Effect of Cold Stress (4ºC) and Combined Cold Stress-starvation on *Campylobacter jejuni* Adherence to Caco-2 Epithelial Cells

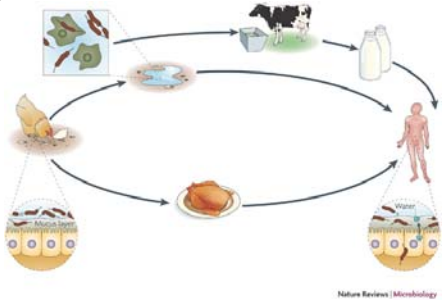
Miriam Kwarteng-Siaw[a], Elisa Mingo[b], Alfonso V. Carrascosa[b], Adolfo J. Martinez-Rodriguez[b]

[a]*Dept of Biological Sciences, Columbia University, New York, New York, USA*

[b]*Dept. of Biotechnology and Microbiology, Institute of Food Science Research (CIAL), CSIC-UAM., Spain*

Introduction

- *Campylobacter jejuni (C. jejuni)* is the most common cause of foodborne bacterial diarrhea in developed nations. 190,566 cases were reported in Europe by the European Food Safety Authority (EFSA) in 2008 with 11.4 for every 100,000 inhabitants in Spain and 13.02 per 100,000 individuals in the U.S according to the CDC in 2009.
- *C. jejuni* is gram negative, microaerophilic (5% O_2, 10% CO_2, 85% N_2) (Garénaux et al, 2008. *Current Microbiology* 56, 293 – 297) and thermophilic (37ºC - 42ºC) (Silva et. al., 2011. *Frontiers in Microbiology* 2, 1 -12.). Once in the body, it travels to the intestines which have these optimal growth conditions.
- Although commensal in other organisms including birds, *C. jejuni* is pathogenic in humans. Its most common modes of transmission to humans are through consumption of contaminated water, milk and chicken.



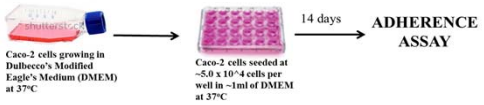
- *Campylobacter* is highly sensitive to desiccation, cold, heat, acid, osmotic and oxidative stress, aeration, starvation, and NaCl concentrations above 2% (Park, 2002. *International Journal of Food Microbiology* 74, 177 – 188) because it lacks specific regulons for stress response. However, its genome plasticity contributes to its capacity to respond to stress and ability to survive in non optimal conditions such as on raw chicken meat and skin for more than 10 days (Davis and Conner, 2007. *Poultry Science* 86, 765–767).
- Once in the body, *C. jejuni* travels to specific sites of the intestinal mucosa using chemotactic signaling (Hugdahl, et al., 1988. *Infection and Immunology* 56, 1560 – 1566) and initially adheres reversibly and non-specifically to the mucous membrane and then irreversible and specifically to intestinal epithelial cells (Konkel et al., 1997. *Molecular Microbiology* 24, 953 – 963) to trigger a reorganization of intestinal cell structure allowing for invasion (Zhu et al., 2006. *Reviews in Medical Microbiology* 17, 39 – 43) and induction of a cytotoxic response (Konkel et al., 2001. *Current Issues in Intestinal Microbiology* 2, 55 – 71).
- With the importance of *Campylobacter* adherence in its intestinal colonization abilities, we are most interested in the factors involved with the adhesion process and ways to curtail adhesion in order to control *Campylobacter* prevalence in chicken and consequently, humans.

Aim/Objective

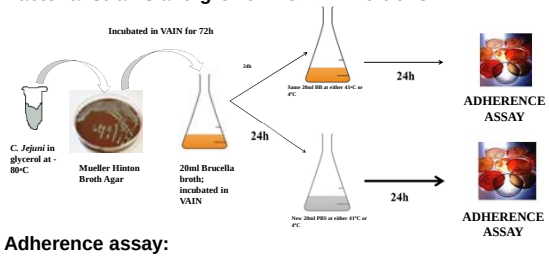
- To investigate the effects of cold stress (temperature of 4ºC) on adherence of two strains of *Campylobacter*, *C. jejuni* 11168 and *C. jejuni* LP1 to Caco-2 epithelial cells
- To investigate the combined effects of cold stress and starvation (growth in nutrient depleted Phosphate Buffered Saline (PBS) media) on adherence of *C. jejuni* 11168 and *C. jejuni* LP1 on Caco-2 epithelial cells

Methods

Caco-2 cell culture

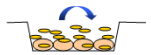


Bacterial strains and growth media conditions

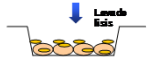


Adherence assay:

4ml of C. jejuni inoculum centrifuged and resuspended in 4ml PBS; 100µl collected for serial dilutions and plating to determine starting concentration as a control



0.5ml of *C.jejuni* resuspended in PBS inoculated into triplicate Caco-2 cell wells and incubated at 37ºC for 1h



Cells are washed with PBS once and lysed open with triton

Serial dilutions of the contents of each well (up to -5 dilution) are plated on MHB agar plates and incubated in VAIN for 48h (refrigerator experiment only) or 72h (refrigerator and starvation experiment)
Colonies are counted to determine control and adherent bacteria concentration

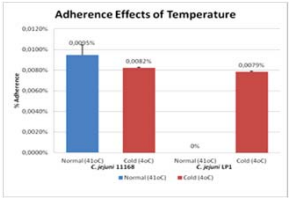
Acknowledgements

This research was funded by the Old Westbury Neurosciece International Program (OWNIP) through an MHIRT grant from the NIMHD (MD001429). Special thanks to all the members of Dr. Mingo's, Dr. Carrascosa's and Dr. Martinez's laboratories for their guidance, training and assistance on the project. Special thanks as well to Dr. Fernando Nieto, OWNIP Director, and Ms. Danielle Benz, OWNIP Coordinator.

Results

Cold stress effects on *Campylobacter jejuni* adherence to Caco-2 cells

- *C. jejuni* LP1
  - No adherent bacteria observed at 41ºC
  - 0.0079 % adherent bacteria observed at 4ºC
  - Increase in adherence with cold stress
- *C. jejuni* 11168
  - 0.0095 % adherent bacteria observed at 41ºC
  - 0.0082 % adherent bacteria observed at 4ºC
  - Slight decrease in adherence with cold stress



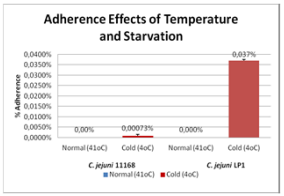
Combined cold stress and starvation effects on adherence

No viable or adherent bacteria observed for both strains at 41ºC in PBS

Adherent bacteria observed for both strains at 4ºC in PBS

0.037 % for LP1; 0.00073 % for 11168

Overall increase in adherence with cold stress and starvation



Conclusion/Future Work

- Cold stress (4ºC) increases the adherence levels of *C. jejuni* LP1 (clinical isolate) to Caco-2 epithelial cells, whereas the collection strain *C. jejuni* 11168 is slightly affected.
  - -Further research is needed for *C. jejuni* 11168 because as a collection strain, it has been shown to lose some virulence and pathogenesis factors that allow for adequate stress response.
- The combination of cold stress and starvation sharply increases the adherence of *C. jejuni* LP1 to Caco-2 epithelial cells. *C. jejuni* 11168 adherence is also increased but at a much lower level.
- Although further research is required, the results advise that some strains of *C. jejuni* could become more virulent increasing adherence to intestinal cell epithelium after cold shock and starvation.
- As *Campylobacter* is subjected to cold stress and starvation on chicken during storage, these results are cause for concern and should be considered in further studies to control *Campylobacter* in the food chain.