T11-09 A Large Scale Survey Describing the Relationship Between Different Animal Reservoirs and Human Campylobacteriosis

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Introduction: *Campylobacter jejuni* is a leading cause of bacterial foodborne gastroenteritis worldwide. *C. jejuni* contaminated poultry meat and meat products are considered the most important sources of disease in humans. Nevertheless, other animal reservoirs must be investigated to elucidate the part not attributed to poultry.

Purpose: The objective of this work is to describe using the comparative genomic fingerprinting (CGF) with 40-gene assay (CGF40) the *C. jejuni* population circulating in the poultry production chain in France and in different animal reservoirs to determine a link with human cases.

Methods: A total of 645 poultry isolates representative of the French poultry industry (farm, slaughterhouse and retail), 455 isolates from dogs and cats, 122 isolates collected from river water and shells and 143 strains from human campylobacteriosis were typed by CGF40 according to Taboada et al. (2012). Isolates were categorized into types based on more than 90% CGF40 fingerprint similarity (CGF-90%). The results were analyzed using Bionumerics software and the genetic diversity of the different strain populations was evaluated using the Simpson Index of Similarity (ID).

Results: A great diversity has been observed with 141 different types among the 1364 isolates typed (ID=0.958). Within the pet isolates (ID=0.895) and the clinical isolates (ID=0.910), the genetic diversity was significantly lower than among poultry isolates (ID=0.961) and water isolates (ID=0.961). The main part of human isolates (57%) were divided into 4 CGF-90% types all found in chicken and pet isolates. Moreover, few human isolates belong to CGF-90% types sharing by water isolates.

Significance: This is the first large scale survey in France involving representative number of isolates from poultry, pets, river water, shells and humans. The results confirm that poultry meat production remain a substantial source of human infections in France. But other animal reservoirs sources, especially pets, could be potential sources of campylobacteriosis.

T11-10 Temporal and Population Dynamics of *Salmonella enterica* ssp. *enterica* Serovar Agona Isolates from a Recurrent Multistate Outbreak

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Introduction: The largest outbreak of *Salmonella* Agona in the United States occurred in 1998. It affected more than 400 patients and was linked to toasted oats cereal. Ten years later, a similar outbreak occurred with the same outbreak strain linked to the same production facility.

Purpose: In this study, whole-genome sequence (WGS) data from a set of *Salmonella* Agona isolates were analyzed to provide insight into the evolutionary relationships among strains linked to two outbreaks of salmonellosis separated by ten years.

Methods: We analyzed WGS sequence data from 46 *Salmonella* Agona isolates. Five out of 46 isolates were associated with the 1998 outbreak and 25 isolates were associated with the 2008 outbreak while 16 isolates were unrelated to both events. We reconstructed a phylogenetic hypothesis of the samples using a reference-free k-mer based method for identifying variable sites. We then tested alternative hypothesis regarding differences in mutation rates and historical fluctuations in effective population size.

Results: Using SNP analyses, we were able to distinguish and separate *Salmonella* Agona isolates from both outbreaks with only a few SNP differences between them. The phylogeny illustrates that the 2008 outbreak involves direct descendants from the 1998 outbreak rather than a second independent contamination event. Estimates of historical fluctuations in population size for the whole dataset and one reduced to only containing the isolates associated with the outbreak showed that the latter had little to no change in effective population size. Further, there are fewer numbers of SNP differences within genes linked to cell mobility, intracellular transport, and transcription within the cereal clade.

Significance: Based on these results, there is evidence supporting the persistence of *Salmonella* over time with little genomic changes and that emerging lineages or clonal swarms may harbor a higher mutational load than observed in the larger population.

T11-11 Comparison of *Listeria monocytogenes* Invasion among the Serotypes Isolated from Foods and Human

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Introduction: *Listeria monocytogenes* is generally isolated from food, especially meat products, and 90% of the isolates are 1/2a, 1/2b and 4b. The pathogen causes foodborne disease by invasion intestinal tissue, and thus, they may have different invasion efficiencies among serotypes.

Purpose: The objective of this study was to evaluate the invasion efficiency of *L. monocytogenes* among serotypes, and between food isolates and human isolates.

Methods: To compare the invasion efficiency, *L. monocytogenes* serotypes (1/2a, 1/2b, 1/2c, 3b, 4b, 4d) isolated from foods (14 strains) and human (11 strains) were used. *L. monocytogenes* inocula were prepared in minimum essential medium (MEM) supplemented with 20% fetal bovine serum to obtain multiplicity of infection of 100. Monolayer (5×10^4 cells/ml) of Caco-2 cell grown in 24-well tissue culture plates was washed twice with phosphate buffered solution. One milliliter of the inocula were inoculated into the cell monolayer of Caco-2 cells, and they were incubated in 5% CO_2 at 37°C for 2 h. After treating Caco-2 cells with 50 µg/ml gentamicin and 0.5%Triton X-100, resulting suspensions were plated on tryptic soy agar with 0.6% yeast extract to enumerate infected *L. monocytogenes*.

Results: The invasion efficiencies of *L. monocytogenes* strains into Caco-2 cell were dependent on serotypes. However, the invasion efficiencies of *L. monocytogenes* food isolates were similar to those of *L. monocytogenes* human isolates.

Significance: This result indicates that different serotypes of *L. monocytogenes* may have different invasion efficiencies into Caco-2 cell.

T11-12 Study of the Potential Zoonotic Transmission of *Clostridium difficile* in Belgian Cattle Farms

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Introduction: Zoonoses are infectious that can be transmitted between animals and humans through direct contact, close proximity or the environment. Since domestic and food animals frequently test positive for the bacterium, it seems plausible that *C. difficile* could be zoonotic. A former study showed that the prevalence in veal calf aged less than 6 months was 22% while in adult cattle population, it was 6.9 %.

Purpose: This study aimed to determine the prevalence and the epidemiology of *C. difficile* in cattle farms and the possible spread of the bacterium among animals and farmers.

Methods: A total of 176 fecal samples of cattle were collected from 5 different Belgian farms (south East Belgium), from November 2015 to February 2016. A stool sample of each farmer was also requested. Detection of *C. difficile* was performed by classical culture on *C. difficile* selective medium (cycloserine cefoxitin fructose cholate). Isolates were characterized by PCR-ribotyping and Genotype Cdiff test (Hain Lifescience), which allows the detection of all toxin genes, mutations in *gyrA* gene and the deletion in the regulator gene *tcdC*. Toxic activity was confirmed by a cytotoxic assay on MRC-5 cells.

Results: *C. difficile* was detected in 14 of 178 (7.9%) animal samples. Isolates were grouped into five different types, including PCR-ribotype 015 (this ribotype is one the most encountered in hospitals in Belgium). The other types were UCL46A, UCL24*, UCL24, UCL33. All of them were identified as toxigenic by cytotoxicity assay and toxin genes profile. In contrast, none of the 5 farmers studied were positive for the bacterium.

Significance: Results obtained indicate that PCR-ribotypes commonly isolated from hospitalized patients are also present in cattle, indicating an animal reservoir. However, a zoonotic transmission could be not demonstrated in this preliminary study.

T12-01 Modeling the Inhibition of *Clostridium botulinum* in Reduced-sodium Pasteurized Process Cheese Products

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Introduction: The 1986 "FRI/Tanaka model" predicts safety of shelf-stable process cheese spread formulations using the parameters of moisture, pH, NaCl and disodium phosphate (DSP) to inhibit toxin production by *Clostridium botulinum*. Although this model is very reliable in predicting safety for standard-of-identity spreads, the effects of additional factors are not considered.

Purpose: To expand the *C. botulinum* food safety predictive model to consider the interactive effect of moisture, pH, potassium-based replacements for NaCl and DSP, fat, and sorbate.

Methods: Eighty formulations were identified using a central composite design targeting seven factors (50-60% moisture, pH 5.4-6.2, 0-0.2% sorbic acid, 10-30% fat, 1.7-2.4% NaCl, 0.8-1.6% DSP, and 0-50% potassium replacement for sodium salt). Treatments were inoculated with 3-log proteolytic *C. botulinum* spores (10-strain mixture) per gram, hot-filled into sterile vials, and stored anaerobically at 27°C. Samples (5/interval) were assayed at 0, 1, 2, 3, 4, 8.5, 17.5, 26, 40 and 56 weeks for presence of botulinum toxin using the mouse bioassay. A parametric survival model was fit to the censored time to toxin data. The model can predict both failure probability at specified times and time to toxicity with specified failure probability.

Results: All linear, quadratic and pairwise effects were considered for model fit. As hypothesized, the effects of pH, moisture, DSP, NaCl and sorbate were significant ($P<0.001$). Fat level and potassium-replacement were significant at $P<0.017$ and 0.053. The model is conservative, consistently predicting failure for toxic samples although it does predict failure for some samples that were not toxic. Comparison with previously collected challenge study data confirms that the model predictions are valid only for combinations within the ranges tested.

Significance: This research adds the factors of salt reduction, fat, and sorbate to the model predicting the botulinum safety of process cheese products. Additional study is required to expand the model to lower moisture and higher phosphate-emulsifier concentrations.

T12-02 Modeling Survival of *Salmonella* Enteritidis during Storage of Yoghurt at Different Temperatures

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❖ ***Developing Scientist Competitor***

Introduction: Yoghurt has an important role in the human diet due to its nutritional value and positive effect on health. This product has been recently included in a vulnerability assessments of food systems developed by Food and Drug Administration (FDA, 2012), suggesting that yoghurt could be a potential target for bioterrorist attack.

Purpose: To investigate the behavior of *Salmonella* Enteritidis in yoghurt at 4, 12, 20, and 25°C and develop predictive microbiology models for vulnerability assessment purposes.

Methods: Survival data were obtained at different temperatures by plate count method and used to fit survival models (Geeraerd model, Weibull model, the modified Weibull model, the trilinear model, the bilinear model) by using the package of nlsMicrobio in R software. To evaluate the effect of storage temperature on kinetic parameters such as inactivation rate (k_{max}) and shoulder (S_l), secondary models were developed by using two empirical models.

Results: According to the survival curves and smaller goodness of fit indices (RMSE, ACC_f), Geeraerd model with shoulder and tailing was selected as the most appropriate model to describe the survival of *Salmonella* in yoghurt during storage at different temperatures. At 4°C, *Salmonella* displayed the lowest inactivation rate (0.05 h^{-1}), whereas at 25°C, the maximum temperature assayed, it showed the highest inactivation rate (0.32 h^{-1}). S_l was the longest in samples stored at 4°C (55.93 h), whereas in samples stored at 25°C it was the shortest (4.28 h). In addition, the tested empirical models were able to accurately *predict Salmonella survival as a function of temperature.*

Significance: Results suggest that contamination by *Salmonella* in yoghurt could pose a significant risk to consumers. The predictive models herein developed could be applied to better support quantitative vulnerability and risk assessment studies, providing more accurate estimates.

T12-03 Behavior of *Staphylococcus aureus* in the Presence of Bacteriocin Producer *Enterococcus faecalis* in Fresh Soft Cheeses

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Introduction: *Staphylococcus aureus* is a pathogen of major concern in foods, especially milk and cheese, due to the production of thermoresistant enterotoxins. Dairy autochthonous microbiota is mainly composed of lactic acid bacteria (LAB) with recognized antagonistic potential over certain pathogenic and spoilage bacteria. Despite this, the impact of bacteriocin-producing LAB over *S. aureus* physiology and virulence in foods is still poorly understood.

Purpose: This study aimed at monitoring *S. aureus* behavior in the presence of bacteriocin-producing *E. faecalis* during the production of a fresh soft cheese.

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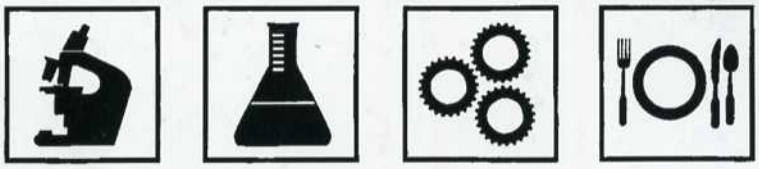

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