Coinfection Mechanisms of *Campylobacter jejuni* and *Escherichia coli* species in Human and Chicken Gastrointestinal Cells

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Introduction

C. jejuni is a Gram-negative bacterium that is the cause of campylobacteriosis; Europe's most common food-related illness with death occurring in severe cases[1]. Further complications during infection have the potential to lead to Guillain-Barre syndrome, ulcerative colitis and Crohn's disease. Although it can be found to contaminate a multitude of different foods and water sources its main vector is poultry products.

Escherichia coli is an opportunistic pathogen, found in the gastrointestinal microbiome of healthy warm-blooded animals[2]. Extraintestinal pathogenic *E. coli* (ExPEC) is the most frequent Gram-negative bacterial pathogen in humans and the leading cause of extraintestinal infections worldwide[2,3].

With the current curiosity surrounding the microbiome and its impact upon human health the understanding the interactions between different microbes has never been so important. Recent research has illustrated the impact and influence that gut microbes can have on one-another and outlines the synergistic relationship of *Escherichia coli* and *Campylobacter jejuni*[4,5,6,7].

Aim: To investigate a potentially synergistic relationship between *C. jejuni* and *E. coli* and determine if the presence of either species increases the invasive potential of the other.

Materials and methods

Immunocytochemistry

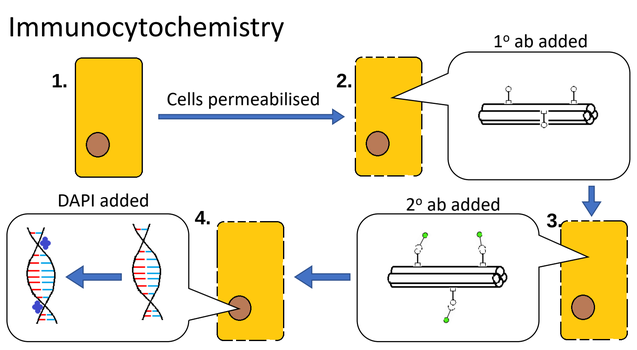

Figure 1: 1. 50,000 8E-11 or Caco-2 cells/ml were placed on a 22x22mm coverslip and allowed to adhere overnight in 2ml antibiotic free media (89% Gibco® DMEM F-12, 10% Gibco® Fetal Bovine Serum and 1% Glutamine). Following a 500µl dose of 10% neutral buffered formalin, each of the coverslips were set to block with 5% Bovine Serum Albumin. **2.** Cells were permeabilised with Triton X-100 and 1 in 33 dilution of mouse pan antibody (ab86734) to Cytokeratin or, for actin filament staining, a solution of 1 in 1000 Phalloidin-iFluor 488 (ab176753) was applied. **3.** Cells were then supplemented with 1 in 33 dilution of Alexa Fluor® 488 goat anti-mouse IgG (H+L) (ab150113) (this step was missed for Phalloidin staining) **4.** Finally, slides were set with Vectashield® Antifade Mounting Medium with approximately 0.0375 µg/ml DAPI in order to visualise the cells nuclei.

Bacteria Culture

The cell culture of *C. jejuni* strains were grown within the Whitley A85 anaerobic workstation, which maintained a microaerophilic environment (5% O_2, 10% CO_2, 85% N_2) at a temperature of 42ºC. *Escherichia coli* strains were grown in aerobic conditions at a temperature of 37ºC

Gentamicin Protection Assay

A 24 well plate with a confluent layer of gastrointestinal cells[8] were inoculated with varying quantities of bacteria set to McFarland standard. The dosed plate was then incubated at 5% O_2 for four hours. A concentration of 0.2mg/ml Gentamicin was applied to each well and the plate was once again incubated for 90 minutes. Cells were then washed with Phosphate buffered solution (PBS). After the final wash, cells were uplifted using Gibco® Tryple express. This Tryple express solution, now containing the gastrointestinal cells, were put into Eppendorf's and centrifuged. The cells were then exposed to to 0.1% Sigma® Triton-X in order to permeabilise the gastrointestinal cells and therefore release the internalised bacterium. A sample of this final solution was taken and plated onto Oxoid Columbia agar base with 5% defibrinated horse blood (CBA) plate. Colonies were then counted after a 24 hour period of incubation.

Results

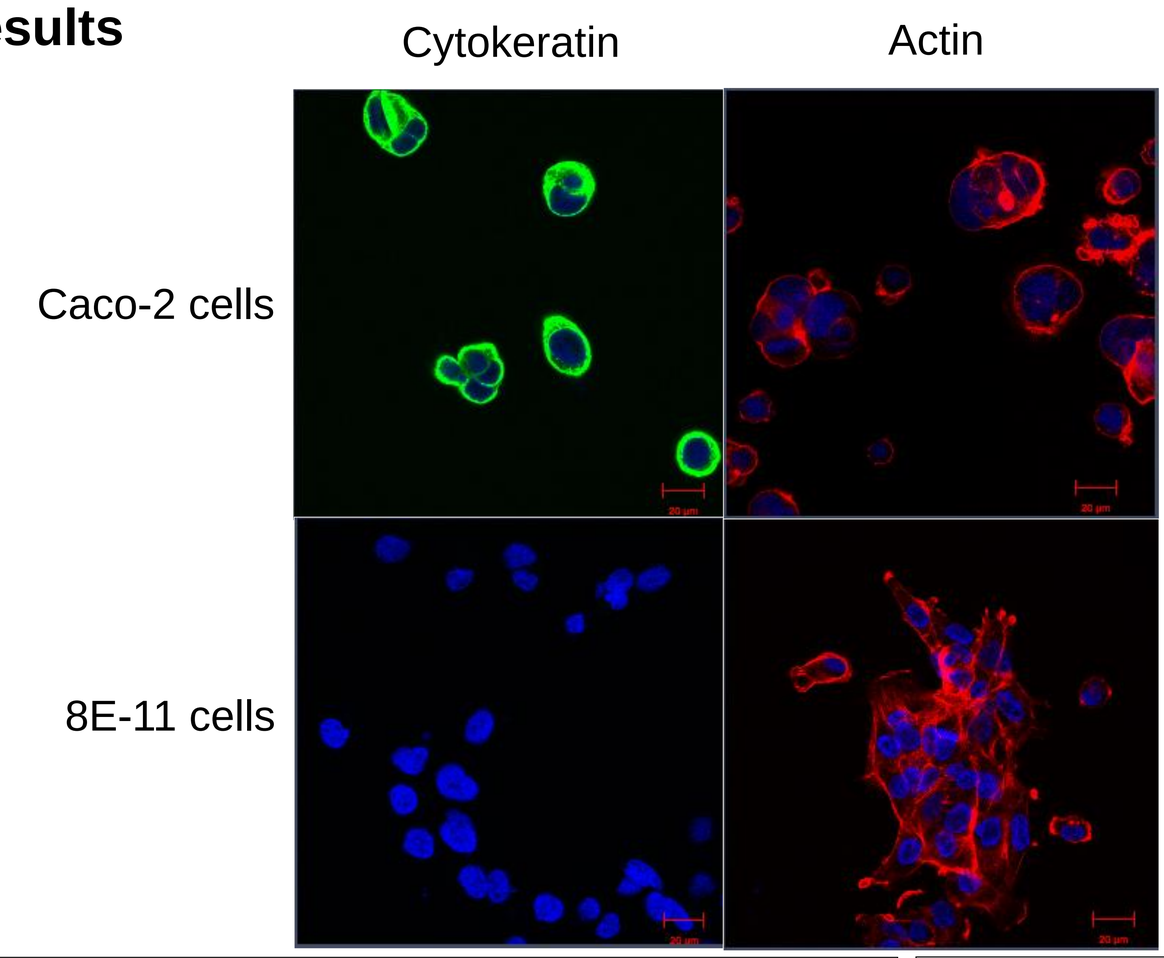

Figure 2: Confocal images of 8E-11 (chicken) and Caco-2 (human) cells.

From left to right (top row): Permeabilised Caco-2 cells stained with mouse pan antibody (ab86734) to Cytokeratin and Alexa Fluor® 488 goat anti-mouse IgG (H+L) (ab150113); Permeabilised Caco-2 cells stained with Phalloidin-iFluor 488 (ab176753).

From left to right (bottom row): Permeabilised 8E-11 cells stained with mouse pan antibody (ab86734) to Cytokeratin and Alexa Fluor® 488 goat anti-mouse IgG (H+L) (ab150113); Permeabilised 8E-11 cells stained with Phalloidin-iFluor 488 (ab176753).

All cells stained with Phalloidin-iFluor 488 (ab176753) are pseudo-coloured red.

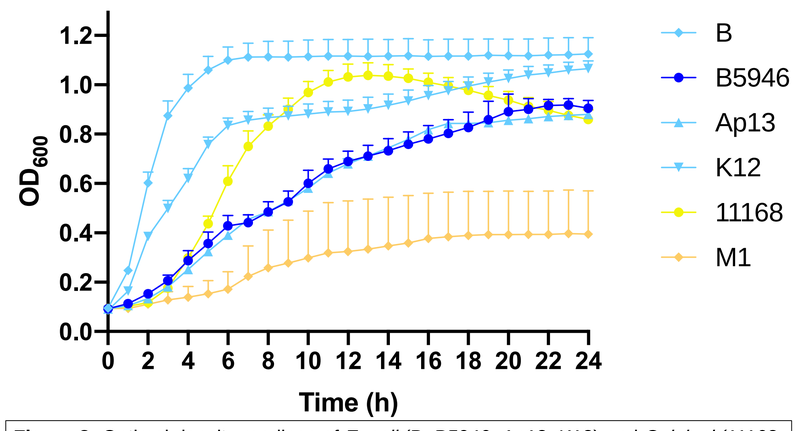

Figure 3: Optimal density readings of *E. coli* (B, B5946, Ap13, K12) and *C. jejuni* (11168, M1) taken at wavelength OD_{600}. Bacteria were grown in Lysogeny Broth over the 24 hour period under assay conditions.

Table 1: Minimal inhibition values for measuring the susceptibility (S) and resistance (R) of *E. coli* (B, B5946, Ap13, K12) and *C. jejuni* (11168, M1) to varying Gentamicin concentratons[9].

Species	Strain	Gentamicin Concentration (mg/ml)	Breakpoint
E. coli	B	N/A	R
	B5946	0.16	S
	Ap13	0.16	S
	K12	0.04	S
C. jejuni	11168	0.04	N/A
	M1	0.04	N/A

Table 2: Initial inoculate of bacteria in Gentamicin protection assays (n=3) calculated from starting counts taken at the time of dosing to gastrointestinal cells set to MacFarland standard.

	Low dose (CFU)		High dose (CFU)	
	11168	B5946	11168	B5946
Caco-2	11,800	252,500	23,600	505,000
8E-11	135,000	75,000	270,000	150,000

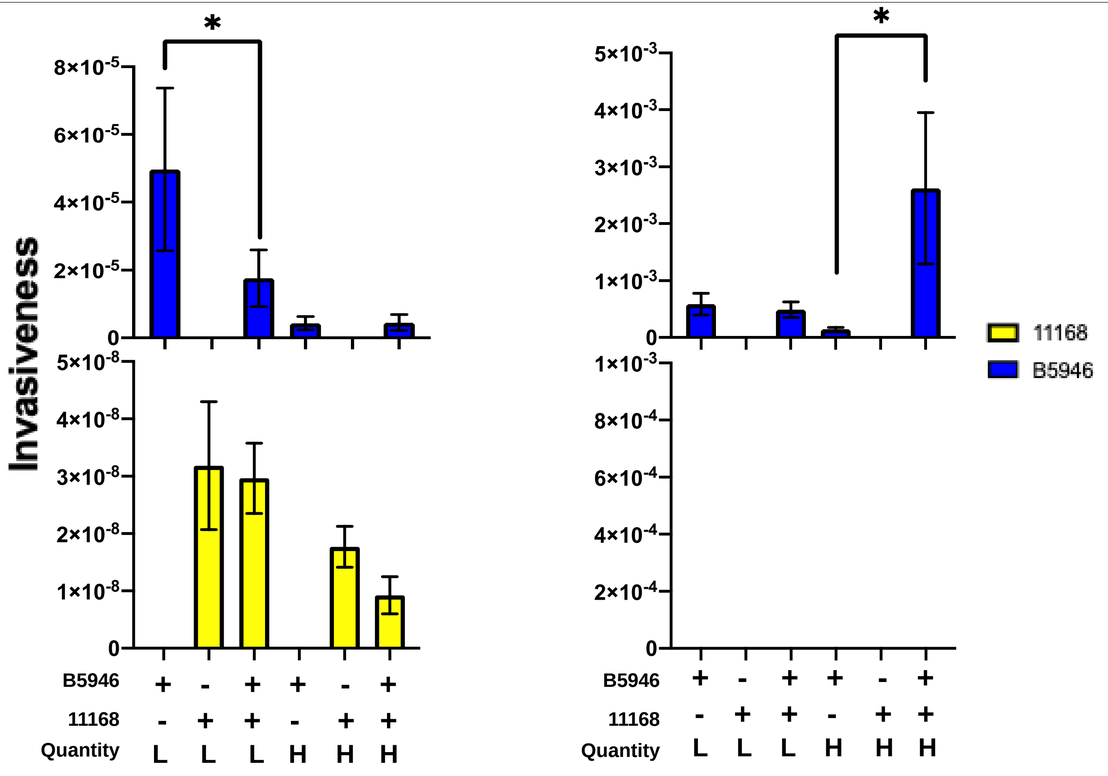

Figure 4: The invasiveness of B5946 and 11168 in 8E-11 (left) and Caco-2 (right) cells as determined by Gentamicin protection assay (n=3). The percentage of bacterial invasion has been corrected for the multiplicity of infection for both low (L) and high (H) bacterial dosage levels. A significance level of $p<0.05$ between groups is indicated by an asterisk.

Discussion

- The characterisation of 8E-11 and the lack of signalling for Cytokeratin outlines a key difference to that of an epithelial cell type.
- Caco-2 cells demonstrated greater resistance to the associated toxicity of 11168.
- Coinfection with *C. jejuni* and *E. coli* leads to differential effects in 8E-11 and Caco-2 cells: *E. coli* invasion of 8E-11 cells decreased in the presence of *C. jejuni*, while in Caco-2 cells *C. jejuni* appears to increase the invasive potential of *E. coli*.

Further work is needed to understand the underlying mechanism that leads to the differential responses in human and chicken gastrointestinal cells.

Literature cited

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