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Short communication

The viability of the genetically diverse *C. jejuni* and *C. coli* strains in the macrophage J774 cell line

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Abstract

The intracellular survival of *Campylobacter* has been described within epithelial cells as well as in macrophages *in vitro*. The goal of this study was to estimate the viability of the genetically diverse *C. jejuni* and *C. coli* strains in the macrophage J774 cell line. Strains selected for analysis differed with regard to the occurrence of genes encoding specific virulence factors. The present work indicates that was no correlation between the source of isolates and relative intracellular survival.

Key words: *Campylobacter*, macrophage J774 cell line

Introduction

Campylobacter jejuni is a zoonotic pathogen and the most common bacterial cause of food-borne diarrheal illness worldwide. *C. jejuni* has established a commensal relationship in the gastrointestinal tract of many animals, but in humans *Campylobacter* infection causes colitis and diarrhea (Coker et al. 2002). The mechanisms of pathogenesis are not yet well defined (Young et al. 2007). Little is known about the molecular mechanisms by which *C. jejuni* enter intestinal epithelial cells. However, *C. jejuni* are internalized by enterocytes in a microtubule-dependent, actin-independent fashion, suggesting that they employ an entry mechanism unlike those reported for other intestinal pathogens (Mixter et al. 2003, Smith et al. 2005, Watson and Galan 2008) *C. jejuni* is able to survive within enterocytes by deviating from the canonical endocytic pathway thus residing in the unique intracellular compartment that does not fuse with lysosomes (Day et al. 2000, Beltinger et. al. 2008, Pogacar et al. 2008). In contrast, in macrophages, *C. jejuni* is delivered to lysosomes and consequently killed (Watson and Galan 2008). This may provide evidence that *C. jejuni* has evolved specific adaptations to survive within host cells. The aim of this study was to establish if the differences in genetic profile of particular *Campylobacter* strains can affect or modify their survival within macrophages.

Materials and Methods

C. jejuni and *C. coli* strains selected for analysis differed with regard to genes encoding specific virulence factors and the presence of plasmids, as well as the phylogenetic group classification (Table 1). J774 macrophages were grown in tissue culture Petri dishes at 37°C in 5% CO_2 atmosphere. For all assays, 24-well tissue culture trays were seeded with $2x10^5$ cells per

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Table 1. Genotypic characterization of *C. jejuni* and *C. coli* strains.

	2p *C. coli*	5p *C. jejuni*	2s *C. jejuni*	12s *C. jejuni*	D1 *C. jejuni*	A4 *C. coli*	C27 *C. jejuni*	K1 *C. jejuni*	81116 *C. jejuni*
Sources	dog	dog	pig	pig	children	poultry	poultry	poultry	poultry
*fla*A	+	+	+	+	+	+	+	+	+
*fla*B	+	+		+	+	+	+	+	+
*cdt*A	–	+			+	+		+	+
*cdt*B	–	+	+		+	+	+	+	+
*cdt*C		+	+		+	+	+	+	+
*cdt*ABC		+			+	+		+	+
*vir*B11	+	+	+	+	+		+	+	
cj0588		+			+			+	+
plazmid pTet	+			+	+			+	
plazmid pVir	+		+	+	+		+	+	
*fla*A-typing*	F	VII	I	I	V	C	IV	VII	IX
*ADSRRS**	9Cc	4Cj	5Cj	11Cj	7Cj	4Cc	18Cj	9Cj	22Cj

* These data were presented as dendrograms in Krutkiewicz and Klimuszko (2010)

Table 2. Number of CFUx10^3/ml of *C. jejuni* and *C. coli* strains reisolated from murine macrophages J774 at different time points after infection.

Strains	1 h	3 h	8 h	12 h	24 h
2p *C. coli*	56.0 ± 10.0	7.50 ± 0.82	3.0 ± 0.12	0.60 ± 0.20	0.060 ± 0.003
5p *C. jejuni*	110.0 ± 27.0	6.40 ± 0.84	5.90 ± 1.60	1.50 ± 0.50	0.080 ± 0.025
2s *C. jejuni*	53.0 ± 3.10	36.0 ± 5.10	13.0 ± 4.90	4.30 ± 1.10	0
12s *C. jejuni*	110.0 ± 3.20	18.0 ± 2.20	1.90 ± 0.51	0.70 ± 0.11	0.180 ± 0.011
D1 *C. jejuni*	79.0 ± 32.0	48.0 ± 27.0	9.50 ± 0.05	6.30 ± 1.90	0
A4 *C. coli*	41.0 ± 6.20	25.0 ± 4.60	14.0 ± 5.90	6.0 ± 0.58	0
C27 *C. jejuni*	51.0 ± 19.0	17.0 ± 4.80	10.0 ± 1.40	6.20 ± 1.0	0
K1 *C. jejuni*	110.0 ± 19.0	37.0 ± 19.0	10.0 ± 19.0	4.50 ± 19.0	0
81116 *C. jejuni*	36.0 ± 6.30	8.30 ± 1.50	6.0 ± 1.0	0.44 ± 0.79	0.080 ± 0.003

Results were considered significant at $p \leq 0.025$

mL and incubated for 24 h. Density of bacterial suspension was determined spectrophotometrically and infection was carried out by inoculating macrophages with *C. jejuni* and *C. coli* at an approximate MOI of 100. Infected macrophage monolayers were incubated for 2h, then medium containing 50 µg/mL of gentamicin was added to kill extracellular bacteria. The invasion period was monitored for 1, 3, 8, 12 and 24 h post infection. Following each time point, the macrophages were lysed with cold, distilled water and the live bacteria released were evaluated by plating serial dilutions on blood agar plates. Simultaneously the number of live macrophages in the culture infected with the appropriate strain of bacteria was evaluated after 1, 3, 8, and 24 hours of incubation. The cells were centrifuged and resuspended in 1 mL of PBS. The cells were then suspended in 0.4% solution of trypan blue and counted in a Neubauers chamber.

Statistics

All values are reported as means ± SEM. Differences are considered statistically significant at $p \leq 0.05$.

Results and Discussion

Results indicated that *C. jejuni* and *C. coli* strains were able to survive within macrophages for over 24 h. After that time live bacteria were not found. Other authors have demonstrated that the time of survival for *Campylobacter* strains significantly differs from

24 to 72h. In the present data, a statistically significant decrease in the number of surviving bacteria was observed, beginning from the first hour after infection. The differences in the number of surviving bacteria were not related to the source of a particular strain. However, the biggest dissimilarity between the strains of different origin was observed 3h after infection 1.5 /C.27/ to 17.5 /12s/ times (Table 2). These results indicate that *Campylobacter* did not reproduce in phagocytic cells. The ability to survive and grow within macrophages is often considered as a determinant of microbial virulence and pathogenicity. In our studies the correlation between genetic profiles of *C. jejuni* and *C. coli*, with the special respect of genes encoding virulence factors, and time of survival within macrophages was not confirmed. Reduction of the survival rate of cultured macrophages did not correlate with the percentage of dead cells, which only insignificantly decreased over the study period. In spite of differences in the number of surviving bacteria, the number of macrophages that survived bacterial infection only insignificantly varied at different time points. In conclusion, we note that neither the presence of selected virulence genes nor the genetic profile of examined *Campylobacte*r spp. strains had an influence on the survival rate of these bacteria in murine J774 macrophage culture.

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References

Beltinger J, del Buono J, Skelly MM, Thornley J, Spiller RC, Stack WA, Hawkey CJ (**2008**) Disruption of colonic barrier function and induction of mediator release by strains of *Campylobacter jejuni* that invade epithelial cells. World J Gastroenterol 14: 7345- 7352.

Cooker AO, Isokpehi RD, Thomas BN, Amisu KO, Obi CL (**2002**) Human campylobacteriosis in developing countries. Emerg Infect Dis 8: 237-244.

Day WA Jr., Sajecki JL, Pitts TM, Joens LA (**2000**) Role of catalase in *Campylobacter jejuni* intracellular survival. Infect Immun 68: 6337-6345.

Krutkiewicz A, Klimuszko D (**2010**) Genotyping and PCR detection of potential virulence genes in *Campylobacter jejuni* and *Campylobacter coli* isolates from different sources in Poland. Folia Microbiol 55: 167-75.

Mixter PF, Klena JD, Flom GA, Siegesmund AM, Konkel ME (**2003**) In vivo tracking of *Campylobacter jejuni* by using a novel recombinant expressing green fluorescent protein. Appl Environ Microbiol 69: 2864-2874.

Pogacar M, Mihaljevic R, Klancnik A, Brumini G, Abram M, Mozina S (**2008**) Survival of stress exposed *Campylobacter jejuni* in the murine macrophage J774 cell line. Int J Food Microbiol 129: 68-73.

Smith CK, Kaiser P, Rothwell L, Humphrey T, Barrow PA, Jones MA (**2005**) *Campylobacter jejuni*-induced cytokine responses in avian cells. Infect Immun73: 2094-2100.

Watson RO, Galan JE (**2008**) *Campylobacter jejuni* survives within epithelial cells by avoiding delivery to lysosomes. PLoS Pathog 4: e 14

Young KT, Lindsay M.D, DiRita VJ (**2007**) *Campylobacter jejuni*: molecular biology and pathogenesis. Nat Rev Microbiol 5: 665-679.