC* gene was evaluated using qPCR. Adhesion and invasion assays were performed in INT-407 cell line.

Results: The tested strains were variable in their motility, *flaC* expression levels, and in adhesion/invasion characteristics. *C. concisus* RMIT-O17 was significantly more mobile than other tested strains. In addition, *flaC* expression level was significantly higher in this strain which was also significantly more invasive compared to other tested strains. Interestingly, the colony of RMIT-O17 possessed a unique golden colour on horse blood agar and was found to produce the highest amount of biofilm.

Conclusions: Although RMIT-O17 was isolated from a healthy child, it exhibited a higher level of virulence when compared with other oral and intestinal strains. Therefore, further studies are needed to characterise other virulence factors in this strain.

P135

Occurrence of *Campylobacter jejuni* in wastewater and receiving river for quantitative microbial risk assessment of drinking water in Japan

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In Japan, several waterborne outbreaks associated with *Campylobacter jejuni* have been reported. In order to maintain microbial safety of drinking water, advanced management based on quantitative microbial risk assessment is required. However, little information on the occurrence of *C. jejuni* in drinking water source is available for assessing microbial risks. The objective of this study is to investigate the occurrence of *C. jejuni* in river water as a drinking water source, treated wastewater and effluent as contaminated sources, and to estimate the $\log_{10}$-reduction requirements by water treatments. The concentrations of *C. jejuni* are measured by an MPN-PCR method with two-stage culture (Bolton and Preston). The $\log_{10}$-reduction requirements were calculated by Monte-Calro simulation using the concentration data in river water (drinking water source) and treated wastewater (the case of direct microbial contamination of water source). The concentration ranges in treated wastewater, wastewater effluent and river water were 20 to 12000, 92 to 1100, 0.011 to 1.5 (MPN/L), respectively. As a result, wastewater effluent had a great impact on the concentration of receiving river. The $\log_{10}$-reduction requirements of mean values in the case of river water and treated wastewater were estimated to be 1.4 and 4.3, respectively. These results indicated that microbiologically safe drinking water with respect to *C. jejuni* is provided by water treatment in Japan.

P136

Metagenomic analysis for gastric microbiota including helicobacters of the patients with chronic gastritis.

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The relationship of *Helicobacter pylori* infection and gastric microbiota was not fully clarified for the pathogenesis of chronic gastritis. 16S metagenomic analysis of chronic gastritis patients with *H. pylori* infection was performed. Eight patients with atrophic gastritis were recruited for this study. The patients were divided into two groups according to the result in the culture of *H. pylori* using gastric biopsy specimens. Four patients were determined as *H. pylori*-positive (current infection) and the remaining four patients were *H. pylori*-negative (past infection). Gastric biopsy specimens were collected from antrum and corpus in each patient. Total DNA was extracted from the specimens and amplified with 6 pairs of primers for super variable regions of 16SrRNA gene. The amplicons were sequenced by next generation sequencer Ion-PGM. *Proteobacteria* or *Firmicutes* was the most dominant phylum in microbiota of *H. pylori*-positive patients with atrophic gastritis. In the gastric microbiota of *H. pylori*-positive patients, *H. pylori* was detected with a higher rate (50 - 99%) in all the corpus specimens. In contrast, in the antrum specimens, *H. pylori* was detected with a higher rate (higher than 70%) in 2 patients, but with a lower rate (1-3%) in 2 patients. The lower detection rate (4.5%- 1.24%) of genus *Helicobacter* was shown in three H. pylori-negative patients. The existence of small number of H. pylori DNA in gastric mucosa has not been clarified from the point of view of ecology. It has been also demonstrated that the higher existence of *Enterobacteriaceae* and *Streptococcaceae* was detected in *H. pylori*-negative patients. It is possible that gastric mucosal environments might be influenced and changed by co-infection with *H. pylori* and gastric microbiota.

P137

Okadaella gastrococcus produce virulence factors DNase and cytotoxicity to CHO Cells

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Background: *Okadaella gastrococcus* is characterized by biological activities including alpha-hemolysis, H_2S gas production, alanine arylamidase, leucine arylamidase, glutamic acid decarboxylase, alpha and beta galactosidases, alkaline phosphatase, alanyl-phenylalanyl-proline arylamidase and arginine aminopeptidase and negativity for urease, catalase and oxidase. The presence of *O. gastrococcus* in the classic gastric carcinogenic cascades suggests their carcinogenic roles. Bacterial cytolethal distending toxins (CDTs) have gained much attention because of their carcinogenic potential.

Objectives: To examine whether *O. gastrococcus* produces DNase and CDT using various cell lines.

Methods: *Okadaella gastrococcus* (ATCC BAA-2258) was used in this investigation. *O. gastrococcus* was cultured at 37°C under anaerobic conditions for 24 h on BHI agar plate containing 5 % sheep blood. Colonies were suspended in 1 ml of PBS and centrifuged at 7000 g at 4°C for 2 min. The OD_{600} adjusted suspension was sonicated and centrifuged at 20,000 g at 4°C for 10 min. The supernatant was filtered (0.22 µm) and 10 µl of serially diluted sonic lysate was added in the well containing Caco-2, CHO, HeLa, Hep-2, Vero and Y-1 cells. Morphological changes of the cells were observed after 48 and 120 h.

Results: The sonic lysate prepared from DNase-positive bacteria induced elongation of CHO cells at 48 and 120 h after exposure, but the other cell lines did not show significant cellular morphological changes.

Conclusions: DNase positive *O. gastrococcus* produced cell elongation activity, but not CDT-like activity, on only CHO cells. It is warranted to investigate the molecular nature and genetic code of the cytotoxin.

P138

Viability of *Okadaella gastrococcus* (ATCC BAA-2258) in acidic conditions

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Background: *Okadaella gastrococcus* is the first Gram-negative coccoid *Streptococcaceae*. *O. gastrococcus* is associated with not only various *Helicobacter pylori* related gastric mucosal pathologies but also the intracellular and intralesional presence of *O. gastrococcus* in the classic carcinogenic cascade has been shown. Objective: To examine the viability of *O. gastrococcus* in acidic conditions and to compare with that of *H. pylori*.

Methods: *O. gastrococcus* and *H. pylori* were used in this experiment. *H. pylori* and *O. gastrococcus* were cultured at 37°C under microaerophilic and anaerobic conditions, respectively, for 24 h on BHI agar plate containing 5 % sheep blood. Bacterial suspensions (500 µl) were incubated with 4.5 ml of PBS adjusted to pH 2.0, 3.0, 4.0 with 1 N HCl and pH 7.4. Samples (10 µl) collected at scheduled times (5, 10, 20, 40, 60, 80, 100 and 120 min) were diluted with 9.99 ml PBS (pH 7.4), subcultured and colony forming unit (CFU) /ml was determined. **Results:** Neither *O. gastrococcus* nor *H. pylori* could tolerate pH 2.0. At pH 3.0, *H. pylori* was viable for 120 min but *O. gastrococcus* was viable for 100 min. At pH 4.0, both of them were viable at least for 120 min.

Conclusions: Although *H. pylori* showed stronger viability than *O. gastrococcus* under the acidic conditions, acid tolerance of *O. gastrococcus* would be sufficient to establish gastric colonization because of the closer pH susceptibility of *O. gastrococcus* to acid tolerant *E. coli* 690. It is warranted to investigate the molecular mechanism by which *O. gastrococcus* adapts to acidic conditions.

P139

The Cj0610c is necessary for peptidoglycan O-acetylation and chicken colonization in *Campylobacter jejuni*

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Background: Through the screening of the chicken colonization factors of *Campylobacter jejuni*, we found that the *cj0610c* mutant of *C. jejuni* exhibited a decreased level of colonization compared to that of wildtype. Whereas *Helicobacter pylori* HP0855 gene which is a homolog of *cj0610c*, has been identified to code for an O-acetyltransferase of peptidoglycan (PG) in recent years, the Cj0610c function is still unknown in *C. jejuni*.

Objectives: The objective of this study is to demonstrate the role of Cj0610c in chicken colonization of *C. jejuni*.

Methods: Isogenic mutants of the *cj0610c* were constructed in *C. jejuni* strains 81-176 and 11-164. Phenotypes of the mutants were compared with those of their wild-type strains.

Results: The levels of motility, biofilm formation, adhesion and invasion of Caco-2 cells of *cj0610c* mutants were significantly reduced. In the chick colonization experiment, *cj0610c* mutant of 11-164 strain was not detected in the cecal content 12 days after inoculation, whereas 11-164 wild-type strain were detected in all samples recovered during the experiment. The levels of PG O-acetylation of *cj0610c* mutants were lower than those of wild-type strains. On the other hand, the mutation of *cj0610c* had no effect on the morphology in TEM image and the Gel electrophoresis profiles of lipooligosaccharide and capsular polysaccharide.

Conclusions: This study suggested that Cj0610c is involved in PG O-acetylation of *C. jejuni*, and the PG O-acetylation affects motility, biofilm formation, cell adhesion and invasion of *C. jejuni* as well as its ability to colonize chicken colonization.

P140

Longitudinal study of Campylobacter in commercial broiler farms

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Campylobacter infection in human is burden disease. Infected broiler plays an important role of the human infection. To our better understanding, Campylobacter in broiler farms was studied. Firstly, the prevalence and the influencing factors of Campylobacter from broiler farms during March 2012 to May 2014 were explored. 46 flocks from 4 commercial farms were isolate for Campylobacter. Influencing factors such as age of chicken, flock size were questionnaire and analyzing by using SPSS package program. The prevalence of Campylobacter of chickens was found 60.87% (28/46) and the percentage of positive Campylobacter of environments was 28.26% (13/46). Binary multivariate logistic regression demonstrated that season (summer & rainy), flock size, chicken age and pool were statistically found as OR 3.24, 1.07, 1.51 and 1.94, consecutively. Secondary, the SIR modeling was performed to determine the reproductive ratio of Campylobacter spreading in 4 broiler farms. 30 cloacal swabs of 4 broiler farms was collected weekly. Positive and negative results as the proportion of infection were record and analyzed using modeling program package (scilab-5.5.1). The result was found that the infection rate (r) was 6.8 and the recovery rate (α) was 2.5. So, the reproductive ratio was the 2.75 when compared to the results of the isolation Campylobacter from the farms. The SIR model indicated that the spreading of the Campylobacter could be found throughout in the broiler farm in this condition.

P141

Risk factors associated with Campylobacter colonization in Thai commercial broiler flocks

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Poultry were considered as the important source of *Campylobacter*, which were frequently involved with human diarrheal disease. In Thailand, the prevalence of *Campylobacter* in broiler flocks was reported ranging from 15 to 90 percent. To reduce the number of *Campylobacter* infection, determination of risk factors associated with *Campylobacter* colonization in broiler flocks is necessary. Therefore, the objective of this study was to investigate risk factors associated with *Campylobacter* colonization in commercial broiler flocks in Thailand. Cecal samples of 432 broiler flocks raised in the central and north eastern regions of Thailand were collected from January 2012 to April 2014. Farm management and flock data of examined broiler flocks were obtained by using the structured questionnaires. Generalized estimated equation (GEE) was used for determination of risk factors associated with *Campylobacter* colonization in broiler flocks. Of 432 broiler flocks, 242 flocks were *Campylobacter* positive (56.02%; 95% CI 51.34, 60.70%). Several variables were significantly related to *Campylobacter* colonization in Thai broiler flocks including farm size (P=0.0123), production capacity (P=0.0004), use of non-treated water (P=0.0028), presence of surface water near/in farm area (P=0.0003), presence of other animals in farm area (P=0.0025), presence of other animals near farm border (P=0.0396), rainfall (P<0.0001) and presence of *Campylobacter* in previous flock (P=0.0026).These findings indicate that intensive biosecurity should be primarily emphasized in order to prevent or reduce *Campylobacter* colonization in Thai broiler flocks.

P142

Evaluation of risk based microbiological criteria for Campylobacter in broiler carcasses in Belgium using TRiMiCri

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Background: Campylobacteriosis is the most frequently reported foodborne zoonosis in EU. A potential solution for the reduction of consumer exposure to *Campylobacter* is establishing a microbiological criterion (MC) for *Campylobacter* on broiler meat.

Objectives: The aim of the present study was to evaluate risk-based microbiological criteria based on the collected *Campylobacter* data in Belgium.

Methods: The freely available (http://tools.food.dtu.dk/) software TRiMiCri was applied to evaluate risk-based microbiological criteria by two approaches: the traditional one that implies microbiological limit (ML-MC) and the second one which is based on the relative risk estimate (RRL-MC). The baseline risk was estimated based on the Belgian baseline data. The input data were *Campylobacter* counts from 28 *Campylobacter* positive batches sampled in 6 slaughterhouses.

Results: Approximately 30% of produced batches in Belgium was not complying with ML-MC (n=5, m=1000, c=0) or with MC-RRL (n=5, RRL=1). Less stringent MC decreased the percentage of non-compliance (NC) but increased the minimum relative residual risks (MRRR – the quotient of the mean risk of all batches complying with the MC and the mean risk of the whole set of batches). An optimum criterion would combine a low MRRR with a low percentage of NC.

Conclusions: TRiMiCri provides user friendly software to evaluate risk based microbiological criteria. The analyses performed offer a tool to make a risk based decision on the choice of the MC. In both approaches, ML-MC and RRL-MC, proportion between MRRR and percentage of NC was rather similar.

P143

The effects of N-linked protein glycosylation on *Campylobacter jejuni* biofilm formation

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Background: *Campylobacter jejuni* proteins are frequently glycosylated with a conserved heptasaccharide which serves a number of functions, including protection of cellular proteins. Currently, the impacts of N-linked protein glycosylation on the environmental survival of *C. jejuni* and on biofilm formation are unknown.

Objectives: This study investigated the effects of N-linked glycosylation of *C. jejuni* proteins on biofilm formation in terms of glycan translocation and the presence heptasaccharide itself, which may highlight the cellular functions important to biofilm formation in *C. jejuni*.

Methods: *C. jejuni* strain 11168-O and isogenic Δ*pglB* and Δ*pglF* mutant strains were utilized in microtitre plate assays to assess gross biofilm formation. SEM and time-lapse microscopy were conducted to determine morphological changes and to investigate the process of biofilm formation. Autoagglutination assays were also conducted to determine changes to intercellular adherence.

Results: Both Δ*pglB* and Δ*pglF* mutants showed a marked increase in biofilm formation in addition to rapid establishment of mature biofilms when compared to wild-type strain. The morphology of biofilm formed by mutants also differed from wild-type strains with mutant strains showing a filamentous architecture. Time lapse analysis suggests these changes are a response to increased propensity for microcolony formation associated with increased autoagglutination.

Conclusions: This work indicates that protein glycosylation may play a role in cell to cell adherence in *C. jejuni* biofilms. In addition, it suggests that the Pgl protein glycosylation system may be involved in mediation of this cellular adherence and thereby, biofilm formation.

P144

Evaluation of average nucleotide identity using MUMmer (ANI-m), ribosomal multi-locus sequence typing (rMLST), and rpoB and atpA gene phylogeny for identification of enteric bacteria, including Campylobacter, by whole genome sequence analysis

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Enteric Diseases Laboratory Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA[1]Agricultural Research Service, United States Department of Agriculture, Albany, CA, USA[2]Applied Maths, Inc., Belgium[3]

Background: An identification database for analysis of whole genome sequence (WGS) data for identification of enteric bacteria including *Campylobacter* is being developed at CDC using BioNumerics v. 7.5 as a software platform. The universally applicable methods Average Nucleotide Identity using MUMmer (ANI-m), Ribosomal Multi-Locus Sequence Typing (rMLST) and *rpoB* gene phylogeny and *Campylobacter*-specific *atpA* gene phylogeny were evaluated.

Methods: These methods were evaluated using a set of genome assemblies: 157 *Campylobacter* genomes, including 125 high quality draft genomes and 32 closed genomes, representing 23 *Campylobacter* species.

Results: Alleles were assigned for all 52 rMLST loci for 125/157 genomes in the validation set, with seven loci inconsistently detected in *C. curvus*, *C. gracilis*, *C. hominis*, *C. mucosalis*, *C. rectus*, *C. showae* and one locus inconsistently found in *C. fetus* and *C. lari*. rMLST appropriately clustered the 157 genomes in the evaluation to the species level. *rpoB* and *atpA* alleles were assigned in most genomes except for *rpoB* in *C. canadensis*, *C. hominis*, *C. gracilis* and *atpA* in *C. canadensis*. The *rpoB* and *atpA* gene phylogenies appropriately clustered the genomes, with a similarity cut-off of at least 91% between species and 95% between subspecies. ANI-m showed identities of ¡Ý95% for members within a species for the six most common clinically relevant *Campylobacter* species and ¡Ü92% for members outside these species.

Conclusions: All approaches tested here show promise for identification of *Campylobacter* but they will need to be evaluated and validated on a larger set of genomes.

P145

Prevalence and characteristics of Campylobacter at poultry farms and their direct environment

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National Institute for Public Health and the Environment (RIVM), the Netherlands[1]

Background: Campylobacter infections are largely considered foodborne, however, exposure through environmental routes is an additional risk factor.

Objectives: Study whether broiler and laying hen farms contribute to the environmental load of Campylobacter.

Methods: Various environmental compartments at or close to laying hen farms (n=5) and broiler (n=3) farms, and caecal material, were examined by applying standard culture

Methods: for Campylobacter. Similarity between Campylobacter from poultry and environment was examined using Multilocus Sequence Typing. Results Campylobacter was prevalent in caecal samples from laying hens (97%) and broilers (93%), and in environmental samples (soil and surface water, but not in dust and flies) taken at or close to laying hen farms (21%) and broiler farms with Campylobacter-positive flocks (26%). In soil, 40% - 100% of isolates had sequence types (STs) identical to those from caecal material. The diversity of STs in surface water adjacent to farms was high, suggesting environmental background load; occasionally, identical STs in broilers and surface water adjacent to broiler farms (11%) were detected.

Conclusions: Poultry farms contribute to the Campylobacter load in soil at the farms, and surface water in the farm environment. Detected concentrations and STs in surface water suggest that broiler farms contribute more to the Campylobacter load in surface water than laying hen farms. The contribution of individual poultry farms to the Campylobacter load in surface water seems limited and not exceeding the environmental background load, but the combined contribution of multiple poultry farms in a watershed may be significant.

P146

Preston Broth and Bolton Broth plus clavulanic acid suppress ESBLs sufficiently in Campylobacter enrichment procedures

Hazeleger, W.C.[1]; Zhang, J.[1]; Jacobs-Reitsma, W.F.[2]; den Besten, H.M.W.[1];
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The presence and increase of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* in food have become important factors that interfere with the isolation of *Campylobacter*, resulting in false-negative detection results. The ISO-protocol for detection of thermotolerant *Campylobacter* spp. in food and animal feeding stuffs (ISO 10272-1, 2006) describes the use of Bolton broth (BB) which is mixed 10:1 with the food sample including a pre-enrichment step at 37°C to resuscitate sublethally damaged cells. Currently, the ISO-protocol is revised and a distinction is made between different food samples, where the more selective Preston Broth (PB) is advised for samples in which high background flora such as ESBLs is suspected. However, detailed growth dynamics of *Campylobacter* and its competitors during enrichment remain unclear, while these would provide a solid base for further improvement of the enrichment procedure of *Campylobacter*. Therefore, growth kinetics were studied in detail using several strains of *C. jejuni* and ESBLs combined and separately in BB, PB and BB supplemented with clavulanic acid (BBc). Also, growth dynamics of *Campylobacter* and ESBLs in naturally contaminated chicken samples were evaluated. No significant differences in growth kinetics were found using a pre-enrichment step of 4 h at 37°C compared to immediate enrichment at 41.5°C. Furthermore, the yields and often the growth rates of *Campylobacter* in co-culture with ESBLs were lower than in pure cultures, indicating severe suppression of *Campylobacter* by ESBLs. PB and BBc, however, successfully inhibited growth of ESBLs and are therefore a better choice as enrichment media for potentially ESBL-contaminated samples.

P147

Everything you always wanted to know about Campylobacter

Jacobs, WF[1]; Hazeleger, WC[2];
RIVM National Institute for Public Health and the Environment, Bilthoven, The Netherlands[1]Laboratory of Food Microbiology, Wageningen University, Wageningen, The Netherlands[2]

Did you know that...
... growth of campylobacters in liquid medium like Heart Infusion Broth is hardly visible, and if nice and thick growth does show this most likely means a contamination in your broth.
... *vented* Petri-dishes should be used to facilitate the growth in micro-aerobic atmosphere, but also help to be able to re-open the dishes after incubation.
... a *Campylobacter*-selective agar plate like CCDA may look like showing no growth, but in practice may contain a thin layer of growth all over the plate and only just giving the impression to be negative for growth. Check with loop on presence of colony mass!
We would like to share our over 25 years of working-with-*Campylobacter*-experience with the audience. Preferably in an interactive way by asking the audience for their own experience and starting-up questions, but at least by presenting a practical "picture" of this ever surprising micro-organism.

P148

Impact of sample size on the Limit of Detection LOD_{50} to detect Campylobacter in foods.

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National Institute for Public Health and the Environment RIVM, Bilthoven, NL[1]Wageningen University, Laboratory of Food Microbiology, Wageningen, NL[2]

The European Commission Mandate M/381 asked the European Committee for Standardization (CEN) to provide full validation of the revised versions of ISO 10272-1/-2 by inter-laboratory studies and to establish the performance characteristics of these ISO methods for detection and enumeration of *Campylobacter* in foods. After finalising the study on *Campylobacter* detection, the elaborated data were inserted in ISO 10272-1. Results are expressed as the limit of detection at 50% (LOD_{50}): the concentration in CFU/sample for which the probability of detection is 50%. No legislative requirements within the EU exist for testing on presence of *Campylobacter* in a specific portion size, in contrast to e.g. the requirement for absence of *Salmonella* in 25 gram (EC 2073/2005). Therefore, for practical and economic reasons, the size of the test portions in the validation study on detection of *Campylobacter* was decided to be 10 gram. As test portions of 25 gram are also commonly used in detection methods, our laboratory compared the LOD_{50} for *Campylobacter* detection in 10 gram and 25 gram test portions of foods. Matrices as used in the inter-laboratory study were tested, using both samples sizes with identical inoculum at 2 different levels (and a blank as control) and the LOD_{50} was calculated for each sample size. Preliminary results indicate better *Campylobacter* detection in 10 gram portions of minced meat and frozen spinach, in contrast to better detection in 25 gram portions of raw milk. Experiments are ongoing to elucidate the possible effect of sample size on the LOD_{50}.

P149

Isolation and distribution of Campylobacter bacteriophages in chickens and the farming environment across varying litter management practices in Australia

Estella, W. [1]; Rodrigues, H.[1]; Weyand , C. [1]; Diallo , I. [1]; Chinivasagam, H. N [1];
Department of Agriculture and Fisheries, Queensland, Australia[1]

Bacteriophage levels (pfu/g) were enumerated across 24 poultry sheds (from 17 farms) adopting four different litter practices. The practices were (a) conventional practice of cleaning out litter after each cycle, (b) the Australian practice of re-using litter and (c) free-range with the adoption of both practices (a) and (b). Bacteriophages were isolated directly using *C. jejuni* culture (PT14), (Atterbury *et al.* 2003) and two different enrichment techniques with *C. jejuni* (PT14) and a *C. jejuni* and *C. coli* farm isolate. The levels of bacteriophages in caeca from direct isolation ranged from a minimum of log 2.3 to a maximum of log 6.6 pfu/g. Enrichment contributed more towards isolation from negative caeca, environmental samples (litter, soil) and carcasses and rather than an increase in bacteriophage levels. Interestingly bacteriophages were not isolated from litter, soil and carcasses when caeca from the same farm were bacteriophage negative. Pulse Field Gel Electrophoresis and Transmission Electron Microscopy enabled the characterisation of a representative number of bacteriophage as belonging to group 3 of the Myoviridae family, which is commonly isolated from poultry. The bacteriophages were distributed across all litter practices studied. Reference: Atterbury, *et al*. (2003) Appl Environ Microbiol 69, 4511-4518

P150

Development of an insect model to assess phage/Campylobacter interactions

Mcglashan, K. [1]; Estella, W. [1]; James, P. [2]; Connerton, I. [3]; Chinivasagam, H.N. [1];
Department of Agriculture and Fisheries, Queensland, Austrialia[1]Queensland Alliance for Agriculture and Food Innovation, University of Queensland, St Lucia Queensland, Australia[2]Division of Food Sciences, Sutton Bonington Campus, University of Nottingham, Leicestershire LE12 5RD, UK[3]

Bio-control of *Campylobacter* in poultry using bacteriophages could be a viable option to manage the levels of the organism in the chicken gut. This requires an understanding of the bacteriophage - *Campylobacter* interaction in relation to a diversity of both *Campylobacter* isolates and bacteriophages. An insect model using the wax moth, *Galleria mellonella* was developed to assess *Campylobacter* - bacteriophage combinations and evaluate suitable doses. *Galleria mellonella* was used because of the sterile environment provided by the insect haemocoel between the gut and the external body wall of the insect which provides an aseptic platform for assessing bacteria phage interactions. *G. mellonella* can be readily maintained as a laboratory colony at 37°C, the optimum temperature for *Campylobacter*, to provide a constant supply and necessary numbers of larvae and offers a more cost effective method than chicken models. The poster describes the steps taken towards validation of this model.

P151

Simultaneous detection and classification of *Campylobacter jejuni* lipooligosaccharide biosynthetic loci from drinking water source in Japan

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Kyoto University[1]Tohoku University[2]

In Japan, *Campylobacter jejuni* is one of the most important pathogens to precede Guillain-Barre syndrome (GBS). Although waterborne disease outbreaks associated with *Campylobacter* infections have been reported, little information is available on health effects related to them. The objectives of this study are to isolate *C. jejuni* strains from the Katsura River, a drinking water source receiving wastewater effluent in Japan, and to identify lipooligosaccharide (LOS) biosynthetic loci of the isolates based on gene analysis. *C. jejuni* strains were isolated from the Katsura River water samples using membrane filtration method with two stage culture (Bolton and Preston). Serotyping of *C. jejuni* isolates was performed using heat-stable Penner antigens by passive hemagglutination. The *C. jejuni* LOS loci were grouped into six classes based on simultaneous detections of the 42 specific genes within the six classes using BioMark HD (Fluidigm). Seventy-five strains of *C. jejuni* were isolated from the water samples. Thirty-two percent of these strains belonged to class E and 4% to class D. From 4 strains belonging to unknown LOS class, cst-II gene was detected. These results indicated that the *C. jejuni* isolates had little risk of developing GBS. Particular LOS classes showed little relationship to Penner serotypes. In conclusion, simultaneous detections of the 42 specific genes by Biomark can be used for rapid determination of major LOS classes.

P152

Classification of three newly identified Campylobacter species isolated from New Zealand birds and water sources

Grange, Z L[1]; Midwinter, A C[1]; French, N P [1]; Balm, M[1]; Bloomfield, S J[1]; Biggs, P J[1];
Massey University[1]

Increasing accessibility of whole genome analyses has expanded our ability to characterise previously unidentified microbial species. Research has traditionally focused on *Campylobacter* species of concern to humans, with many cultured isolates classified as unknown. As part of source attribution and pathogen dynamic studies, cryptic *Campylobacter* isolates were cultured using conventional methods from native birds, introduced birds and waterways in New Zealand. Exploratory phenotypic and genomic typing was conducted to describe and characterise unidentified isolates. Core genome and ribosomal multi-locus sequence typing of the isolates and comparison to published *Campylobacter* species, identified the isolates as belonging to three clusters which were related to *Campylobacter jejuni* and *Campylobacter coli* but genomically distinct. These putative new species have been provisionally named as *Campylobacter* species *nova 1* (*C. aotearoa*), *C.* sp. *nova 2* (*C. gallirallus*), and *C.* sp. *nova 3* (*C. zealandensis*). *C. gallirallus* and *C. aotearoa* are morphologically indistinguishable from *C. jejuni* and grow optimally under standard culture conditions. *C. gallirallus* can be differentiated by the production of H2S and an inability to reduce nitrate. *C. aotearoa* can be identified by PCR targeting a gene thought to only be present in this species. *C. zealandensis* is morphologically distinct under standard conditions, with preferential growth conditions of 37°C and a hydrogen-enriched atmosphere. Our research supports the identification of the cryptic isolates as three novel *Campylobacter* species. At present, none of the three species have been isolated from human campylobacteriosis cases, livestock, poultry or companion animals in New Zealand.

WHILE AT THE CONFERENCE…

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The registration desk will be attended each day from 7.30am for registration and assistance. Registration desk contact phone number: 021 217 8298

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CHRO 2015 – PROGRAMME OUTLINE

MONDAY 2ND NOVEMBER

0900-0920	WELCOME TO NEW ZEALAND, ROTORUA AND CHRO 2015 – Mayor of Rotorua, CEO of ESR, CHRO 2015 Convenor		
0920-1000	OPENING LECTURE – Professor Brendan Wren, UK: The compare and contrast of Helicobacter pylori/Campylobacter jejuni pathogenesis		
1000-1040	PLENARY LECTURE – Professor Rick Peek, USA: Gastric cancer, stem cells, microbial oncoproteins		
1110-1230	Descriptive and Analytical Epidemiology	Taxonomy and emerging species	Helicobacter pathogenesis and evolution
1330-1410	PLENARY LECTURE – Professor Mike Konkel, USA: Modulation of Campylobacter gene expression during human infection		
1415-1530	Molecular Epidemiology - 1	Host pathogen interactions	Guillain Barré syndrome
1600-1740	Genomic and Analytical Epidemiology	Campylobacter pathogenesis	Genetic and proteomic responses for adaptation - Campylobacter
1800	Evening social function: Traditional Campylobacter vs Helicobacter Football match – SPORTSDROME, EEC		

TUESDAY 3RD NOVEMBER

0815-	PLENARY LECTURE – Dr Bill Hanage, USA: New developments in genomic epidemiology		
	Comparative genomics and genomic epidemiology (sponsored by Applied Maths)	Pathogenomics and sequelae	Government and regulatory aspects
1110-1230	Comparative 'omics: Campy	Emerging species - focus on Arcobacter	Helicobacter - pathogenesis and sequelae
1330-1410	PLENARY LECTURE – Professor Ichizo Kobayashi, Japan: Genome/epigenome dynamics in the short- and long-term evolution of H. pylori		
1415-1530	Attribution workshop	Advances in identification and subtyping	Helicobacter - genomics and models
1600-	Attribution workshop (continued)	Advances in detection methods	Environmental survival
1815-	End of day / poster session / joint social function with NZ Microbiological Society		

WEDNESDAY 4TH NOVEMBER

JOINT PROGRAMME WITH THE NEW ZEALAND MICROBIOLOGICAL SOCIETY

0815-0900	PLENARY LECTURE – NZMS – Professor E. Peter Greenberg, USA – Sociomicrobiology	
0900-0945	PLENARY LECTURE – CHRO - Professor Sarah O'Brien, UK - Campylobacter: epidemiology of an enigmatic organism	
1000-1040	Control strategies for Campylobacter (sponsored by the UK Society for Applied Microbiology)	Non-poultry sources of Campylobacter spp.
1330-1410	Plenary LECTURE - CHRO – Dr Hanne Rosenquist, Denmark - Government and regulatory efforts to control Campylobacter	
1415-1530	Roundtable discussion on poultry control - international perspective	Molecular Epidemiology -2
1600-1645	PLENARY LECTURE – NZMS – Professor GwangPyo Kyo, Korea - Comparative phylogenetic and functional analyses of human microbiota in Korean twins	
1650-1815	Roundtable discussion on control - international perspective part 2: Non-poultry sources.	Gene regulation and metabolism
1930-2330	CHRO 2015 Gala Dinner / announcement of CHRO 2017 host	

THURSDAY 5TH NOVEMBER

0815-0900	PLENARY LECTURE – Professor Ernst Kuipers, The Netherlands – Control and management of H. pylori - the state of the art	
0910-1035	Control strategies for Campylobacter - 2	H pylori diagnosis and control
1110-1230	Antimicrobial Resistance (AMR)	Vaccines
1315-1405	Control strategies for Campylobacter - 3	Late breakers session
1410-1500	PLENARY LECTURE – Professor Christine Szymanski, Canada - The Campylobacter jejuni N-linked protein glycosylation pathway and its engineering to create an effective chicken vaccine	
1500	CONFERENCE CLOSING AND PASSING OF THE CHRO TORCH	