

FSRRN ANNUAL MEETING ABSTRACTS

Vaccination against <i>Salmonella</i> and the Association with Measures of <i>Salmonella</i> Prevalence in Live and Slaughtered Swine—A Systematic Review	2
Systematic Review of <i>Salmonella</i> Intervention Strategies in Broilers	3
Interventions to Control <i>Campylobacter</i> in Poultry: A Systematic Review	4
Colonizing Potential of Select <i>Campylobacter jejuni</i> Mutants in the Avian Gastrointestinal Tract	5
Genotypic Characterization of <i>Campylobacter jejuni</i> Poultry Isolates	6
Identification of <i>Campylobacter</i> Genes Differentially Expressed in Chickens by DNA Microarray	7
Comparison of <i>Campylobacter jejuni</i> Genes Differentially Expressed in Animal Hosts by Microarray Analysis	8
Association of Pathogen Load in Cattle with Retail Beef Contamination	9
Application of DNA Aptamers As Capture Molecules to Detect <i>Salmonella Enterica</i> serovar Typhimurium from Complex Matrices	10
Evaluating Cost-Effective Food Safety Risk Reduction Strategy at Retail	11
A Mathematical Model of the Dynamics of <i>Salmonella</i> Cerro Infection in a U.S. Dairy Herd	13
Synthesizing Data from Multiple Studies Designs—An Example Using Interventions Associated with <i>Salmonella</i> In Swine	15
Differential Response of <i>Campylobacter jejuni</i> Strains to Deoxycholate	16
Repetitive Sequence Polymerase Chain Reaction (Rep-PCR) Analysis of <i>Campylobacter</i> Isolates from Poultry Reared in Different Production Environments	17
Addition of Fumaric Acid to Cattle Water Troughs to Inactivate <i>Escherichia coli</i> O157:H7	18
Bovine Gene Expression in Acute <i>Salmonella enterica</i> Serovar Typhimurium Infection Analyzed by MPSS and Microarray	19
Human and Animal Enteric Caliciviruses in Oysters from Different U.S. Coastal Regions	21
CONTACT INFORMATION.....	22

Vaccination against *Salmonella* and the Association with Measures of *Salmonella* Prevalence in Live and Slaughtered Swine—A Systematic Review

Denagamage, T.¹, A.M. O'Connor¹, J. Sargeant², A. Rajic³, J. McKean¹.

¹*Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011, USA.*

²*Department of Clinical Epidemiology and Biostatistics, Health Sciences Centre, McMaster University, Hamilton, Ont., L8S 4K1, Canada.*

³*Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ont., N1G 3W4, Canada.*

Abstract

A systematic review was carried out to critically appraise and synthesize the existing literature on vaccination and its effectiveness to control *Salmonella* in swine. A four step procedure was followed to conduct the review. (1) The review topic was the association between vaccination against *Salmonella* and *Salmonella* prevalence in swine at slaughter. The population was defined as market weight finisher swine. The outcome of interest was indicators of *Salmonella* exposure as detected by culture, from ante-mortem or post-mortem animals. Any intervention designed to stimulate a systematic immune response to protect against *Salmonella* was considered relevant. (2) A search of 13 electronic online databases was conducted. Hand searching was conducted on three conference proceedings. Reference lists of included studies were checked for additional references. (3) Methodological quality of all relevant studies was assessed by two reviewers independently. Components of the quality assessment included objectives and study population, intervention, withdrawals and loss to follow-up, outcome assessment, and data analysis. (4) Data extraction included the characteristics of population, intervention, outcome, statistical analysis, and results. The search identified over 7000 abstracts. 276 manuscripts described an association between management practices and *Salmonella* in swine. 23 studies discussed the use of vaccination to reduce *Salmonella* in swine, only 4 were randomized clinical trials that evaluated *Salmonella* in market weight swine. One study provided sufficient detail to evaluate the study. This study reported that vaccination was effective at reducing *Salmonella* in market age pigs. None of the 4 randomized clinical trials used blinding in the assessment of the outcome or randomized at the individual pig level.

Systematic Review of *Salmonella* Intervention Strategies in Broilers

Wills, R.W.¹, R.H. Bailey¹, K.M. Clements¹, J. Sargeant².

¹*Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762, USA.*

²*McMaster University, Hamilton, Ont., L8S 4K1, Canada, and Public Health Agency of Canada, Guelph, Ont., N1G 3W4, Canada.*

Abstract

Systematic reviews are a method of identifying effective treatments or processes based on the available evidence from a variety of sources. They differ from traditional narrative or critical reviews of literature by using a replicable, scientific methodology to collect all available information on a subject. We are conducting a formal systematic review to identify evidence for effectiveness of interventions for *Salmonella* in the production and processing of broilers. In order to accommodate multiple study questions, the initial literature search was purposely broad. An initial search using keywords of salmonella and broiler or layer or laying or poultry or chicken resulted in 13,099 citations. A total of 2781 unique citations addressing *Salmonella* and broiler or laying chickens was identified. In the first level of relevance screening of abstracts from the scientific literature, the abstracts were categorized by type of poultry, segment of production, type of intervention strategy, and whether or not it was primary research. After this initial screening, a focused study question on vaccination as an intervention strategy in broilers was developed. It utilized 54 papers screened from 811 citations that addressed broilers. Quality assessment, data extraction, and data synthesis were then conducted.

Interventions to Control *Campylobacter* in Poultry: A Systematic Review

Sargeant, J.M.^{1,2}, L. Waddell², A.M. O'Connor³, R.W. Wills⁴ R.H. Bailey⁴.

¹*Department of Clinical Epidemiology and Biostatistics, Health Sciences Centre, McMaster University, Hamilton, Ont., L8S 4K1, Canada.*

²*Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ont., N1G 3W4, Canada.*

³*Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA.*

⁴*Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762, USA.*

Abstract

In September 2006, we initiated a systematic review to investigate the evidence for the efficacy of interventions to control *Campylobacter* in poultry. The initial tasks consisted of forming an advisory team to ensure expertise in *Campylobacter*, the poultry industry, and systematic review methodology. The specific questions to be addressed by the review were developed in consultation with this group. Progress on this ongoing systematic review, including challenges and solutions, will be presented.

Colonizing Potential of Select *Campylobacter jejuni* Mutants in the Avian Gastrointestinal Tract

Theoret, J.R.¹, B.F. Law¹, Q. Zhang², M.E. Konkel³, B.A. White⁴, and L.A. Joens¹.

¹Department of Veterinary Science and Microbiology, University of Arizona, Tucson, AZ 85721, USA.

²Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA.

³School of Molecular Biosciences, Washington State University, Pullman, WA 99164, USA.

⁴Department of Animal Sciences, University of Illinois, Urbana, IL 61801, USA.

Abstract

Depending on the host species, *Campylobacter jejuni* can manifest as a pathogenic infection or a commensal colonization. In commercial broiler chickens, *Campylobacter* forms a commensal relationship with the host, resulting in lifelong colonization and providing a major source of infection for humans. Recent microarray analysis of *Campylobacter jejuni* harvested from the ceca of broiler chickens has yielded many genes that are over-expressed as compared to planktonic growth. Two highly over-expressed genes, *Cj 1534c* and *Cj FlaA/B* were selected and knockout mutagenesis was performed. Colonization studies were then carried out using commercial broiler chicks and the two *Campylobacter jejuni* NCTC 11168 mutants. It was determined that both the *FlaAB* and *1534c* knockout mutants were decreased in their ability to colonize avian hosts. This data provides preliminary evidence for further study of over-expressed genes as potential targets for intervention of colonization by *C. jejuni*.

Genotypic Characterization of *Campylobacter jejuni* Poultry Isolates

Lane, A.B.¹, W.G. Miller², L.A. Joens³, Q. Zhang⁴, B.A. White⁵, M.E. Konkel¹.

¹*School of Molecular Biosciences, Washington State University, Pullman, WA 99164.*

²*U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Produce Safety and Microbiology Unit, Albany, CA 94710. USA.*

³*Department of Veterinary Science and Microbiology, University of Arizona, Tuscon, AZ 85721, USA.*

⁴*Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA.*

⁵*Department of Animal Sciences, University of Illinois, Urbana, IL 61801, USA.*

Abstract

Campylobacter jejuni, a commensal organism of chickens, is the leading bacterial cause of foodborne gastroenteritis in the United States. Epidemiological case studies have demonstrated a link between *C. jejuni*-mediated enteritis and the handling and consumption of raw or undercooked poultry meats. Two molecular typing techniques, pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST), were used to determine the genetic relatedness of 113 *Campylobacter* isolates recovered from poultry processing plants located in Kansas, Iowa, and Washington state, and from cecal droppings collected at different broiler houses. The species of each isolate was determined using multiplex PCR for the *lpxA* gene. Of the 113 isolates, 100 isolates were found to be *C. jejuni* and the remaining 13 *C. coli*. All 113 isolates were typed using PFGE, which involves digestion of chromosomal DNA with *Sma*I, a rare cutter for *Campylobacter*, and subsequent separation of the large fragments by electrophoresis. The resulting macrorestriction profiles (MRP) were used to identify isolates that are genetically related or identical. From the 100 *C. jejuni* isolates, 25 distinct MRP groups were observed, and each was given a numerical or alphabetical designation. The number of isolates in each MRP group ranged from one to twelve. Three isolates, 41, G9a, and G11a, were non-typable using *Sma*I PFGE. Thus far, multi-locus sequence typing (MLST) has been used to assess the genetic relatedness of 48 of the 113 *Campylobacter* isolates. This technique involves determining the sequence of seven housekeeping genes and comparing them to a database of known sequences. Thirty-seven *C. jejuni* isolates were classified into ten known and five previously undescribed ST groups. Thirteen *C. coli* isolates belonged to two different known ST groups. The PFGE MRP groupings and MLST ST groupings correlated, with the exception that in a few cases, isolates which were of identical ST could be differentiated into distinct MRP groups using PFGE. These data suggest that PFGE is more discriminatory than MLST in the determination of genetic relatedness for *C. jejuni* isolates.

Identification of *Campylobacter* Genes Differentially Expressed in Chickens by DNA Microarray

Barton, E.¹, P. Plummer¹, L.A. Joens², M.E. Konkel³, B.A. White⁴, and Q. Zhang¹.

¹Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA.

²Department of Veterinary Science and Microbiology, University of Arizona, Tuscon, AZ 85721, USA.

³School of Molecular Biosciences, Washington State University, Pullman, WA 99164, USA.

⁴Department of Animal Sciences, University of Illinois, Urbana, IL 61801, USA.

Abstract

In three independent experiments, 10-day-old broiler chickens were inoculated with *Campylobacter jejuni* 700819 (NCTC 11168). Five days after the inoculation, the birds were sacrificed and cecal contents were collected for the isolation of *Campylobacter* RNA. The RNA extracted from the chickens as well as *in vitro* cultures were used for cDNA synthesis and labeling. Three competition hybridization experiments were performed using DNA microarray slides purchased from a commercial supply. The obtained data were processed using Lowess normalization, scale normalization, and median centering using the R statistical package. The normalized data were then subjected to a mixed-linear model analysis using the SAS statistical package. This analysis yielded 38 genes (35 upregulated and 3 downregulated) that were differentially expressed ≥ 2 -fold ($P < 0.05$) in the chicken host. The upregulated genes encode products of multiple function groups including amino acid transport and utilization, carbohydrate transport and metabolism, cell envelope biogenesis, biosynthetic pathways, energy production, and adaptation to pH fluctuation. These changes are consistent with the environmental conditions in the gastrointestinal tract, in which *Campylobacter* must utilize alternative energy sources (primarily amino acids) and be able to cope with various host defense mechanisms. Confirmation of the differentially expressed genes is being performed using real-time PCR. Some of the identified genes will be disrupted by insertional mutagenesis to determine their actual contribution to *Campylobacter* colonization in chickens.

Comparison of *Campylobacter jejuni* Genes Differentially Expressed in Animal Hosts by Microarray Analysis

Law, B.¹, M.E. Konkel², B.A. White³, Q. Zhang⁴, and L.A. Joens¹.

¹Department of Veterinary Science and Microbiology, University of Arizona, Tuscon, AZ 85721, USA.

²School of Molecular Biosciences, Washington State University, Pullman, WA 99164, USA.

³Department of Animal Sciences, University of Illinois, Urbana, IL 61801, USA.

⁴Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA.

Abstract

C. jejuni genes differentially expressed in two animal models to those of *C. jejuni* genes expressed on plates were identified using microarray analysis. Then, comparisons were made between genes that were up-regulated in both animal models. Hybridization experiments were performed on Combimatrix DNA microarray slides. After normalization of the data, 18 genes were up-regulated (≥ 5 -fold) in *C. jejuni* colonizing broiler chickens and 23 genes were up-regulated in the piglet model. The encoded gene products included hypothetical proteins, transporters, periplasmic proteins, biosynthetic proteins, DNA binding proteins and iron binding proteins. In addition, six genes were up-regulated (≥ 4 -fold) in both the broiler chickens and the piglets. The gene products included those as listed above and also a unique gene product, a bacterioferritin that our lab has identified as a probable pilus antigen. The genes up-regulated in both species will be mutated to examine their role in *C. jejuni* pathogenesis.

Association of Pathogen Load in Cattle with Retail Beef Contamination

Abley, M.¹, T. Wittum¹, H. Zerby², J. Bard², and J. Funk³.

¹*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH 43210, USA.*

²*Department of Animal Sciences, The Ohio State University, Columbus, OH 43210, USA.*

³*National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI 48824, USA.*

Abstract

INTRODUCTION: *Salmonella* and *Campylobacter* are estimated to cause 3.9 million illnesses annually in the United States, and most of these illnesses are food-related. Cattle can be sub-clinically infected with these pathogens and fecal contamination of meat during processing is a food safety risk. Quantitative measures of foodborne safety risk are rarely reported and are a critical data gap for development of quantitative risk assessments. The goal of this study was to determine the association between the concentration of *Salmonella* and *Campylobacter* in cattle feces with concentrations in meat.

METHODS: Samples were collected 5 times from 98 individually identified cattle during the peri-harvest period. Feces were collected on farm and in lairage. A hide swab was collected before removal and the entire carcass was swabbed immediately before chilling. For each individually identified carcass a meat sample was collected. *Salmonella* and *Campylobacter* were cultured and quantified at each stage using the Most Probable Number Method.

PRELIMINARY RESULTS: *Salmonella* was not cultured from any sample. The proportion (%) of samples that were *Campylobacter* positive was 76.5, 81.6, 96.9, 55.1, and 12.2 for farm, lairage, hide, carcass and meat samples respectively. The mean *Campylobacter* concentration for each sample type was: farm, 36753.6 cfu/g; lairage, 160828.6 cfu/g; hide, 0.9 cfu/cm²; carcass 8.7 cfu/half carcass; and meat 1.1 cfu/g. Analysis of the association between pre- and post-harvest *Campylobacter* concentrations are pending.

Application of DNA Aptamers As Capture Molecules to Detect *Salmonella Enterica* serovar Typhimurium from Complex Matrices

Joshi, R.², V.C. Lopes², K.V. Nagaraja², L. Jaykus³, J. Schefers¹, and S. Sreevatsan^{1,2}.

¹Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA.

²Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA.

³Department of Food Science, North Carolina State University, Raleigh, NC, USA.

Abstract

The objective of this research was to evaluate single stranded nucleic acid oligonucleotides (DNA aptamers), as high affinity ligands for capture of whole cell *Salmonellae* and subsequent detection on a Real Time PCR platform. DNA aptamers were selected to bind outer membrane preparations (OMPs) of *Salmonella enterica* serovar Typhimurium using Systematic Evolution of Ligands by Exponential Enrichment (SELEX) using a 40-mer randomized library. A counter-SELEX approach was employed to eliminate aptamers cross-reactive to lipopolysaccharides (LPS) and OMPs of a closely related enterobacterium, *E. coli*. The process yielded a total of 66 aptamer candidates. Specificity of the aptamers was evaluated by gel-shift analysis against *Salmonella* Typhimurium OMP, LPS, and crude extract (CE) and *E. coli* OMP, CE, and LPS. Five candidate aptamers with specificity to *S. Typhimurium* OMP were selected for further characterization. Serial dilutions of pure culture of *Salmonella* Typhimurium followed by aptamer capture narrowed down the list to two candidates with low end detection limits between 10-40 organisms. These two candidates, aptamer 33 and aptamer 45, were further analyzed for sensitivity using a magnetic bead based capture protocol using *S. Typhimurium* cultures and spiked bovine fecal samples. Real time PCR was used to analyze the DNA isolated from the beads after the capture. *Salmonella* was detectable at levels as low as 13 organisms from 0.25g of fecal samples. Cross reactivity studies with other enterobacteria revealed high levels of avidity and specificity to *Salmonella*. This is the first report where aptamers have been used as high affinity ligands for the detection of bacteria without any pre-enrichment steps. This has the potential to be developed into a portable assay suitable for laboratory and field based testing.

Evaluating Cost-Effective Food Safety Risk Reduction Strategy at Retail

Nganje, W. and L. Lehrke.

Department of Agribusiness and Applied Economics, North Dakota State University, Fargo, ND 58105, USA.

Abstract

Consumers make choices about food products based on several factors such as product price, quality and safety. In an efficient market, choices would be made with full information about product attributes. The real world market for food products is not necessarily efficient, and food safety problems complicate consumer decision making. Food safety is a credence attribute. This means consumers cannot tell with certainty the level of microbial pathogens that are present in meat and poultry products at the time of purchase, or even thereafter. There exists a breakdown in market structure due to unavailability of relevant information on food safety, and this implies a need for government intervention.

In 1996, the Food Safety Inspection Service (FSIS) introduced new mandatory food safety regulations following repeated discoveries of *E. coli* and *Salmonella* in the US food supply in the 1980s and early 1990s. The new regulations called Pathogen Reduction/Hazard Analysis and Critical Control Points (PR/HACCP) mandated the establishment of critical control points (CCPs) in food production and processing operations and established testing routines for food products while ensuring the safety of meat and poultry products. By 2000 these regulations had been adopted by meat and poultry processors. Data from CDC FoodNet (April, 2006), reveals that pathogen levels have decreased after the adoption of mandatory PR/HACCP in meat and poultry processing.

In spite of the documented success of PR/HACCP at the processing level, farm and retail level applications are optional. One critical observation is that a dichotomy exists between decreasing pathogen levels and increasing outbreaks from retail facilities. Currently, food service and retail facilities are implementing various forms of intervention, including PR/HACCP. We use stochastic optimization and stochastic dominance methods, which incorporate multiple risk factors, to evaluate cost-effective strategy at retail. Retail data on pathogen prevalence for *E. coli*, *Salmonella*, and *Campylobacter* (collected by North Dakota State University Microbiologists at North Dakota Retail facilities); and the cost of intervention for three alternative strategies were evaluate for beef, chicken, turkey and pork. The associated risks, costs and benefits of alternative mitigation strategies¹ are evaluated jointly in this model.

¹ These strategies involve different combinations of testing by the USDA and/or outside firms, standard operating procedures (SOPs) done internally, and testing done by the retail firm itself.

Preliminary results suggest that the probabilities of *E. coli* contamination were high across all meat types and that optimal intervention strategies varied by meat type and pathogen. Beef especially, showed a need for pathogen testing at the retail level. Poultry are normally sold as packaged from processing facilities and undergo limited transformation or additional processing at retail. The stochastic dominance analysis revealed that tightening tolerance levels to 5 percent prevalence are cost effective at retail. This is a marked distinction from processing where tolerance below 10 percent will tend to increase intervention costs exponentially.

A Mathematical Model of the Dynamics of *Salmonella* Cerro Infection in a U.S. Dairy Herd

Chapagain, P.P.¹, J.S. Van Kessel², J.S. Karns², D.R. Wolfgang³, E. Hovingh³, K.A. Nelen³, Y.H. Schukken¹, and Y.T. Grohn¹.

¹*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA.*

²*Environmental Microbial Safety Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705, USA.*

³*Department of Veterinary and Biomedical Science, Pennsylvania State University, University Park, PA 16802, USA.*

Abstract

We developed a mathematical model of the transmission dynamics of salmonella to describe an outbreak of *S. Cerro* infection that occurred in a Pennsylvania dairy herd. The data were collected as part of a cooperative research project between the Regional Dairy Quality Management Alliance and the Agricultural Research Service. After the initial detection of a high prevalence of *S. Cerro* infection in the herd, a frequent and intensive sampling was conducted and the outbreak was followed for one year. The data showed a persistent presence of *S. Cerro* with a high prevalence of infection in the herd. The dynamics of host and pathogen was modeled using a set of non-linear differential equations. A more realistically distributed (gamma distributed) infectious period using multiple stages of infection was considered. The basic reproduction number was calculated and relevance to the intervention strategies is discussed.

POSTER SESSION ABSTRACTS

Synthesizing Data from Multiple Studies Designs—An Example Using Interventions Associated with *Salmonella* In Swine

O'Connor, A.M.¹, T. Denagamage¹, J. Sargeant², A. Rajic³, and J. McKean¹.

¹*Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011, USA.*

²*Department of Clinical Epidemiology and Biostatistics, Health Sciences Centre, McMaster University, Hamilton, Ont., L8S 4K1, Canada.*

³*Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ont., N1G 3W4, Canada.*

Abstract

An effective method of synthesizing data is meta-analysis, however meta-analysis is usually used when the outcome of studies are similar. Using the association between feed characteristic and *Salmonella* prevalence we will present an approach to combining data from studies that use multiple outcomes from multiple studies designs. The standard procedures for a systematic review of the literature were followed until the synthesis component i.e. review question design, search, relevance screening, quality assessment and data extraction. To combine the evidence we modified of the Interim Evidence Ranking System for Scientific. Each study was characterized as one of 5 study design types based on the hierarchical scale for evidentiary value. After classifying the studies by type, the studies were considered collectively across the evidence base in order to rate the strength of the body of evidence. The rating system was based on two factors: quantity and consistency. The quantity ranking had 3 levels and considered the number of studies, the total number of individuals studied and the generalizability of the findings to the target population. The consistency ranking also had 3 levels and considered whether studies with both similar and different designs report similar findings. After ranking the body of evidence for quality and consistency, an overall ranking was assigned for the strength of the evidence. The final ranking system had four levels. For example, **the highest rank of scientific evidence**, reflects a high level of comfort among qualified scientists that the association/relationship is scientifically valid. This level ranked relationship would be considered to have a very low probability of significant new data overturning the conclusion that the relationship is valid or significantly changing the nature of the relationship. This approach to combining data in a transparent manner from multiple sources may be a method of informing policy makers when a large amount of heterogeneous literature is available about a topic.

Differential Response of *Campylobacter jejuni* Strains to Deoxycholate

Malik-Kale, P.¹, A.B. Lane¹, R. Hare-Sanford¹, B.A. White², Q. Zhang³, L.A. Joens⁴, and M.E. Konkel¹.

¹Department of Microbiology, School of Molecular Biosciences, Washington State University, Pullman, WA 99164, USA.

²Department of Animal Sciences, University of Illinois, Urbana, IL 61801, USA.

³Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA.

⁴Department of Veterinary Science and Microbiology, University of Arizona, Tuscon, AZ 85721, USA.

Abstract

Campylobacter jejuni, which is commensal organism in chickens, is a leading cause of foodborne illness in the U.S. Despite the organism's prevalence in poultry, our understanding of this organism's ability to adapt to different environmental niches is in its infancy. Several pathogenic bacteria, including *Salmonella* spp., *Shigella* spp. and *Vibrio cholerae*, have been found to respond to bile salts by modulating gene expression. The primary components of bile salts include cholic acid, deoxycholic acid (DOC), glycocholic acid, and taurocholic acid. DOC is present at 0.2 to 2% in the human intestinal tract and at 0.01% to 0.7% in the chicken intestinal tract. Work in our lab indicates that the co-culturing *C. jejuni* with DOC triggers the organism's virulence potential. More specifically, culturing *C. jejuni* with DOC induces the expression of genes that encode for the *Campylobacter* invasion antigens (Cia proteins). The Cia proteins are secreted from the bacteria, and enable the bacteria to invade epithelial cells. β -galactosidase reporter assays have revealed at least one gene that encodes a secreted protein, CiaB, is induced by DOC. We hypothesize that the ability of *C. jejuni* to respond to DOC may differ from one strain to another, thereby providing certain strains with an advantage within a host. The present study addresses whether: 1) Different strains of *Campylobacter* exhibit different sensitivities to DOC; and 2) If a difference in the induction of *ciaB* promoter activity in these strains exists. We are currently testing the sensitivity of 116 poultry isolates to different concentrations of DOC and monitoring the kinetics of *ciaB* induction using a *ciaB*_{Promoter}-*lux* reporter vector. These results will provide a foundation to better define the adaptive response of *C. jejuni* to DOC, which is a major component of the bile salts found in the human and chicken intestinal tract.

Repetitive Sequence Polymerase Chain Reaction (Rep-PCR) Analysis of *Campylobacter* Isolates from Poultry Reared in Different Production Environments

Wilson, M.K.¹, B. Law², L.A. Joens², M.E. Konkel³, and B.A. White¹.

¹Department of Veterinary Science and Microbiology, University of Arizona, Tuscon, AZ 85721, USA.

²Department of Veterinary Science and Microbiology, University of Arizona, Tuscon, AZ 85721, USA.

³School of Molecular Biosciences, Washington State University, Pullman, WA 99164.

Abstract

Campylobacter jejuni is the leading cause of foodborne illness in the United States. Molecular methods are often a food safety interest for identifying sources of infection and pathways of transmission, and can also identify clades that may facilitate the development of intervention strategies. Repetitive sequence polymerase chain reaction (Rep-PCR; REP, ERIC and BOX) was used to determine the genetic profiles and diversity of 63 *Campylobacter* isolates from chickens reared in different production environments (conventional, organic, and free-range) in different states. PCR fingerprints were subjected to computer-assisted pattern analysis. ERIC- and BOX-PCR yielded the highest number of PCR products and greatest reproducibility. Three UPGAMA trees were generated. The composite tree (combined REP-, ERIC-, and BOX-PCR) gives greater discriminatory power than the ERIC tree and BOX tree. In general, there were two major UPGAMA groupings based on flock and production environment. Interestingly, two amplicons (one ERIC and one BOX) appeared in over 93% of the reactions. The BOX amplicon was sequenced and BLASTn indicated the *C. jejuni* subsp. *jejuni* 16S ribosomal RNA (*rrs*) gene.

Addition of Fumaric Acid to Cattle Water Troughs to Inactivate *Escherichia coli* O157:H7

Heidenreich, J.M. and F. Diez-Gonzalez.

Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55108, USA.

Abstract

Water is a transmission vector for *Escherichia coli* O157:H7 in cattle populations. The use of fumaric acid (FA) as an additive in cattle water troughs to inactivate *E. coli* O157:H7 in cattle feces-contaminated water was investigated. Different concentrations of FA were tested at various pH values to determine their effect against *E. coli* O157:H7 in the presence of manure. Manure inoculated with a three strain *E. coli* O157:H7 mixture was tested in FA solutions (0.1%, 0.2%, 0.25%, 0.3%) adjusted to a pH 1.5 or 2.0. After 60 minutes of treatment without agitation, the reduction of *E. coli* O157:H7 was less than 1.7 log CFU/g in most treatments, but a 0.1% FA solution adjusted to pH 1.5 reduced the viable count 6.5 log CFU/g by 60 minutes. When the manure suspension was maintained agitated, a 4 to 5 log CFU/g reduction was achieved after 60 minutes with all FA concentrations at pH 2.0 while treatments at pH 1.5 had a 6 log reduction. Based on these results a FA solution at a concentration of 0.1% or higher and at pH 1.5 or 2.0 could be used to decrease fecal *E. coli* O157:H7 contamination in a cow water trough if the water was agitated. Addition of FA in cattle water troughs could potentially lead to an effective pre-harvest treatment to reduce spread of *E. coli* O157:H7 in cattle herds.

Bovine Gene Expression in Acute *Salmonella enterica* Serovar Typhimurium Infection Analyzed by MPSS and Microarray

Lawhon, S.D.¹, S. Khare¹, C.A. Rossetti¹, C.L. Galindo⁴, R.E. Everts³, J.F. Figueiredo¹, J.E.S. Nunes¹, T. Gull¹, C. Haudenschild⁶, G.S. Davidson⁵, H.R. Garner⁴, K. Drake⁷, H.A. Lewin³, A.J. Bäuml², and L.G. Adams¹.

¹*Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4467, USA.*

²*Department of Microbial and Molecular Pathogenesis, College of Medicine, Texas A&M University System Health Science Center, College Station, TX 77843-1114, USA.*

³*Department of Animal Sciences, University of Illinois, Urbana, IL, 61801, USA.*

⁴*Eugene McDermott Center for Human Growth and Development, The University of Texas Southwestern Medical School, Dallas, TX 75390-8591, USA.*

⁵*Sandia National Laboratories, Computation, Computers and Mathematics Center, Albuquerque, NM 87123, USA.*

⁶*Solexa, Inc., Hayward, CA 94545, USA.*

⁷*Seralogix, Austin, TX 78730, USA.*

Abstract

Salmonella enterica Serovar Typhimurium (*S. Typhimurium*) is the leading cause of death in humans due to foodborne illness in the United States. Infection in humans and young calves is characterized by polymorphonuclear cell influx to the intestinal mucosa and diarrhea. The clinical similarities between human and bovine infection make the calf an ideal model for human salmonellosis. Previous studies have shown that fluid secretion caused by *S. Typhimurium* is caused by effector proteins secreted by the Type III Secretion System (T3SS), specifically SipA, SopA, SopB, SopD, and SopE2. Loss of the genes encoding these effectors reduces not only fluid secretion in bovine ligated ileal loops but also Interleukin 8 (IL-8) gene expression. The objective of this study was to create a profile of host gene expression during acute infection with wild type *S. Typhimurium* and also an isogenic *sipA sopABDE2* mutant. This was accomplished using microarray analysis and Massively Parallel Signature Sequencing (MPSS). A microarray time-course study was performed by collecting and analyzing samples from bovine ligated ileal loops over seven time points, 15 minutes, 30 minutes, 1 hour, 2, 4, 8, and 12 hours. MPSS analysis measures signatures from individual mRNA sequences. MPSS was performed on pooled samples from 1 hour, 2 hours, and 4 hours and identified 5,460 unique signatures. Signatures included those for genes previously identified in host response to *Salmonella* such as chemokine (C-C motif and C-X-C motif) ligand, tumor necrosis factor receptor superfamily (member A1 and 5), prostaglandin E synthase, lymphotoxin- β and fibronectin 1 as well as signatures for genes with previously unrecognized roles in host response to *Salmonella* infection including

dipeptidylpeptidase IV (adenosine deaminase complexing protein-2), angiotensin receptor 1, angiotensin II receptor-associated protein, thyroid receptor interacting protein, and adhesion molecule syndecan-4. MPSS also revealed evidence of extensive antisense transcript expression. Signatures identified only in the wild type inoculated loops may serve as potential biomarkers specific for early infection.

Human and Animal Enteric Caliciviruses in Oysters from Different U.S. Coastal Regions

Costantini, V.¹, F. Loisy², L. Joens³, F. Le Guyader², L. Saif¹.

¹FAHRP, OARDC, Ohio State University, Wooster, OH 44691, USA.

²Lab. de Microbiol, IFREMER, Nantes, France.

³Vet. Sci. and Microbiol. Dept., University of Arizona, Tucson, AZ 85721, USA.

Abstract

It is estimated that 50-66% of all foodborne illnesses of known etiology and 52% of gastroenteritis associated with consumption of raw or partially cooked shellfish are attributable to human noroviruses (NoVs). Enteric caliciviruses, including both NoVs and sapoviruses (SaVs) persist in the environment and the genetic and antigenic relatedness between human and animal strains suggests a possibility for interspecies transmission. Our objective was to determine the occurrence of enteric caliciviruses in U.S. market oysters. Samples were collected from 45 different bays along the 3 U.S. coasts during summer and winter of 2002-2003. Pooled oysters from each bay were analyzed by RT-PCR and results were confirmed by hybridization and sequence analysis. Nine samples (20%) were positive for human NoV GII after hybridization and 5/9 were related to the NoV GII-4 previously implicated in diarrhea outbreaks. Seven samples (15%) were positive for porcine NoV GII and one sample was positive for porcine SaVs after hybridization and sequencing. Bovine NoVs were detected in two samples and confirmed by sequencing. Different seasonal and state distributions were detected. The simultaneous detection of human and animal enteric caliciviruses in oysters raises concerns for human infection or co-infection with human and animal strains that could result in emergence of new strains.

CONTACT INFORMATION

Abley, Melanie
614-292-3412
abley.1@osu.edu
Department of Veterinary Preventive Medicine, The Ohio State University,
Columbus, OH 43210, USA.

Adams, L. Garry
979-845-5092
gadams@cvm.tamu.edu
Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M
University, College Station, TX 77843-4467, USA.

Bailey, R. Hartford
662-325-7729
rhbailey@cvm.msstate.edu
Department of Pathobiology and Population Medicine, College of Veterinary
Medicine, Mississippi State University, Mississippi State, MS 39762, USA.

Bard, Jaime
614-688-5696
bard.13@osu.edu
Department of Animal Sciences, The Ohio State University, Columbus, OH 43210,
USA.

Barton, Eva
515-294-2381
bartony@iastate.edu
Department of Veterinary Microbiology and Preventive Medicine, Iowa State
University, Ames, IA 50011, USA.

Bäumler, A.J.
Phone
ajbaumler@ucdavis.edu
Department of Microbial and Molecular Pathogenesis, College of Medicine, Texas
A&M University System Health Science Center, College Station, TX 77843-1114,
USA.

Chapagain, Prem P.
305-348-6266
prem.chapagain@fiu.edu
Department of Physics, Florida International University, Miami, FL 33199, USA.

Clements, Kris M.

Phone

Email

Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762, USA.

Costantini, Veronica

330-263-3744

costantini.3@osu.edu

FAHRP, OARDC, Ohio State University, Wooster, OH 44691, USA.

Davidson, G.S.

505-844-7902

gsdavid@sandia.gov

Sandia National Laboratories, Computation, Computers and Mathematics Center, Albuquerque, NM 87123, USA.

Denagamage, Thomas

515-294-3837

thomasde@iastate.edu

Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011, USA.

Diez-Gonzalez, Francisco

612-624-9756

fdiez@umn.edu

Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55108, USA.

Drake, Ken

512-828-7955

drake@seralogix.com

Seralogix, Inc., 6034 West Courtyard Drive, Austin, TX 78730, USA.

Everts, Robin E.

217-333-3623

everts@uiuc.edu

Department of Animal Sciences, University of Illinois, Urbana, IL, 61801, USA.

Figueiredo, Josely F.

979-845-9814

jfigueiredo@cvm.tamu.edu

Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4467, USA.

Funk, Julie
517-432-3100
funkj@msu.edu
National Food Safety and Toxicology Center, Michigan State University, East
Lansing, MI 48824, USA

Galindo, Cristi L.
409-939-6886
cristi.galindo@utsouthwestern.edu
Department of Animal Sciences, University of Illinois, Urbana, IL, 61801, USA.

Garner, Harold R.
214-648-1661
harold.garner@utsouthwestern.edu
Eugene McDermott Center for Human Growth and Development, The University of
Texas Southwestern Medical School, Dallas, TX 75390-8591, USA.

Grohn, Yrjo
607-253-3571
ytg1@cornell.edu
Department of Population Medicine and Diagnostic Sciences, College of Veterinary
Medicine, Cornell University, Ithaca, NY 14853, USA.

Gull, T.
979-845-9814
tgull@cvm.tamu.edu
Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M
University, College Station, TX 77843-4467, USA.

Hare-Sanford, Rebekah
509-335-3522
***email**
School of Molecular Biosciences, Washington State University, Pullman, WA 99164,
USA

Haudenschild, C.
***Phone**
chrish@solexa.com
Solexa, Inc., Hayward, CA 94545, USA.

Heidenreich, Jessie M.
651-212-0372
heid0129@umn.edu
Department of Food Science and Nutrition, University of Minnesota, St. Paul,
Minnesota 55108, USA.

Hovingh, Ernest
814-863-8526
eph1@psu.edu
Department of Veterinary and Biomedical Science, Pennsylvania State University,
University Park, PA 16802, USA.

Jaykus, Lee-Ann
919-513-2074
leeann_jaykus@ncsu.edu
Department of Food Science, North Carolina State University, Raleigh, NC, USA.

Joens, Lynn A.
520-621-4687
joens@ag.arizona.edu
Department of Veterinary Science and Microbiology, University of Arizona, Tucson,
AZ 85721, USA.

Joshi, Raghavendra
*phone
joshi044@umn.edu
Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine,
University of Minnesota, St. Paul, MN, USA.

Karns, Jeffrey S.
301-504-6493
karnsj@ba.ars.usda.gov
Environmental Microbial Safety Laboratory, Agricultural Research Service, USDA,
Beltsville, MD 20705, USA.

Khare, Sangeeta
979-845-9814
skhare@cvm.tamu.edu
Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M
University, College Station, TX 77843-4467, USA.

Konkel, M.E.
509-335-5039
konkel@mail.wsu.edu
School of Molecular Biosciences, Washington State University, Pullman, WA 99164,
USA.

Lane, Alison B.
509-335-3522
ablane@mail.wsu.edu
School of Molecular Biosciences, Washington State University, Pullman, WA 99164.

Law, Bibiana F.
520-621-4148
bibiana@email.arizona.edu
Department of Veterinary Science and Microbiology, University of Arizona, Tucson,
AZ 85721, USA.

Lawhon, Sara D.
979-845-9814
slawhon@cvm.tamu.edu
Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M
University, College Station, TX 77843-4467, USA.

Le Guyader, Françoise S.
33 2 40 37 40 52
sleguyad@ifremer.fr
Laboratoire de Microbiologie, IFREMER, Nantes, France.

Lehrke, Linda
*phone
linda.lehrke@ndsu.edu
Department of Agribusiness and Applied Economics, North Dakota State University,
Fargo, ND 58105, USA.

Lewin, Harris A.
217-244-3034
h-lewin@uiuc.edu
Eugene McDermott Center for Human Growth and Development, The University of
Texas Southwestern Medical School, Dallas, TX 75390-8591, USA.

Loisy, Fabienne
33 (0)2 40 37 40 43
Fabienne.Loisy@ifremer.fr
Lab. de Microbiol, IFREMER, Nantes, France.

Lopes, Vanessa C.
612-625-1737
lopes002@umn.edu
Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine,
University of Minnesota, St. Paul, MN, USA.

Malik-Kale, Preeti
509-335-3522
*Email
School of Molecular Biosciences, Washington State University, Pullman, WA 99164,
USA.

McKean, James
515-294-8792
x2mckean@iastate.edu
Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011, USA.

Miller, William G.
510-559-5992
bmiller@pw.usda.gov
U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Produce Safety and Microbiology Unit, Albany, CA 94710. USA.

Nagaraja, Kakambi V.
612-625-9704
nagar001@umn.edu
Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA.

Nelen, Kimberly A.
814-865-5630
kab208@psu.edu
Department of Veterinary and Biomedical Science, Pennsylvania State University, University Park, PA 16802, USA.

Nganje, William
701-231-7459
wnganje@ndsuxext.nodak.edu
Department of Agribusiness and Applied Economics, North Dakota State University, Fargo, ND 58105, USA.

Nunes, Jairo E.S.
979-845-9814
jnunes@cvm.tamu.edu
Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4467, USA.

O'Connor, Annette M.
515-294-5012
oconnor@iastate.edu
Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011, USA.

Plummer, Paul
515-294-5513
pplummer@iastate.edu
Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA.

Rajic, Andrijana
519-826-2980
Andrijana_rajic@phac-aspc.gc.ca
Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ont.,
N1G 3W4, Canada

Rossetti, C.A.
979-845-9814
crossetti@cvm.tamu.edu
Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M
University, College Station, TX 77843-4467, USA.

Saif, Linda J.
330-263-3742
saif.2@osu.edu
FAHRP, OARDC, Ohio State University, Wooster, OH 44691, USA.

Sargeant, Jan
905-525-9140
sargeaj@mcmaster.ca
Department of Clinical Epidemiology and Biostatistics, Health Sciences Centre,
McMaster University, Hamilton, Ont., L8S 4K1, Canada.

Schefers, Jeremy M.
612-625-8780
sche0201@umn.edu
Department of Veterinary Population Medicine, College of Veterinary Medicine,
University of Minnesota, St. Paul, MN, USA.

Schukken, Ynte H.
607-255-8202
yhs2@cornell.edu
Department of Population Medicine and Diagnostic Sciences, College of Veterinary
Medicine, Cornell University, Ithaca, NY 14853, USA.

Sreevatsan, Srinand
612-625-3769
sreev001@umn.edu
Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine,
University of Minnesota, St. Paul, MN, USA.

Theoret, James R.
520-621-4148
theoret@email.arizona.edu
Department of Veterinary Science and Microbiology, University of Arizona, Tucson,
AZ 85721, USA.

Van Kessel, Jo Ann S.
301-504-8287
jkessel@anri.barc.usda.gov
Environmental Microbial Safety Laboratory, Agricultural Research Service, USDA,
Beltsville, MD 20705, USA.

Lisa Waddell
519-826-2347
lisa_waddell@phac-aspc.gc.ca
Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ont.,
N1G 3W4, Canada.

White, Bryan A.
217-333-2091
bwhite44@uiuc.edu
Department of Animal Sciences, University of Illinois, Urbana, IL 61801, USA

Wills, Robert
662-325-9718
wills@cvm.msstate.edu
Department of Pathobiology and Population Medicine, College of Veterinary
Medicine, Mississippi State University, Mississippi State, MS 39762, USA.

Wittum, Thomas
614-292-8157
wittum.1@osu.edu
Department of Veterinary Preventive Medicine, The Ohio State University,
Columbus, OH 43210, USA.

Wolfgang, David R.
814-863-5849
drw12@psu.edu
Department of Veterinary and Biomedical Science, Pennsylvania State University,
University Park, PA 16802, USA.

Zhang, Qijing
515-294-2038
zhang123@iastate.edu
Department of Veterinary Microbiology and Preventive Medicine, Iowa State
University, Ames, IA 50011, USA

Zerby, Henry
614-688-4584
zerby.8@osu.edu
Department of Animal Sciences, The Ohio State University, Columbus, OH 43210,
USA.