STRAIN-SPECIFIC VIRULENCE SIGNATURES OF *CAMPYLOBACTER JEJUNI* ASSOCIATED WITH WATERY VERSUS BLOODY DIARRHEA IN THE NEONATAL PIGLET MODEL

Jennifer M. Bosquez[1], Ben Pascoe[2,3], Craig T. Parker[4], Kerry K. Cooper[1, 5*]

[1]School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ 85721, USA

[2]Ineos Oxford Institute for Antimicrobial Research, Department of Biology, University of Oxford, Oxford, United Kingdom

[3]Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand

[4]Produce Safety and Microbiology Research Unit, Agricultural Research Service, United States Department of Agriculture, Albany, CA 94710, USA

[5]BIO5 Institute, University of Arizona, Tucson, AZ 85719, USA

*Corresponding Author: Kerry K. Cooper, School of Animal and Comparative Biomedical Sciences, 1117 E. Lowell St., University of Arizona, Tucson, AZ, kcooper@arizona.edu, Phone: (520) 621-3342

Keywords: watery diarrhea, neonatal piglet model, inflammatory diarrhea, bloody diarrhea, virulence, phenotypes

Short title: Strain specific virulence signatures of *Campylobacter jejuni*

Abstract

Campylobacter jejuni is the leading cause of bacterial gastroenteritis globally, presenting with either watery or bloody/inflammatory diarrhea. Despite this clinical variability, the underlying mechanisms driving these distinct diarrheal manifestations remain unclear. The neonatal piglet model provides a unique opportunity to investigate these differences using strains with defined disease manifestations. We evaluated ten *C. jejuni* strains previously shown to cause consistent diarrheal manifestations in neonatal piglets: five strains associated with watery diarrhea and five associated with bloody/inflammatory diarrhea. The study found significant strain to strain variation among most assays, but overall strains associated with bloody/inflammatory diarrhea exhibited significantly higher levels of epithelial cell invasion, intracellular survival, macrophage survivability, and disruption of intestinal barrier integrity compared to watery diarrhea strains. Notably, both groups produced similar levels of cytolethal distending toxin (CDT), and elicited comparable IL-8 responses. While both groups facilitated translocation of commensal *E. coli*, only bloody/inflammatory strains caused increased paracellular permeability, as shown by TEER and FITC-dextran assays. Variation in adherence was observed between groups depending on the cell line used. This study reveals that there is significant variation in the virulence phenotype of *C. jejuni* strains and identifies several characteristics consistently associated with a specific diarrheal manifestation group. These findings provide new insights into the strain-specific pathogenic mechanisms of *C. jejuni*, with implications for understanding host-pathogen interactions and informing targeted diagnostic and therapeutic strategies.

Author Summary

Campylobacter jejuni is one of the most common causes of bacterial diarrhea worldwide, affecting millions each year. Infected individuals may experience either watery or bloody/inflammatory diarrhea, but the reasons why different strains cause different types of illness have remained a mystery. In this study, we used a neonatal piglet model, which accurately mimics human diarrheal disease, to investigate why certain *C. jejuni* strains cause more severe symptoms than others. We compared strains known to cause either watery or bloody diarrhea and found key differences in their ability to invade intestinal cells, survive inside immune cells, and damage the intestinal barrier. While both groups of strains could trigger inflammation and translocate *E. coli* through the intestinal lining, only the strains linked to bloody diarrhea caused significant barrier disruption and had increased intracellular survival. These findings reveal that different *C. jejuni* strains follow distinct paths during infection, which helps explain why they produce different symptoms. Understanding these differences could lead to better diagnostics, treatments, and possibly vaccines to reduce the burden of campylobacteriosis around the world.

67 **Introduction**

68 *Campylobacter jejuni* is the leading cause of bacterial gastroenteritis worldwide,
69 responsible for over 550 million cases annually[1]. In the United States alone, it affects more than
70 1.9 million people per year, imposing significant economic and public health burdens[2]. While
71 typically self-limiting, *C. jejuni* infection can lead to long-term sequelae such as post-infectious
72 irritable bowel syndrome (PI-IBS), reactive arthritis, and Guillain-Barré syndrome[3-5].
73 Increasing evidence also links *Campylobacter* to growth stunting in children from low- and
74 middle-income countries due to chronic enteric infections[6-8].

75 Clinically, *C. jejuni* infection presents as watery or bloody/inflammatory diarrhea, with up
76 to 27% of U.S. cases involving visible blood in stools[9]. Bloody/inflammatory diarrhea suggests
77 deeper intestinal invasion and inflammation, often associated with more severe outcomes[10].
78 Despite decades of reporting this clinical distinction, the mechanisms driving these divergent
79 diarrheal manifestations remain poorly understood. Several virulence factors have been proposed
80 to influence disease severity, including enterotoxin production, cytotoxins like cytolethal
81 distending toxin (CDT), and bacterial invasiveness[11-13]. However, no studies have definitively
82 linked these traits to specific diarrheal outcomes. Clinical data are often confounded by subjective
83 patient reporting and variable strain backgrounds, complicating direct comparisons. Additionally,
84 many *Campylobacter* studies rely heavily on just a few clinical *C. jejuni* strains, most commonly
85 NCTC11168, 81-176, or 81116[14, 15], which may unintentionally bias findings, given the well-
86 documented heterogeneity in virulence factors across *C. jejuni* strains virulence factors[16-18].

87 The neonatal piglet model provides a robust platform for studying *C. jejuni*-induced
88 diarrhea[19]. Unlike murine models, piglets exhibit human-like and can consistently replicate both
89 watery and bloody/inflammatory diarrheal manifestations following infection with specific *C.*

90 *jejuni* strains[20, 21]. Piglets are recognized as strong alternatives to both canines or non-human
91 primates for non-rodent evaluations that are required by regulatory agencies[22, 23] and have
92 intestinal functions physiologically similar to humans[24-26]. Utilization of this model allows for
93 controlled, strain-specific comparisons with minimized host variation. Healthy piglets typically do
94 not develop disease when exposed to *C. jejuni*[27]. However, colostrum-deprived neonatal piglets
95 consistently develop clinical symptoms such as large intestinal lesions and inflammation along
96 with diarrhea lasting up to six days depending on the *C. jejuni* strain used for infection[28].
97 Similarly, gnotobiotic piglets infected with *C. jejuni* also display symptoms of diarrhea and
98 sloughing of epithelial cells from the mucosa in the cecum and colon closely resembling human
99 campylobacteriosis pathologies[20, 29]. Despite these advantages, the use of piglets in *C. jejuni*
100 research remains limited due to the high costs and specialized housing requirements, leading many
101 researchers to favor more accessible models such as mice, ferrets, guinea pigs, or rats.
102 In this study, we leveraged the neonatal piglet model to investigate phenotypic differences between
103 *C. jejuni* strains that cause watery diarrhea compared to those that cause bloody/inflammatory
104 diarrhea. Using ten strains classified by the resultant diarrheal manifestation, we assessed virulence
105 traits including invasion, intracellular survival, epithelial barrier disruption, and
106 immunostimulation. Our goal was to evaluate these virulence and pathogenicity traits that may
107 relate to the differences in diarrheal presentation, advancing our understanding of *C. jejuni*
108 pathogenesis and informing new approaches to diagnosis or intervention.
109
110
111
112

113 **Materials and Methods**

114 **Bacterial Culturing and Growth**

115 *C. jejuni* strains used in this study (**Table 1**). All strains were grown and maintained on Mueller-
116 Hinton agar plates supplemented with 5% defibrinated bovine blood (MHB; Becton, Dickinson
117 and Company Difco) for 48 hours at 37°C with 10% CO_2. Strains for assays that required liquid
118 culture were grown in Mueller – Hinton broth (MH; Becton, Dickinson and Company Difco) with
119 constant shaking (100 rpm) for 16-18 hours at 37°C with 10% CO_2. Assay control strains
120 *Escherichia coli* DH5α and *Salmonella enterica* subspecies *enterica* serovar Typhimurium str.
121 RM6835 were both grown aerobically either on Luria – Bertani (LB) agar (Fisher Bioreagents) at
122 37°C for 24 hours or in LB broth (Fisher Bioreagents) with constant shaking (100 rpm) at 37°C
123 for 24 hours. For selective isolation of *Campylobacter* in select assays, *C. jejuni* strains were
124 cultured on Campy CEFEX agar, a modified MH agar with the addition of vancomycin (20 mg/L),
125 polymyxin B (0.35 mg/L), trimethoprim (26.4 mg/L), sodium cefoperazone (20 mg/L) and
126 amphotericin B (10 mg/L).

127

128

129

130

131

132

133

134

135

136 **Table 1.** *Campylobacter jejuni* strains utilized in this study

Strain	Source	Sequence Type (ST)	Clonal Complex	Diarrheal Manifestation
NCTC11168	Clinical	ST43	ST-21	Bloody and/or Inflammatory
81-176	Clinical	ST604	ST-42	Bloody and/or Inflammatory
M129	Clinical	ST353	ST-353	Bloody and/or Inflammatory
A9a	Poultry	ST2827	ST-460	Bloody and/or Inflammatory
D33a	Poultry	ST459	ST-42	Bloody and/or Inflammatory
RM1221	Poultry	ST354	ST-354	Watery
S3	Poultry	ST354	ST-354	Watery
D42a	Poultry	ST21	ST-21	Watery
A14a	Poultry	ST1212	ST-607	Watery
A13a	Poultry	ST1212	ST-607	Watery

137

138

139

140

141 **Cytolethal Distending Toxin (CDT) Assay**

142 CDT activity was measured using a modified protocol from Abuoun et al[30]. Briefly, *C. jejuni*
143 strains were grown overnight on MHB plates at either 37°C or 42°C under microaerobic conditions
144 (10% CO_2). Bacterial growth was harvested by resuspending the entire plate in 5 ml of Brucella
145 broth (Becton, Dickinson and Company, BBL). The suspension was centrifuged at 10,000 × g for
146 20 minutes at 4°C. The supernatant was discarded, and the bacterial pellet was resuspended in 3
147 ml of Eagle's Minimum Essential Medium (EMEM; Corning) supplemented with 10% fetal
148 bovine serum (FBS; Corning). The OD_{600} of the suspension was adjusted to 1.0 using EMEM +
149 10% FBS. Lysates were prepared by sonicating the suspension on ice using a QSonica sonicator
150 (20-second pulses, 20-second rests, five cycles total). The lysate was centrifuged at 10,000 × g for
151 20 minutes at 4°C, and the supernatant was filter-sterilized using a 0.22 µm syringe filter (Fisher
152 Scientific) to remove viable bacteria. Sterile lysates were serially diluted two-fold (1:1 to 1:128)
153 in EMEM + 10% FBS. HeLa cells (ATCC CCL-2) were seeded into 96-well plates at 2×10^4
154 cells/ml in 100 µl of EMEM + 10% FBS and incubated for 3 hours at 37°C with 5% CO_2.
155 Subsequently, 100 µl of each dilution of filtered cell lysate was added to the HeLa cells. Plates
156 were incubated at 37°C with 5% CO_2 for five days. Following incubation, cells were fixed with
157 10% formalin and stained with 0.8% crystal violet. The CDT titer was defined as the reciprocal of
158 the highest dilution showing >50% cell distension. *E. coli* DH5α was used as a negative control.
159 Each strain was tested in three biologically independent assays (n = 3).

160

161 **Attachment Assays**

162 Attachment was assessed using three epithelial cell lines: non-polarized INT407 (ATCC CCL-6),
163 and polarized Caco-2 BBE (ATCC HTB-37) and T84 (ATCC CCL-248) cells. Cell lines were

164 seeded in 24-well tissue culture-treated plates (1 ml per well at 1×10^6 cells/ml) and incubated for
165 18 hours at 37°C with 5% CO_2. *Campylobacter* cultures were grown overnight in MH broth with
166 shaking (100 rpm) at 37°C in 10% CO_2. The OD_{600} was adjusted to 0.1 in the appropriate medium
167 for each cell line: EMEM + 10% FBS for INT407, EMEM + 20% FBS for Caco-2 BBE, and
168 DMEM/F-12 + 5% FBS for T84. Each well was washed twice with sterile PBS, then inoculated
169 with 1 ml of *C. jejuni* inoculum (approximately 1×10^8 CFU, multiplicity of infection (MOI) 100).
170 To enhance bacterial contact with host cells, plates were centrifuged at 600 × g for 5 minutes then
171 incubated for 1 hour at 37°C with 5% CO_2. Wells were then washed twice with PBS to remove
172 non-adherent bacteria. Host cells were lysed by adding 1 ml of 0.1% Triton X-100 in PBS and
173 incubating for 10 minutes at room temperature. Lysates were serially diluted and plated on MHB
174 agar for CFU enumeration after incubation at 37°C in 10% CO_2 for 48 hours. Attachment
175 percentage was calculated as: (CFU recovered / CFU inoculated) × 100. Each strain was tested in
176 technical duplicates in three independent experiments (n = 3). Negative controls included two
177 wells inoculated with only sterile PBS. *E. coli* DH5α was used as a positive control. Due to
178 insufficient T84 cell growth, *C. jejuni* strain 81-176 was unable to be included in attachment assays
179 using this line.
180
181 **Invasion assays**
182 Enterocyte invasion was assessed using INT407, Caco-2 BBE, and T84 cells, following the same
183 seeding and infection conditions described for attachment assays. After overnight growth of *C.*
184 *jejuni* in MH broth at 37°C with 10% CO_2 and shaking (100 rpm), cultures were diluted to OD_{600}
185 = 0.1 in the appropriate medium for each cell line to achieve a MOI of 100. Each well of the 24-
186 well plates was washed twice with PBS and then inoculated with 1 ml of the bacterial suspension.

187 Plates were centrifuged at 600 × g for 5 minutes and incubated for 3 hours at 37°C with 5% CO_2
188 to allow for bacterial invasion. Following incubation, wells were washed twice with PBS, and 1
189 ml of cell line-specific medium containing gentamicin (250 µg/ml; Acros Organics) was added to
190 each well. Plates were incubated for an additional hour to eliminate extracellular bacteria. After
191 gentamicin treatment, wells were washed twice with PBS and then lysed with 1 ml of 0.1% Triton
192 X-100 in PBS. The lysates were serially diluted and plated on MHB agar, which was incubated at
193 37°C with 10% CO_2 for 48 hours. The percent invasion was calculated as: (CFU recovered after
194 gentamicin / CFU in initial inoculum) × 100. Each strain was assessed in technical duplicates in
195 three biologically independent experiments (n = 3). *Salmonella* Typhimurium strain RM6835 was
196 used as a positive control, and *E. coli* DH5α as a negative control. Every assay included two
197 uninoculated wells and were processed exactly as the positive and negative controls. Due to
198 insufficient T84 cell growth, *C. jejuni* strain 81-176 was unable to be included in invasion assays
199 using this cell line.
200
201 **Macrophage Survivability**
202 The ability of the different *C. jejuni* strains to survive intracellularly in macrophages was assessed
203 using J774A.1 mouse macrophages (ATCC TIB-67). Cells were seeded into 24-well plates at 1 ×
204 10^6 cells/ml in 1 ml per well and incubated for 18 hours at 37°C with 5% CO_2 to establish
205 monolayers. Overnight MH broth cultures of each *C. jejuni* strain were prepared, adjusted to OD_{600}
206 = 0.1 (~1 × 10^8 CFU/ml) in DMEM + 10% FBS (J7774A.1 culture media), and added to each well
207 at an MOI of 100. Two wells were left uninoculated as negative controls. Plates were incubated at
208 37°C with 5% CO_2 for 24, 48, or 72 hours. At each time point, wells were washed twice with PBS
209 and then treated with 1 ml of gentamicin (250 µg/ml in DMEM + 10% FBS) for 1 hour to eliminate

210 extracellular bacteria. After treatment, wells were washed again, and macrophages were lysed by
211 adding 1 ml of 0.1% Triton X-100 in PBS. Lysates were serially diluted and plated on MHB agar,
212 incubated at 37°C with 10% CO_2 for 48 hours, and colonies enumerated. Each strain was tested in
213 two technical replicates across three independent biological assays (n=3). *C. jejuni* strain 81-176
214 was included as a positive control due to its known interaction with macrophages[31].
215
216 **IL-8 ELISA and 24-hour Intracellular Survivability**
217 T84 cells were seeded into 24-well plates at 5.5×10^5 cells/well and cultured for approximately 7
218 days until reaching 100% confluence. *C. jejuni* strains were grown overnight in MH broth and
219 diluted to $OD_{600} = 0.1$ in DMEM/F-12 with 5% FBS (T84 medium). Each well was infected with
220 1 ml of bacterial suspension (~1×10^8 CFU, MOI 100) and incubated at 37°C with 5% CO_2 for 3
221 hours. Following infection, wells were washed with PBS and treated with 1 ml of gentamicin (250
222 µg/ml in T84 medium) for 1 hour. Wells were then washed again, fresh medium was added, and
223 plates were incubated for 24 hours at 37°C with 5% CO_2. After 24 hours, media from each well
224 was collected, centrifuged at 12,000 × g for 1 minute, and the supernatant stored at –80°C for
225 cytokine analysis. Human IL-8 levels were measured using the BD OptEIA™ ELISA kit (BD
226 Biosciences) per the manufacturer's instructions. To assess intracellular bacterial survival,
227 epithelial cells were lysed after supernatant removal by adding 1 ml of 0.1% Triton X-100 in PBS.
228 Lysates were serially diluted and plated on MHB agar. Plates were incubated at 37°C in 10% CO_2
229 for 48 hours, and colonies were counted. Each strain was evaluated in two technical replicates per
230 assay, with three independent biological replicates (n = 3). Due to insufficient T84 cell growth, *C.*
231 *jejuni* strain 81-176 was unable to be included in IL-8 and intracellular assays using this cell line.
232

233 **Transepithelial Electrical Resistance (TEER)**

234 To evaluate epithelial barrier integrity, transepithelial electrical resistance (TEER) was measured
235 using Caco-2 BBE cells. Cells were seeded at 2×10^5 cells per well into 0.4 µm pore-size Transwell
236 inserts (Falcon, Corning) placed in 24-well tissue culture-treated plates. Cells were cultured in
237 EMEM with 20% FBS at 37°C with 5% CO_2 until a confluent monolayer formed (TEER > 250
238 $\Omega \cdot cm^2$), typically by day 14. Monolayer integrity was assessed microscopically and with an
239 EVOM2 volt/ohm meter (World Precision Instruments). Following confluence, cells were
240 maintained for an additional 120 hours to promote tight junction maturation. Prior to infection,
241 TEER was recorded, and a cell count was performed using a hemocytometer to calculate the
242 bacterial concentration needed for MOI 100. *C. jejuni* cultures were prepared by growing strains
243 overnight in MH broth, adjusting to $OD_{600} = 0.1$, and diluting in Caco-2 BBE medium. From each
244 Transwell, 50 µl of media was removed from the apical compartment and replaced with 50 µl of
245 the corresponding *C. jejuni* inoculum (~5×10^6 CFU; MOI 100). Each strain was tested in duplicate
246 Transwells, with two uninoculated wells serving as negative controls (sterile medium only).
247 Infected plates were incubated at 37°C with 5% CO_2 for 72 hours. TEER measurements were taken
248 at 24, 48, and 72 hours using Millicell ERS-2 electrodes (Millipore). Electrodes were disinfected
249 and rinsed between each well using the following sequence: 10% bleach, sterile water (×2), 70%
250 ethanol, sterile water (×2), and pre-warmed media. Each *C. jejuni* strain was tested in duplicates
251 during three independent biological experiments (n = 3).

252

253 **FITC-Dextran Translocation**

254 To assess paracellular permeability to small molecules, a 4-kDa fluorescein isothiocyanate (FITC)-
255 dextran probe (Millipore Sigma) was applied to mature Caco-2 BBE monolayers grown on 0.4 µm

256 Transwell inserts in phenol red-free MEM (Gibco). Cells were seeded at 2×10^5 cells per insert
257 and cultured to confluence (TEER > 250 Ω·cm²), then maintained for an additional 120 hours to
258 allow tight junction maturation. Overnight *C. jejuni* cultures were adjusted to $OD_{600} = 0.1$ (~$5 \times$
259 10^6 CFU) and used to inoculate the apical compartments (50 µl per insert, MOI 100). Two wells
260 were left as negative controls (sterile medium only). After 72 hours of incubation at 37°C with 5%
261 CO_2, 100 µl of media was removed from the apical compartment and replaced with 100 µl of 4-
262 kDa FITC-dextran solution (10 mg/ml in PBS). Plates were incubated for 3 hours, then 100 µl was
263 collected from the basolateral compartment and transferred to a black walled 96-well plate for
264 fluorescence measurement. Fluorescence was measured using a SpectraMax M2 plate reader
265 (Molecular Devices) with excitation at 490 nm and emission at 530 nm. Relative fluorescence
266 units (RFUs) were blanked against sterile Caco-2 BBE medium. Each *C. jejuni* strain was tested
267 in three biological experiments with two technical replicates each (n = 3).
268
269 **Commensal Bacteria Translocation**
270 The ability of *C. jejuni* to disrupt the epithelial barrier and promote translocation of non-invasive
271 commensal bacteria was assessed using *E. coli* DH5α as a model commensal. Two experimental
272 approaches were performed: (1) a 6-hour co-inoculation and (2) a 72-hour pre-infection with *C.*
273 *jejuni* followed by 3-hour *E. coli* exposure. For both approaches, Caco-2 BBE cells were seeded
274 at 1.5×10^5 cells/ml into 3 µm pore-size Transwell inserts and cultured to confluence (TEER >
275 250 Ω·cm²), with daily monitoring of TEER.
276 ***6-hour co-culture:*** Overnight cultures of *C. jejuni* and *E. coli* DH5α were grown and adjusted to
277 $OD_{600} = 0.1$ (~5×10^6 CFU). The apical compartment was inoculated with 50 µl of each strain
278 (MOI 100 per organism). After 6 hours of incubation, media from the basal compartment was

collected and plated on MacConkey agar for *E. coli* and CEFEX agar for *C. jejuni*. CEFEX agar was incubated at 37°C with 10% CO_2 for 48 hours; MacConkey plates were incubated aerobically at 37°C for 24 hours. Each strain was tested in three independent biological experiments with two technical replicates each (n = 3).

72-hour pre-infection + 3-hour E. coli exposure: Caco-2 BBE cells were infected with *C. jejuni* as described for the TEER assay. After 72 hours, 50 µl of *E. coli* DH5α (MOI 100) was added to the apical compartment and incubated for an additional 3 hours. Basolateral media was collected and plated as described above. Each strain was tested in three independent biological experiments with two technical replicates per experiment (n = 3). To assess intracellular *C. jejuni* and *E. coli*, the apical compartment was treated with gentamicin (250 µg/ml) for 1 hour, washed, and lysed with 0.1% Triton X-100. Lysates were plated on CEFEX and MacConkey agar for quantification. Translocation efficiency was calculated as: (CFU in basal compartment / CFU inoculated) × 100.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism (v10.4.1) for comparisons between diarrheal manifestations or RStudio (v2022.07.1) for comparisons between strains. All data were tested for normality (Shapiro–Wilk test) and equal variance (Levene's test). For comparisons between strains associated with watery and bloody/inflammatory diarrhea (n = 15 per group), the non-parametric Mann–Whitney U test was applied. Statistical significance was defined as $p \leq 0.05$. For comparisons across multiple strains or conditions, non-parametric Kruskal–Wallis tests with Dunn's post hoc test were used. Compact Letter Display (CLD) notation was used to visualize the results of the pairwise comparisons. Strains that do not share a letter are significantly different from each other ($p < 0.05$), while strains sharing the same letter are not significantly different.

302 Letters were assigned using the multcompView package in R, based on adjusted p-values.
303 Statistical significance was defined as $p \leq 0.05$. Each assay included a minimum of three
304 independent biological replicates per strain (n = 3 per group), with technical replicates as noted.
305 Data are presented as mean ± SEM unless otherwise stated.
306

307 **Results**

308 ***C. jejuni strains associated with bloody/inflammatory diarrhea exhibit increased invasion and***
309 ***intracellular survival in epithelial cells.*** To assess whether *C. jejuni* strains associated with
310 distinct diarrheal phenotypes exhibit differences in virulence potential both on a strain-to-strain
311 level and between groups, we compared five strains associated with watery diarrhea and five
312 associated with bloody/inflammatory diarrhea (**Table 1**). All ten strains have previously been used
313 in the neonatal piglet model to confirm their diarrheal manifestations *in vivo* (**Fig 1A-B**). Invasion
314 assays were conducted using INT407, Caco-2 BBE and T84 cells. Significant differences were
315 observed between several strains, notably, A9a was a significantly better invader of all three cell
316 lines than all other nine strains (Note: 81-176 was not tested with T84 cells), whereas all five of
317 the watery diarrhea associated strains were poor invaders regardless of the cell line tested (**Fig 2A,**
318 **Supplementary Fig S1A, Supplementary Fig S2A**). Invasion assays conducted in polarized
319 Caco-2 BBE cell revealed significantly greater invasion by bloody/inflammatory associated strains
320 compared to watery diarrhea strains ($p < 0.0001$; **Fig 2B**) Similar trends were observed using non-
321 polarized INT407 cells ($p = < 0.0001$; **Supplementary Fig S1B**) and T84 cells ($p = 0.0035$;
322 **Supplementary Fig S2B**).

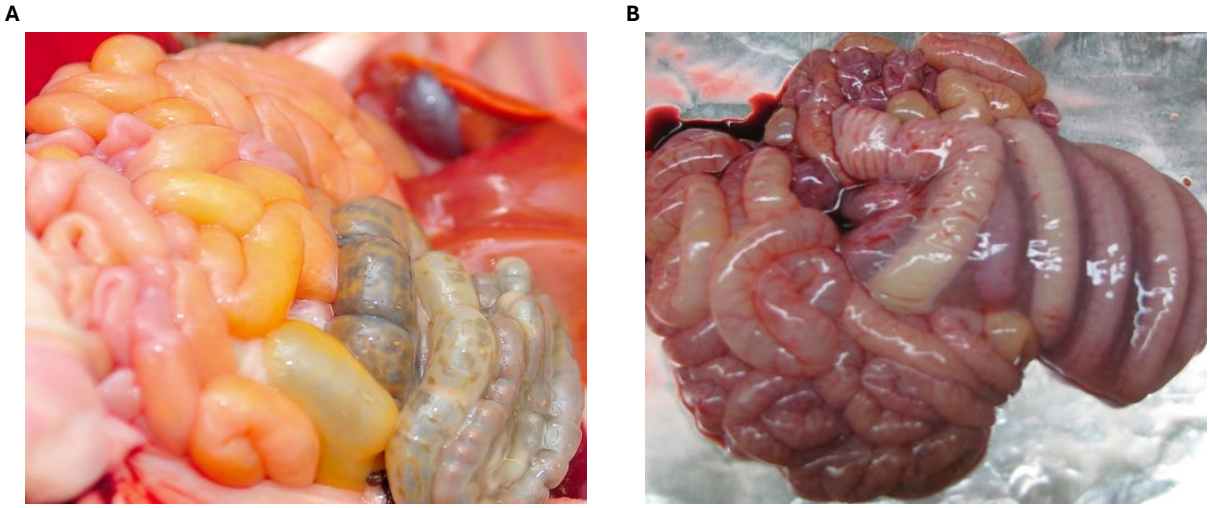

Fig 1. Gross pathology associated with different diarrheal outcome caused by different strains of *Campylobacter jejuni* based on previous challenge in colostrum deprived neonatal piglet model.

(A) Gross pathology of the intestinal tract of neonatal piglets infected with *C. jejuni* strains associated with watery diarrhea manifestation. (B) Gross pathology of the intestinal tract of neonatal piglets infected with *C. jejuni* strains associated with bloody and/or inflammatory diarrhea manifestation. Piglet studies were conducted prior to this study and were described previously in Law et al and Cooper et al[21, 32].

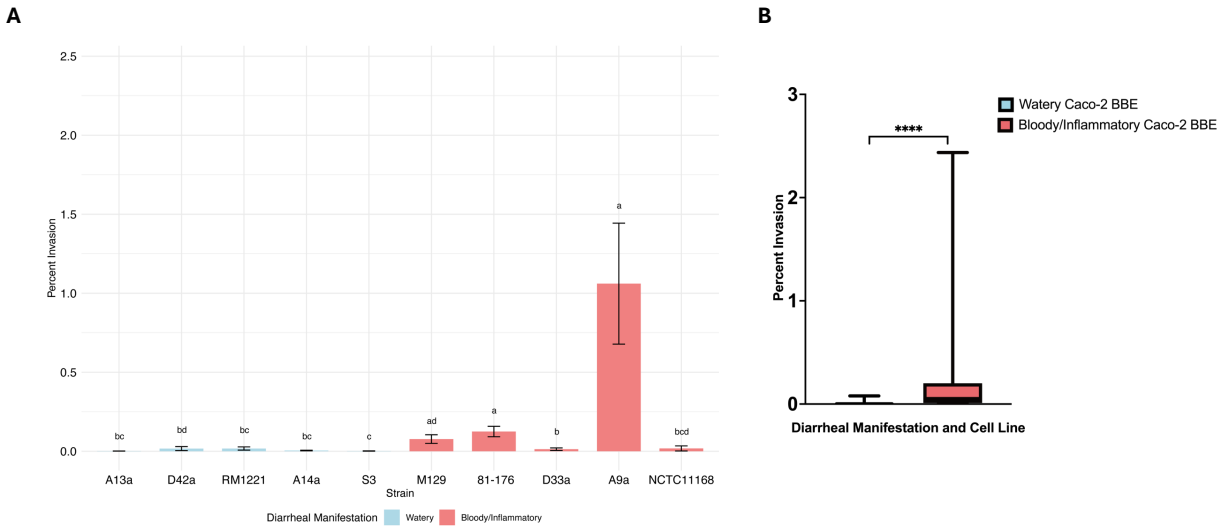

Fig 2. Average percent invasion of intestinal epithelial cell line Caco-2 BBE.

The capacities of all ten *C. jejuni* strains to invade Caco-2 BBE cells at a multiplicity of infection of 100 were examined *in vitro*. Invasiveness is expressed as the percentage of bacteria which had invaded cultured intestinal epithelial cells following a 3-hour incubation and 1-hour gentamicin exposure, compared to the inoculum. (A) Invasion across all ten *C. jejuni* strains. Bars labeled with different letters indicate statistically significant differences ($p < 0.05$) based on Dunn's post hoc test following a Kruskal-Wallis analysis. Shared letters denote groups that are not significantly different from each other. (B) Invasiveness of Caco-2 BBE cells by *C. jejuni* strains grouped by diarrheal manifestation. There was a significant difference between watery and bloody/inflammatory diarrhea associated strains and their invasion of Caco-2 BBE cells (****, $p < 0.0001$). Invasion was calculated from three biologically independent assays with two technical replicates per strain ($n = 15$).

352 To evaluate whether increased invasion correlated with improved intracellular persistence,
353 we measured viable intracellular bacteria at 24 hours post-infection in T84 cells. Strain to strain
354 variation was observed among bloody/inflammatory strains M129 and A9a, where they displayed
355 significantly higher intracellular survival than all other strains (**Fig 3A**). As a group,
356 bloody/inflammatory diarrhea associated strains exhibited significantly higher intracellular
357 survival than watery diarrhea associated strains ($p = 0.0007$; **Fig 3B**).
358
359
360
361
362
363
364
365
366
367

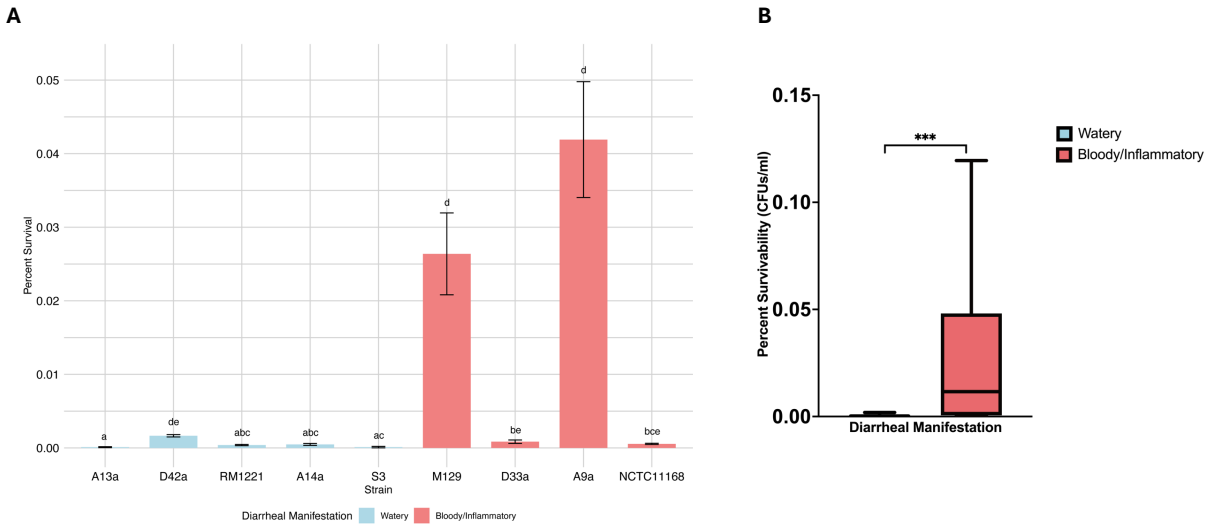

Fig 3. Intracellular survival over 24 hours in T84 cells.

The ability to persist within T84 cells at a multiplicity of infection of 100 over 24-hours was examined *in vitro*. (A) Average percent survival within T84 cells by strain. Bars labeled with different letters indicate statistically significant differences ($p < 0.05$) based on Dunn's post hoc test following a Kruskal-Wallis analysis ($n = 3$). Shared letters denote groups that are not significantly different from each other. (B) Percent T84 24-hour intracellular survival by *C. jejuni* strains grouped by diarrheal manifestation. There was a significant difference between watery and bloody/inflammatory diarrhea associated strains (***, $p = 0.0007$). Average 24-hour intracellular survival was calculated from three biologically independent assays with two technical replicates ($n = 12$). Strain 81-176 is not included in this data set.

381 ***Differential attachment of C. jejuni strains across intestinal cell lines.*** To determine whether
382 differences in invasion were due to upstream differences in adherence, we assessed attachment
383 efficiency after a 1-hour infection of epithelial cell lines INT407, Caco-2 BBE and T84. Significant
384 differences were observed between strains across INT407 (**Fig 4A**) and Caco-2 BBE cells (**Fig**
385 **4C**) but not T84 cells (**Supplementary Fig S3A**). There were significant differences in attachment
386 between watery and bloody/inflammatory diarrhea associated strains, but the differences depended
387 on the cell line used. Bloody/inflammatory associated strains adhered significantly better to the
388 non-polarized INT407 cells (p = 0.028; **Fig 4B**), whereas watery diarrhea associated strains
389 adhered significantly better to the polarized Caco-2 BBE (p = 0.023; **Fig 4D**), while there was no
390 significant difference between the groups with polarized T84 cells (p = 0.93; **Supplementary Fig**
391 **S3B**).

392

393 ***CDT production does not distinguish between watery and bloody/inflammatory strains.*** All *C.*
394 *jejuni* strains in this study produced detectable CDT activity, as measured by the HeLa cell
395 distension assay. We did not observe any strain variation in CDT production between watery and
396 bloody/inflammatory diarrhea associated strains at either 37°C or 42°C (**Supplementary Fig**
397 **S4A)**. When compared as groups at 37°C, mean CDT titers were comparable between watery
398 (geometric mean = 0.094) and bloody/inflammatory diarrhea associated strains (geometric mean
399 = 0.086; p = 0.74; **Supplementary Fig S4B**). Due to the commensal relationship between poultry
400 and *C. jejuni*, CDT production was also measured at the poultry internal body temperature of 42°C
401 and here mean CDT titers were different between watery (geometric mean = 0.10) and
402 bloody/inflammatory strains but not statistically significant (geometric mean = 0.041; p = 0.086;
403 **Supplementary Fig S4B**).

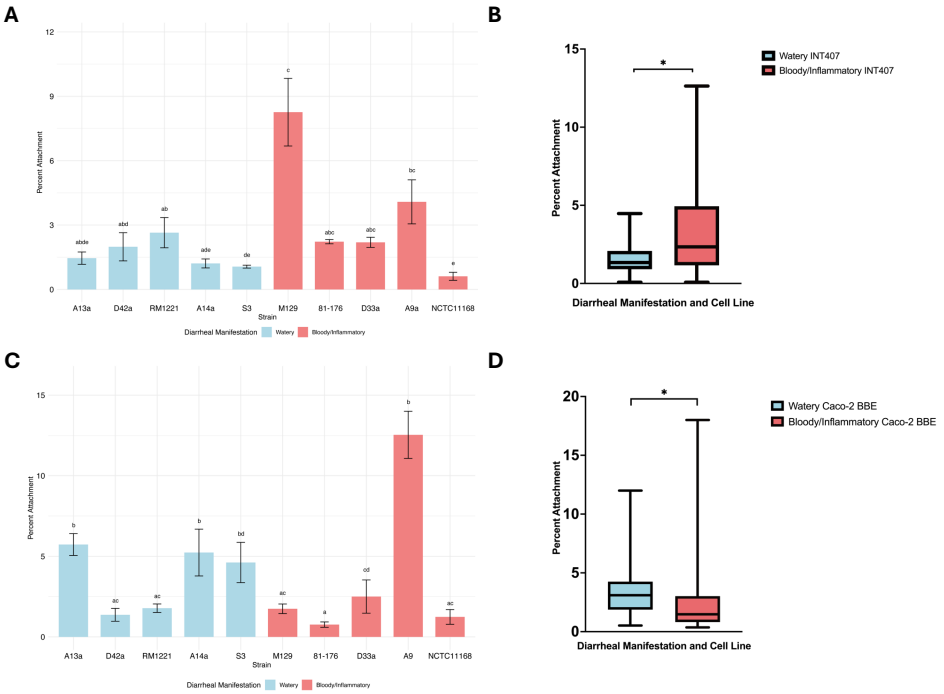

Fig 4. Adherence to intestinal epithelial cell lines .

Adherence is expressed as the percentage of bacteria which had either adhered to or invaded cultured intestinal epithelial cells following a 1-hour incubation, compared to the inoculum. (A) Adherence of each *C. jejuni* strain to INT407 cells. Bars labeled with different letters indicate statistically significant differences ($p < 0.05$) based on Dunn's post hoc test following a Kruskal-Wallis analysis ($n = 3$). Shared letters denote groups that are not significantly different from each other. (B) Adherence to INT407 cell by *C. jejuni* strains grouped by diarrheal manifestation. There was a significant difference between watery and bloody/inflammatory diarrhea associated strains and their attachment to INT407 cells (*, $p = 0.028$). Average percent attachment was calculated from three biologically independent assays with two technical replicates ($n = 15$). (C) Adherence of each *C. jejuni* strain to Caco-2 BBE cells ($n = 3$). (D) Adherence to Caco-2 BBE cells by *C. jejuni* strains grouped by diarrheal manifestation. There was a significant difference between watery and bloody/inflammatory diarrhea associated strains and their attachment to Caco-2 BBE

418 cells (*, p = 0.023). Average percent attachment was calculated from three biologically
419 independent assays with two technical replicates (n = 15).
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440

441 ***Bloody/inflammatory strains exhibit greater 24-hour intracellular survival in T84 cells but***
442 ***induce similar IL-8 responses.*** To evaluate whether differences in intracellular persistence
443 affected epithelial inflammatory signaling, we quantified human IL-8 production in T84 cell
444 supernatants at 24 hours post-infection and measured viable intracellular *C. jejuni* following
445 gentamicin treatment and lysis. IL-8 production was significantly higher among strains M129 and
446 A9a compared to strains D33a, S3, A14a, and A13a (**Supplementary Fig S5A**). Strain M129
447 induced the highest IL-8 levels (141.2 pg/ml), whereas S3 (a watery strain) produced the lowest
448 (62.54 pg/ml), highlighting strain-level variability. Strains associated with bloody/inflammatory
449 diarrhea demonstrated significantly greater intracellular survival at 24 hours than watery diarrhea
450 strains ($p = 0.0007$; **Fig 3B**). Despite this, IL-8 secretion was not significantly different between
451 groups: mean concentrations were 80.92 pg/ml for watery strains and 78.33 pg/ml for
452 bloody/inflammatory strains ($p = 0.19$; **Supplementary Fig S5B**).
453
454 ***Bloody/inflammatory strains more profoundly disrupt epithelial barrier function over time.*** To
455 assess whether *C. jejuni* strains differ in their capacity to disrupt epithelial barrier integrity, we
456 measured TEER in polarized Caco-2 BBE monolayers at 24-, 48-, and 72-hours post-infection.
457 Across 24-,48- and 72-hours, strains A14a, A13a and S3 had the lowest changes in TEER while
458 strains A9a, M129 and 81-176 had the highest changes in TEER (**Fig 5A**). When grouped by
459 diarrheal manifestation, bloody/inflammatory diarrhea associated strains caused a significantly
460 greater reduction in TEER at all time points. At 24 hours, TEER decreased by 15.6% compared to
461 4.03% in watery diarrhea associated strains ($p < 0.0001$; **Fig 5B**). Differences remained significant
462 at 48 hours (27.1% verses 10.7%; $p < 0.0001$) and 72 hours (41.7% verses 18.3%; $p < 0.0001$).

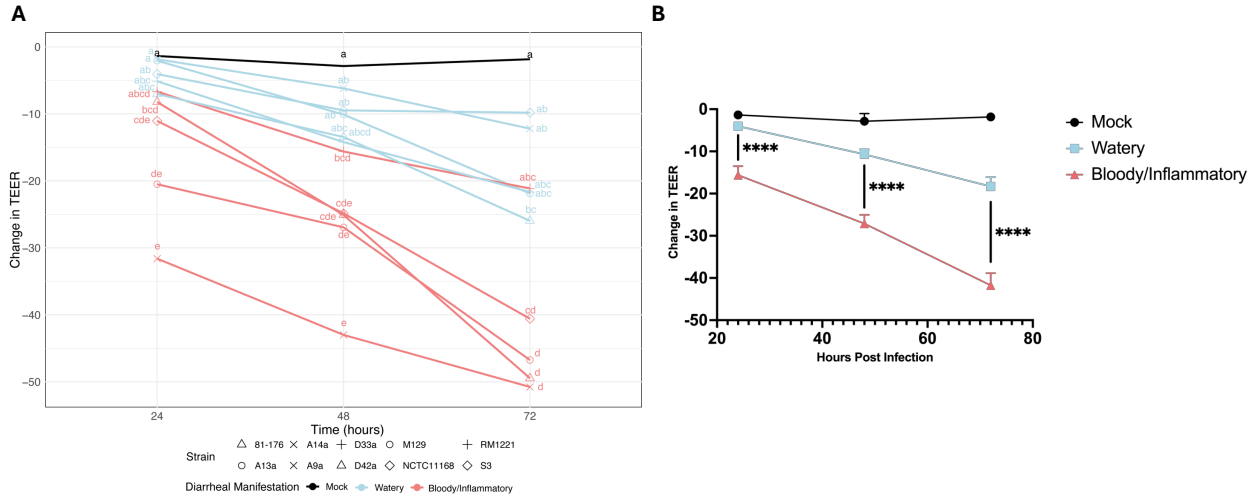

Fig 5. Change in the transepithelial electrical resistance (TEER) over 24, 48 and 72 hours. Change in TEER was calculated based on the initial pre-infection TEER for each strain. (A) Change in TEER for each *C. jejuni* strain over 24-, 48- and 72-hours. Those labeled with different letters indicate statistically significant differences ($p < 0.05$) based on Dunn's post hoc test following a Kruskal-Wallis analysis ($n = 3$). Shared letters denote groups that are not significantly different from each other. (B) Change in TEER across *C. jejuni* strains grouped by diarrheal manifestation. There was a significant difference between the watery and bloody/inflammatory diarrhea associated strains at 24 hours (****, $p < 0.0001$) 48 hours (****, $p < 0.0001$) and at 72 hours (****, $p < 0.0001$). Average change in TEER was calculated from three biologically independent assays with two technical replicates ($n = 15$). Mock infection represents Caco-2 BBE cells that contained only sterile cell culture media.

476 ***Bloody/inflammatory strains increase paracellular permeability to small molecules.*** To directly
477 measure functional barrier permeability, we quantified apical-to-basolateral flux of 4-kDa FITC-
478 dextran across polarized Caco-2 BBE monolayers following 72-hour infection. Strain-specific
479 permeability values were observed to be highest among strain A9a (a bloody/inflammatory strain)
480 while the lowest was observed among strain A13a (a watery diarrhea associated strain; **Fig 6A**).
481 Infection with bloody/inflammatory strains resulted in a 2-fold higher FITC-dextran translocation
482 compared to watery strains ($p < 0.0001$; **Fig 6B**).
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498

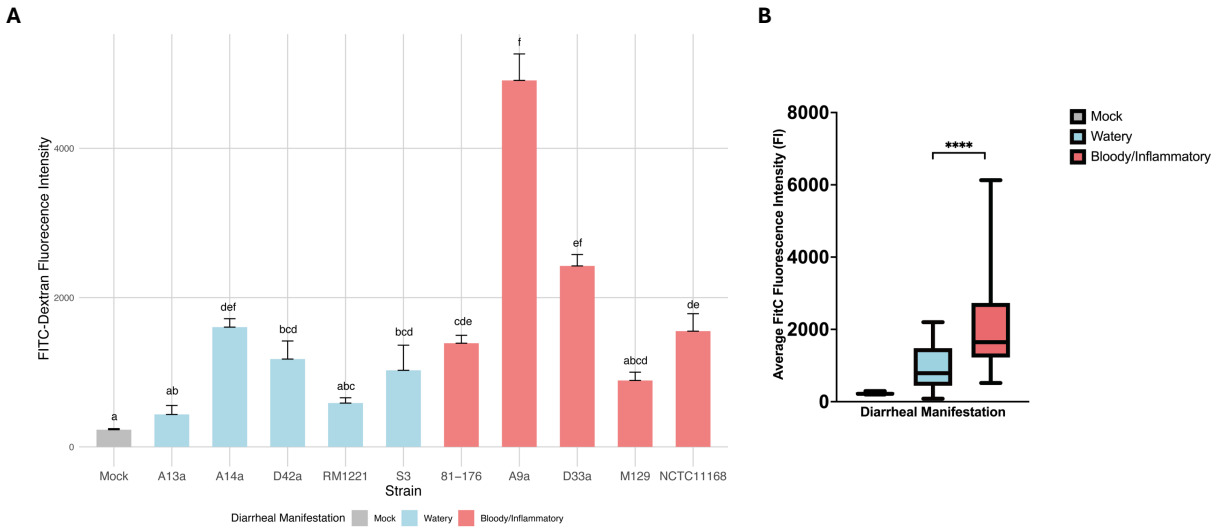

Fig 6. The fluorescence of FITC-Dextran flow through after 72 hours of infection with *C. jejuni* strains.

(A) Average FITC-Dextran fluorescence intensity of each *C. jejuni* strain. Bars labeled with different letters indicate statistically significant differences ($p < 0.05$) based on Dunn's post hoc test following a Kruskal-Wallis analysis ($n = 3$). Shared letters denote groups that are not significantly different from each other. (B) There was a highly significant difference between the watery and bloody/inflammatory diarrhea associated strains (****, $p < 0.0001$). The average FITC-Dextran fluorescence was calculated based on three biologically independent assays per strain with two technical replicates ($n = 15$). Mock infection represents Caco-2 BBE cells that contained only sterile cell culture media.

513 ***C. jejuni promotes translocation of commensal E. coli across the epithelial barrier.*** We next
514 assessed whether barrier disruption by *C. jejuni* strains altered host susceptibility to microbial
515 translocation. Using polarized Caco-2 BBE monolayers in Transwell inserts, we evaluated whether
516 a non-invasive commensal strain of *E. coli* (DH5α) could cross the epithelial barrier during or after
517 infection with *C. jejuni* strains. Two experimental approaches were tested: (i) co-inoculation of *C.*
518 *jejuni* and *E. coli* for 6 hours, and (ii) pre-infection with *C. jejuni* for 72 hours followed by 3-hour
519 exposure to *E. coli*. In both models, *E. coli* translocation was observed among all strains and there
520 were no significant differences between strains (**Supplementary Fig S6A, S6C**). During co-
521 inoculation, the mean *E. coli* CFU recovered from the basal chamber was not significantly different
522 between bloody/inflammatory and watery diarrhea associated strains (p = 0.57; **Supplementary**
523 **Fig S6B**). In the sequential infection model, basal recovery of *E. coli* was more pronounced, but
524 no significant difference was observed between bloody/inflammatory and watery diarrhea
525 associated strains (p = 0.13; **Supplementary Fig S6D**).
526

527 ***Strains associated with bloody/inflammatory diarrhea demonstrate enhanced intracellular***
528 ***survival in macrophages.*** To investigate whether there was strain variation in the interactions with
529 innate immune cells, we measured intracellular survival of the *C. jejuni* strains in J774A.1 murine
530 macrophages at 24-, 48-, and 72-hours post-infection. There were significant differences between
531 strains at all time points. Notably, strain A9a had the highest percent survivability across all time
532 points while 81-176 had the lowest (**Fig 7A, 7C, 7E**). At 24 hours, viable intracellular *C. jejuni*
533 was 2.6-fold higher among bloody/inflammatory diarrhea associated strains but there was no
534 significant difference compared to watery diarrhea associated strains (p = 0.36; **Fig 7B**). At 48
535 hours post infection, bloody/inflammatory diarrhea associated strains showed significantly greater

536 intracellular survivability (p = 0.046; **Fig 7D**). However, by 72-hours post infection, there was
537 again no significant difference between bloody/inflammatory and watery diarrhea associated
538 strains (p = 0.62; **Fig 7F**).

539

540

541

542

543

544

545

546

547

548

549

550

551

552

553

554

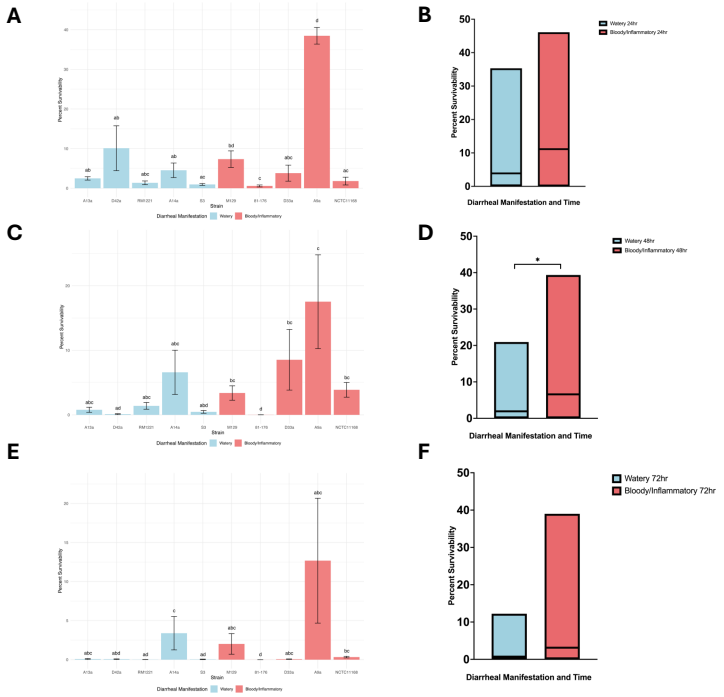

Fig 7. Survivability of *C. jejuni* strains in J774A.1 macrophages over 24, 48 and 72 hours. (A) Survivability by each *C. jejuni* strain over 24 hours within J774A.1 macrophages. Bars labeled with different letters indicate statistically significant differences ($p < 0.05$) based on Dunn's post hoc test following a Kruskal-Wallis analysis. Shared letters denote groups that are not significantly different from each other. Average percent survivability was calculated based on three biologically independent assays with two technical replicates (n = 3). (B) Macrophage survivability of *C. jejuni* strains grouped by diarrheal manifestation over 24 hours. There was no significant difference in survivability between the watery and bloody/inflammatory diarrhea associated strains at 24 hours ($p = 0.36$). Average percent survivability was calculated based on three biologically independent assays with two technical replicates (n = 15). (C) Survivability by each *C. jejuni* strain over 48-hours within J774A.1 macrophages. Average percent survivability was calculated based on three biologically independent assays with two technical replicates (n = 3). (D) Percent survivability within J774A.1 macrophages across *C. jejuni* strains grouped by diarrheal manifestation. At 48-

569 hours post infection there was a difference between bloody/inflammatory and watery diarrhea
570 associated strains (*, p = 0.046). Average percent survivability was calculated based on three
571 biologically independent assays with two technical replicates (n = 15). (E) Survivability of *C.*
572 *jejuni* strains in J774A.1 macrophages over 72 hours. Average percent survivability was calculated
573 based on three biologically independent assays with two technical replicates (n = 3). (F)
574 Macrophage survivability of *C. jejuni* strains grouped by diarrheal manifestation over 72 hours.
575 There was no significant difference in survivability between the watery and bloody/inflammatory
576 diarrhea associated strains at 72 hours (p = 0.62). Average percent survivability was calculated
577 based on three biologically independent assays with two technical replicates (n = 15).
578
579
580
581
582
583
584
585
586
587
588

589 **Discussion**

590 Clinical outcomes associated with different *C. jejuni* strains vary widely, from mild, self-
591 limiting watery diarrhea to more severe presentations involving bloody and/or inflammatory
592 diarrhea, abdominal cramping, and fever[10]. While host factors such as immune status and
593 microbiota composition influence disease severity, accumulating evidence suggests that bacterial
594 strain differences also contribute significantly to clinical heterogeneity[6, 33]. However,
595 identifying phenotypic or molecular markers that distinguish high- and low-virulence *C. jejuni*
596 strains has proven challenging[34]. While group-level differences were clear, we observed notable
597 variation within each group, consistent with strain-to-strain variations from studies mentioned
598 previously. Some watery strains exhibited moderate invasion or barrier disruption, and not all
599 bloody/inflammatory strains displayed the most extreme phenotypes. A study by Law et al
600 performed invasion, attachment and macrophage survivability assays using six out of the 10 *C.*
601 *jejuni* strains used in this study[21]. However, several differences exist between the prior study
602 and ours in the methodology and over all approach. In contrast to our study which used human
603 intestinal epithelial cells (Caco-2 BBE, INT-407 and T84), that study used non-polarized human
604 epithelial cells (HEp-2) that don't form tight junctions for attachment and invasion assays. Our
605 study has shown that different cell lines can provide very different results among *C. jejuni* strains
606 particularly during attachment assays. Furthermore, macrophage survivability was only performed
607 at 72 hours post infection, missing significant differences 48 hours that exist between watery and
608 bloody/inflammatory diarrhea associated strains. This study dramatically expands from the Law
609 et al study by examining a more detailed phenotypic analysis between *C. jejuni* strains, in addition
610 to approaching the strains as two groups with distinct diarrheal manifestations confirmed in
611 neonatal piglets.

612 Using a neonatal piglet model, which closely recapitulates human *C. jejuni* associated
613 diarrheal disease[21, 29, 35], *C. jejuni* strains can be classified into two groups that consistently
614 induce a particular diarrheal manifestation of either watery diarrhea or bloody/inflammatory in the
615 neonatal piglet model[21, 32, 35]. The strains used in this study were chosen for their known
616 diarrheal manifestation as well as their varied source background of either clinical or poultry
617 sources. Given that poultry are the primary reservoirs of *C. jejuni*, our strains represent both mild
618 and severe infections that can arise from poultry. Two strains observed to cause
619 bloody/inflammatory diarrhea in piglets were sourced from poultry while the remaining three were
620 sourced human clinical cases. In contrast, all the watery diarrhea associated strains in this study
621 were isolated from poultry, which likely reflects a surveillance bias. Individuals experiencing
622 milder, self-limiting symptoms such as watery diarrhea may be less likely to seek medical
623 attention, leading to underreporting in clinical settings. By comparing *in vitro* virulence traits, we
624 found significant strain to strain variation both within and between diarrheal manifestation groups,
625 particularly among strains associated with bloody/inflammatory diarrhea which consistently
626 exhibited enhanced invasion, intracellular persistence, epithelial barrier disruption and
627 macrophage survivability, each a hallmark of a more invasive and inflammatory disease
628 mechanism.

629 Adherence to intestinal epithelial cells is one of the primary steps to initiating *C. jejuni*
630 infection[36, 37]. Here we found that bloody/inflammatory diarrhea associated strains had higher
631 levels of adherence to small intestinal INT407 cells than watery diarrhea associated strains. Non-
632 polarized cells such as INT407 cells lack a defined brush border and other features such as tight
633 junctions that are more representative of the epithelial cells of the intestinal tract[38]. The
634 increased adherence of bloody/inflammatory diarrhea-associated strains to INT407 cells may be

635 attributed to the absence of tight junctions in this cell line, which has more easily accessible
636 basolateral adhesion molecules like fibronectin, a recognized target for the bacterial adhesins CadF
637 and FlpA[39]. Multiple studies have observed higher INT407 adherence among *C. jejuni* strains
638 associated with febrile diarrhea than strains or those from clinical sources compared to strains from
639 patients without diarrhea/fever or from environmental sources[40, 41], suggesting that adherence
640 to INT407 cells by *C. jejuni* strains may be a feature of more virulent strains. In contrast, adherence
641 patterns shifted when using Caco-2 BBE cells, a differentiated epithelial cell line originating from
642 the human colon[42]. Here, adherence was higher among watery diarrhea associated strains and
643 there was greater strain to strain variation, particularly between groups, where 81-176 had lower
644 levels of adherence than watery diarrhea associated strains A13a, A14a and S3. Polarized cells
645 such as Caco-2 BBE cells are able to form polarized monolayers with microvilli, and tight
646 junctions, in addition to displaying brush border proteins that, despite being of colonic origin, are
647 characteristic of small intestinal villus cells, including villin, fimbrin and the terminal web proteins
648 fodrin and myosin II[43-46]. Watery diarrhea associated strains may have higher affinity for the
649 adhesions displayed by Caco-2 BBE cells, but this is not yet supported by any studies and requires
650 future investigation. However, adherence to T84 cells was similar across all *C. jejuni* strains and
651 no significant differences were observed between diarrheal manifestation groups or between
652 strains. T84 cells have been derived from a human colonic carcinoma and are highly polarized
653 with short microvilli, tight junctions and desmosomes between cells[47]. T84 cells offer a more
654 accurate model of colonocytes due to their retention of key colonic features, including high
655 expression of colonic markers and the presence of short microvilli characteristic of
656 colonocytes[48]. Variation in the adherence of *C. jejuni* strains with defined diarrheal

657 manifestations among polarized and non-polarized tissue culture cell lines requires more
658 investigations into the mechanisms driving these different responses.

659 Invasion has long been a regarded as a hallmark of *C. jejuni* pathogenesis and has been
660 studied extensively in tissue culture models[37, 49-52]. In the present study we found that
661 bloody/inflammatory diarrhea associated strains demonstrated significantly higher invasiveness of
662 both non-polarized and polarized intestinal epithelial cells. While the individual invasiveness of
663 bloody/inflammatory diarrhea strains varied, there were few significant differences between
664 strains from the same diarrheal manifestation, as all bloody/inflammatory diarrhea associated
665 strains were invasive and all watery diarrhea associated strains were poorly invasive or non-
666 invasive. Several groups have also observed variation in the invasiveness of *C. jejuni* strains.
667 Everest et al (1992) found that *C. jejuni* clinical isolates from patients with colitis were more
668 invasive than isolates from patients with non-inflammatory diarrhea[53]. Strain dependent
669 invasiveness has also been observed using environmental strains compared to clinical strains,
670 where clinical strains were found to be significantly more invasive[41]. Even intra-strain
671 invasiveness has been found, particularly among continuously passed laboratory strains. For
672 example, the clinical strain 81-176 has been reported to range in its invasiveness from 0.8 to
673 1.8%[54-56], while the genome sequenced clinical strain NCTC11168 (NCTC11168-GS) had
674 diminished invasiveness and translocation abilities compared to the original isolate (NCTC11168-
675 O), highlighting the potential effects of high laboratory passage on strain pathogenicity and
676 colonization[57]. Furthermore, Cooper et al (2013) found that NCTC11168-GS had decreased
677 disease production in the neonatal piglet model compared to NCTC11168-O[32]. Taken together,
678 the consistent invasiveness observed among bloody/inflammatory diarrhea associated strains in
679 our study, regardless of cell line used, supports invasion as a defining characteristic of the

680 bloody/inflammatory diarrheal manifestation. The lack of invasiveness among watery diarrhea-
681 associated strains may reflect alternative pathogenic strategies, such as growth capabilities and
682 metabolic adaptability, that do not rely on epithelial invasion. These differences in invasive
683 capacity likely contribute to the distinct clinical presentations observed between strain groups and
684 reinforce the need for broader strain inclusion in *C. jejuni* pathogenesis research.

685 Bloody/inflammatory strains not only exhibit greater invasion of both polarized and non-
686 polarized epithelial cells but also demonstrate enhanced intracellular persistence. Intracellular
687 survival of *C. jejuni* has been linked to proinflammatory signaling and immune evasion in both *in*
688 *vitro* and *in vivo* models [31, 58], but this is the first study to demonstrate that such phenotypes are
689 heightened in strains associated with a consistent bloody/inflammatory diarrheal manifestation *in*
690 *vivo*. Through macrophage survivability assays, we observed a connection to immune evasion by
691 bloody/inflammatory diarrhea associated strains where these strains exhibited prolonged
692 persistence within macrophages up to 48 hours post-infection. This suggests that not only are
693 bloody/inflammatory diarrhea associated strains able to survive for at least 24 hours within
694 intestinal epithelial cells, but they also have an ability to evade phagocytic killing for several days.
695 Strain variation in macrophage survivability was most pronounced between bloody/inflammatory
696 diarrhea strains, particularly among strain A9a at 24 hours where it had higher levels of
697 survivability compared to strains 81-176, D33a and NCTC11168 as well as all watery diarrhea
698 associated strains. Consistent with our study, Day et al (2000) and Law et al (2009) found that
699 M129 was able to be recovered from J774A.1 macrophages after 72-hours, while the later study
700 also observed survivability among A9a, A13a, A14a and D42a[21, 59]. Survivability differences
701 between watery and bloody/inflammatory diarrhea associated strains at 48 hours, but not at 24 or
702 72 hours, suggests a transient but critical window during which certain *C. jejuni* strains,

703 particularly those associated with bloody or inflammatory diarrhea, are better equipped or contain
704 alternative mechanisms to persist slightly longer within host immune cells. *C. jejuni* contains one
705 catalase enzyme, encoded by the *katA* gene, which aids in protection from reactive oxygen species
706 generated during the oxidative burst once the bacterium is phagocytosed within macrophages[60,
707 61]. The *katA* gene has been found to be critical for *C. jejuni* macrophage survivability, where
708 wildtype strain M129 was shown to be able to persist at levels significantly higher than a M129
709 *katA* mutant at 24, 48 and 72 hours within J774A.1 macrophages[59]. Another study found that
710 the down-regulation of *katA* in *C. jejuni* strain NCTC11168 through a mutation in a MarR-type
711 transcriptional regulator (Cj1556) was also reduced J774A.1 macrophage survivability[62, 63].
712 Future studies should investigate catalase expression or activity contributes to the prolonged
713 intracellular persistence observed in bloody/inflammatory diarrhea-associated strains.

714 Disruption of epithelial barrier function further differentiated the two diarrheal
715 manifestation groups. Bloody/inflammatory strains induced greater reductions in TEER and
716 increased paracellular flux of FITC-dextran across polarized monolayers - both indicators of tight
717 junction impairment[64, 65]. Other studies evaluating the changes to the TEER *in vitro* utilizing
718 invasive *C. jejuni* strains, particularly 81-176 and NCTC11168, did find a significant drop in the
719 TEER. However, none of these studies utilized strains with defined diarrheal manifestations or
720 compared them to watery diarrhea associated strains[66-69]. This disruption in barrier integrity
721 was significantly more pronounced in bloody/inflammatory strains and our study is the first to
722 directly link this phenotype with reproducible diarrheal manifestations across multiple isolates.
723 The effects we observed on the integrity of the Caco-2 BBE monolayers, regardless of defined
724 diarrheal manifestation, translated into increased translocation of a non-invasive commensal *E.*
725 *coli* strain, providing evidence that the barrier compromise facilitated microbial movement across

726 the epithelium. The translocation of noninvasive bacteria such as *E. coli* during *C. jejuni* infection
727 has been previously investigated *in vitro* with varying results. Konkel et al (1992) observed no
728 change in the TEER or translocation of *E. coli* DH5α up to 6 hours following inoculation of Caco-
729 2 cell monolayers using bloody/inflammatory strain M129 and strains of unknown diarrheal
730 manifestations; F38011, 81116 and 78-27[70]. In our study, despite watery diarrhea associated
731 strains being poorly invasive or non-invasive, we observed *E. coli* translocation at similar levels
732 to bloody/inflammatory strains, suggesting alternate means for *E. coli* translocation other than
733 invasion. Translocation of *E. coli* was found to not correspond with *C. jejuni* invasiveness in one
734 study that used invasion-defective *C. jejuni* flagella mutant strains, with lipid raft-mediated
735 endocytosis suggested to induce this effect[71]. The use of M cells to promote transcytosis of non-
736 invasive bacteria was also observed using bloody/inflammatory diarrhea associated strain 81-176
737 but was not investigated using any other strains[72]. Both lipid rafts and M cells should be further
738 investigated as potential mechanisms behind *E. coli* translocation by invasive and non-invasive *C.*
739 *jejuni* strains.

740 Not all measured pathogenicity phenotypes differed between the two manifestation groups.
741 Our study found that CDT production levels were not altered both between strains and across
742 diarrheal manifestation groups at either temperature, suggesting CDT is not a discriminating factor
743 for diarrheal severity. CDT is widespread among *C. jejuni* isolates and contributes to host cell
744 cycle arrest, DNA damage, and epithelial disruption[73], however the specific contributions of
745 CDT in *C. jejuni* pathogenesis remains unclear[74, 75]. There is limited evidence that CDT
746 expression is critical to disease but it was found that CDT contributes to greater adherence and
747 invasion of epithelial cells along with more progressive gastritis and proximal duodenitis in two
748 *in vivo* mouse models[76, 77]. However, the role of CDT in driving different diarrheal symptoms

749 is not well understood and previous studies have assessed CDT production with HeLa cells among
750 *C. jejuni* strains from clinical or poultry sources and have reported variability, although none had
751 a diarrheal manifestation associated with any of the strains[78-80]. Additionally, IL-8 secretion, a
752 hallmark of initial epithelial response to *C. jejuni* contact[81-83] and a potent chemoattractant and
753 cellular activator[82] also did not differ significantly between diarrheal manifestation groups at 24
754 hours post-infection. On a strain to strain level, more variation was observed among
755 bloody/inflammatory strains than watery diarrhea associated strains, with both M129 and A9a
756 being significantly different from D33a, while only M129 was significantly different from watery
757 diarrhea associated strains S3, A14a, and A13a. Only watery diarrhea associated strain RM1221
758 and D42a showed significantly higher IL-8 secretion than other strains within its group and one
759 bloody/inflammatory diarrhea associated strain D33a. Few studies have looked at differences in
760 IL-8 secretion across *C. jejuni* strains and this is the first study to assess IL-8 secretion by *C. jejuni*
761 strains with known diarrheal manifestations in the neonatal piglet model. One study using
762 bloody/inflammatory diarrhea associated strain NCTC11168 and human colitis strain L115
763 showed no difference in IL-8 secretion by T84 cells[84], while another study looked at IL-8
764 secretion between *C. jejuni* strains from poultry and human sources and found no difference in IL-
765 8 secretion by T84 cells between groups[85]. However, IL-8 secretion is not based solely on the
766 invasive or adherent capability of *C. jejuni* strains, as it can also be activated by CDT
767 production[82, 83], indicating that early chemokine responses may not correlate with longer-term
768 invasive or inflammatory phenotypes. Furthermore, our findings that both bloody/inflammatory
769 and watery diarrhea associated strains produce similar levels of CDT and elicit comparable IL-8
770 responses despite significant differences in invasion. This variation highlights the importance of

771 considering strain-level differences when evaluating the inflammatory potential of *C. jejuni* and
772 suggests that multiple mechanisms may drive IL-8 secretion in the host.

773 Strain A9a, originally recovered from a poultry processing plant in Kansas, USA[86], is
774 associated with bloody/inflammatory diarrhea in the neonatal piglet model. Its phenotypic
775 responses *in vitro* highlight the wide variation in virulence factors between bloody/inflammatory
776 diarrhea associated strains as well as compared to watery diarrhea associated strains. A9a
777 demonstrated the highest levels of adherence to intestinal epithelial cells, particularly to Caco-2
778 BBE and T84 cells, and also the highest levels of invasion in both non-polarized and polarized
779 cells along with enhanced intracellular survival compared to all other *C. jejuni* strains. While its
780 CDT production was comparable to many of the other *C. jejuni* strains, A9a showed the highest
781 stimulation of IL-8 secretion from T84 cells, results that support IL-8 production due to high
782 adherence and invasion even with similar levels of CDT. A9a also caused the most pronounced
783 disruption to epithelial barrier integrity, with the steepest decline in TEER, the highest FITC-
784 dextran permeability, and the greatest translocation of *E. coli* DH5α across Caco-2 BBE
785 monolayers over 72 hours. Finally, A9a had the highest survivability within macrophages across
786 all time points, reflecting its resilience against innate immune responses. Despite its strong
787 virulence potential, A9a was not hypervirulent in the piglet model[21]. Instead, it produced
788 bloody/inflammatory diarrhea similar to the other strains in this study, 81-176, M129, D33a and
789 NCTC11168, despite the differences in virulence capabilities, a finding that suggests that multiple
790 factors drive the diarrheal manifestations. *C. jejuni* strain variation has been in other studies, such
791 as Ex114, a *C. jejuni* strain that was found to be 25 times more invasive than reference strain
792 81116, which is a relatively low invader[16]. Consistent with our findings, the same study reported
793 that increased invasiveness was not linked to enhanced adhesion, and motility levels were similar

794 between highly and minimally invasive strains. In other studies, stain 81-176, sourced from a raw
795 milk outbreak,[87] is widely regarded as a highly invasive clinical isolate and has been shown to
796 cause robust epithelial invasion in cell lines[54, 88] and multiple animal models[89] as well as
797 produce disease in humans[90]. Yet its performance varies between laboratories depending on
798 passage history[54, 56, 91]. Repeatedly passages reduced the invasiveness and serum survival of
799 81-176[92]. Similarly, while strain NCTC11168 is a reference strain in *C. jejuni* research, its
800 virulence is known to diminish with laboratory passage, leading to reduced motility, invasion and
801 colonization capacity *in vitro*[57] in addition to altering *in vivo* virulence in the neonatal piglet
802 model[32]. Our findings support these previous reports that strain-dependent variation plays a key
803 role in pathogenicity and is highlighted by the high variation associated with strain A9a in most of
804 the phenotypic assays performed in this study.

805 This strain to strain variations observed in this study, even among strains associated with a
806 particular consistent diarrheal manifestation in the neonatal piglet model, highlight the inherent
807 complexity and plasticity of *C. jejuni* virulence[93-96]. This study also underscores the need for
808 population-scale studies integrating phenotypic data with comparative genomics to identify
809 combinations of traits that drive disease severity. One such study that performed a comparative
810 genomic analysis on 67 *C. jejuni* and 12 *C. coli* strains, identified that the *Campylobacter jejuni*-
811 integrated elements (CJIEs) originally identified in *C. jejuni* strain RM1221, were variably present
812 across other *Campylobacter* strains[97]. These genomic elements, some of which included
813 prophages and mobile genetic elements, contribute to functional differences that may affect
814 virulence, stress response, or host interaction. Notably, Parker et al reported that coding sequences
815 within CJIEs were highly variable, highlighting a potential genomic mechanism for the phenotypic
816 diversity observed in our assays[97]. This supports our findings that even within clinically defined

817 groups, virulence phenotypes can differ, potentially due to underlying genomic heterogeneity. The
818 consistency of our group-level findings despite intra-group variability supports the biological
819 relevance of the phenotypes we observed. One limitation of this study is that the number of strains
820 included was limited, but they were well-characterized and associated with consistent clinical
821 phenotypes in a validated *in vivo* neonatal piglet model. Our *in vitro* assays cannot fully replicate
822 host complexity, but the consistency of results across multiple models strengthens confidence in
823 the observed trends. Future work should integrate whole-genome sequencing, transcriptomic
824 profiling, and *in vivo* validation to identify the molecular determinants responsible for these
825 virulence phenotypes.

826 In summary, *C. jejuni* strains associated with bloody/inflammatory diarrhea exhibit a
827 distinct virulence profile characterized by epithelial invasion, intracellular survival, barrier
828 disruption, and macrophage persistence (**Fig 8**). Together, enhanced invasion and intestinal
829 damage, intracellular survival, and macrophage survivability provide a functional explanation for
830 the more severe pathology observed in the piglet model by bloody/inflammatory strain and, by
831 extension, potentially in human infections. By linking *in vivo* disease phenotypes to mechanistic
832 *in vitro* traits, our findings advance understanding of *C. jejuni* pathogenesis and provide a
833 framework for developing targeted diagnostics, risk-based screening tools, and novel therapeutic
834 interventions. Furthermore, the strain-to-strain variation observed in this study, even within groups
835 associated with the same *in vivo* diarrheal manifestation, present the caution that needs to be taken
836 when interpreting the results of experiments that utilize only a few *C. jejuni* strains.

837

838

839

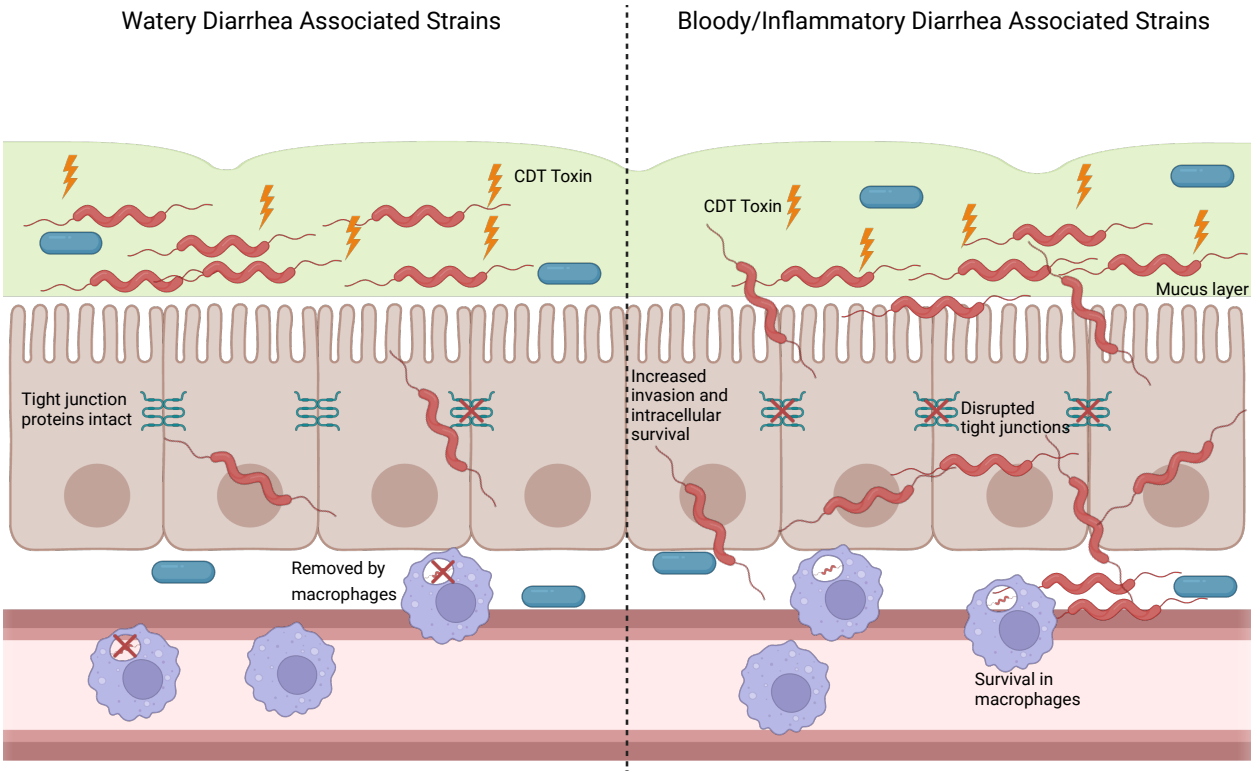

Fig 8. Proposed lifestyles of *C. jejuni* strains associated with watery or bloody/inflammatory diarrhea.

Watery diarrhea associated strains live an extracellular lifestyle where they attach to enterocytes and produce CDT toxin. They are poor invaders, disrupting the tight junctions between epithelial cells to a minimal degree, although commensal bacterial such as *E. coli* are translocated. Those that do weakly invade, do not survive long term intracellularly and are effectively removed by macrophages. Bloody/inflammatory diarrhea associated strains support an intracellular lifestyle where they attach to and invade enterocytes, significantly disrupting the tight junctions between those enterocytes. Bloody/inflammatory diarrhea associated strains can evade killing by macrophages for longer periods of time, promoting infection and contributing to more severe patient symptoms. Figure created in BioRender.

Acknowledgments

The authors thank all members of the Cooper laboratory for providing critical technical assistance in sample processing and other aspects of the study.

Ethics statement

This study did not involve the use of animals or human participants. The *Campylobacter jejuni* strains used in this study were previously characterized in a neonatal piglet model under approved animal use protocols, as described in Law et al and Cooper et al. No new *in vivo* experiments were conducted for this study.

Data availability statement

All relevant data are within the manuscript and its supplementary files.

Author Contributions

JB: Conceptualization, methodology, experimentation, formal analysis, writing – original draft, and review & editing. **KKC:** Conceptualization, methodology, resources, funding acquisition, project administration, supervision, writing, and review & editing. **CP:** Writing, and review & editing. **BP:** Writing, and review & editing.

Funding statement

This work was supported in part by Start-up funds provided to Kerry Cooper by the University of Arizona, and Technology and Research Initiative Fund (TRIF) award CALS_ACBC_Cooper_2101696 provided to Kerry Cooper by the University of Arizona. No

funding agency had any role in the study design, data collection, data analysis, or preparation of the manuscript.

Competing interests

The authors declare no competing interests.

881 **Supplementary Figure Captions**

882 **Supplementary Fig S1. Invasion of INT407 cells.**

883 The capacities of all ten *C. jejuni* strains to invade INT407 cells at a multiplicity of infection of
884 100 were examined *in vitro*. Invasiveness is expressed as the percentage of bacteria which had
885 invaded cultured intestinal epithelial cells following a 3-hour incubation and 1-hour gentamicin
886 exposure, compared to the inoculum. (A) Invasion across all ten *C. jejuni* strains. Bars labeled
887 with different letters indicate statistically significant differences ($p < 0.05$) based on Dunn's post
888 hoc test following a Kruskal-Wallis analysis. Shared letters denote strains that are not significantly
889 different from each other. (B) Invasiveness of INT407 cells by *C. jejuni* strains grouped by
890 diarrheal manifestation. There was a significant difference between watery and
891 bloody/inflammatory diarrhea associated strains and their invasion of INT407 cells (****, $p <$
892 0.0001). Invasion was calculated as from three biologically independent assays with two technical
893 replicates per strain ($n = 15$).

894

895 **Supplementary Fig S2. Average percent invasion of intestinal epithelial cell line T84.** The
896 capacities of all ten *C. jejuni* strains to invade T84 cells at a multiplicity of infection of 100 were
897 examined *in vitro*. Invasiveness is expressed as the percentage of bacteria which had invaded
898 cultured intestinal epithelial cells following a 3-hour incubation and 1-hour gentamicin exposure,
899 compared to the inoculum. (A) Invasion of T84 cells across all ten *C. jejuni* strains. Bars labeled
900 with different letters indicate statistically significant differences ($p < 0.05$) based on Dunn's post
901 hoc test following a Kruskal-Wallis analysis. Shared letters denote groups that are not significantly
902 different from each other. (B) Invasiveness of *C. jejuni* strains grouped by diarrheal manifestation.
903 There was a significant difference between watery and bloody/inflammatory diarrhea associated

904 strains and their invasion of T84 cells (**, p = 0.0035). Invasion was calculated from three
905 biologically independent assays with two technical replicates per strain (n = 15).
906
907 **Supplementary Fig S3. Adherence to T84 cells.**
908 (A) Adherence to T84 cells by *C. jejuni* strains. Bars labeled with different letters indicate
909 statistically significant differences ($p < 0.05$) based on Dunn's post hoc test following a Kruskal-
910 Wallis analysis. Shared letters denote groups that are not significantly different from each other (n
911 = 3). Strain 81-176 was not included in this data set. (B) Adherence to T84 cells by *C. jejuni* strains
912 grouped by diarrheal manifestation. There was no significant difference between watery and
913 bloody/inflammatory diarrhea associated strains and their attachment to T84 cells (p = 0.93).
914 Average percent attachment was calculated from three biologically independent assays with two
915 technical replicates (n = 12). Strain 81-176 is not included in this data set.
916
917 **Supplementary Fig S4. Cytolethal Distending Toxin (CDT) production at 37°C and 42°C.**
918 CDT toxin production was measured using HeLa cells and reported as the reciprocal of the highest
919 dilution with < 50% abnormal cells. (A) CDT toxin production at 37°C and 42°C by *C. jejuni*
920 strains. CDT production was measured in three biologically independent assays per strain (n = 3).
921 (B) CDT production *C. jejuni* strains grouped by diarrheal manifestation. No significant difference
922 was found in CDT production when grouped by diarrheal manifestation at either 37°C (p = 0.74)
923 or 42°C (p = 0.086). CDT production was not significantly different when performed at 37°C
924 compared to 42°C for watery diarrhea associated strains (p = 0.65) or bloody/inflammatory
925 diarrhea associated strains (p = 0.12). Average CDT was calculated from three biologically
926 independent assays with two technical replicates (n = 15).

927

928 **Supplementary Fig S5. IL-8 Production following 24-hour incubation with *C. jejuni* strains.**
929 (A) IL-8 production in pg/ml of *C. jejuni* strains. Bars labeled with different letters indicate
930 statistically significant differences ($p < 0.05$) based on Dunn's post hoc test following a Kruskal-
931 Wallis analysis. Shared letters denote groups that are not significantly different from each other.
932 Average IL-8 production was calculated from three biologically independent assays with two
933 technical replicates ($n = 3$). Strain 81-176 is not included in this data set. Mock infection represents
934 Caco-2 BBE cells that contained only sterile cell culture media. (B) IL-8 production in pg/ml of
935 *C. jejuni* strains grouped by diarrheal manifestation. There was no significant difference between
936 watery and bloody/inflammatory diarrhea associated strains ($p = 0.19$) Average IL-8 production
937 was calculated from three biologically independent assays with two technical replicates ($n = 15$).
938 Mock infection represents Caco-2 BBE cells that contained only sterile cell culture media. Strain
939 81-176 is not included in this data set.

940

941 **Supplementary Fig S6. Translocation of *Escherichia coli* through Caco-2 BBE cell**
942 **monolayers.**

943 (A) 6-hour co-culture incubation with *C. jejuni* strains. Bars labeled with different letters indicate
944 statistically significant differences ($p < 0.05$) based on Dunn's post hoc test following a Kruskal-
945 Wallis analysis. Shared letters denote groups that are not significantly different from each other.
946 Average 6-hour *E. coli* translocation was calculated from three biologically independent assays
947 with two technical replicates ($n = 3$). (B) 6-hour co-culture incubation with *C. jejuni* strains
948 grouped by diarrheal manifestation ($n = 15$). There was no significant difference between the
949 watery and bloody/inflammatory diarrhea-associated *C. jejuni* strains ($p = 0.57$). Mock infection

950 represents Caco-2 BBE cells that contained only sterile cell culture media. (C) Pre-infection with
951 *C. jejuni* for 72 hours followed by 3-hour exposure to *E. coli* through Caco-2 BBE cells by strain
952 (n = 3). (D) Pre-infection with *C. jejuni* for 72 hours followed by 3-hour exposure to *E. coli* through
953 Caco-2 BBE grouped by diarrheal manifestation (n = 15). There was no significant difference in
954 the translocation of *E. coli* between the watery and bloody/inflammatory diarrhea-associated *C.*
955 *jejuni* strains (p = 0.13).
956
957
958

959 **References**

960 1. Organization WH. Global View of Campylobaceriosis, Report of an Expert Consultation.
961 2012.

962 2. Walter EJS, Cui Z, Tierney R, Griffin PM, Hoekstra RM, Payne DC, et al. Foodborne
963 Illness Acquired in the United States—Major Pathogens, 2019. Volume 31, Number 4. 2025.
964 PubMed PMID: April.

965 3. Schorling E, Knorr S, Lick S, Steinberg P, Brüggemann DA. Probability of sequelae
966 following *Campylobacter* spp. infections: Update of systematic reviews and meta-analyses.
967 Public Health Challenges. 2023;2(4):e145. doi: 10.1002/puh2.145.

968 4. Nyati KK, Nyati R. Role of *Campylobacter jejuni* infection in the pathogenesis of
969 Guillain-Barré syndrome: an update. Biomed Res Int. 2013;2013:852195. Epub 20130813. doi:
970 10.1155/2013/852195. PubMed PMID: 24000328; PubMed Central PMCID:
971 PMCPMC3755430.

972 5. Peters S, Pascoe B, Wu Z, Bayliss SC, Zeng X, Edwinson A, et al. *Campylobacter jejuni*
973 genotypes are associated with post-infection irritable bowel syndrome in humans. Commun Biol.
974 2021;4(1):1015. Epub 20210830. doi: 10.1038/s42003-021-02554-8. PubMed PMID: 34462533;
975 PubMed Central PMCID: PMCPMC8405632.

976 6. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al.
977 Burden and aetiology of diarrhoeal disease in infants and young children in developing countries
978 (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet.
979 2013;382(9888):209-22. Epub 20130514. doi: 10.1016/s0140-6736(13)60844-2. PubMed PMID:
980 23680352.

981 7. Investigators TM-EN. The MAL-ED Study: A Multinational and Multidisciplinary
982 Approach to Understand the Relationship Between Enteric Pathogens, Malnutrition, Gut
983 Physiology, Physical Growth, Cognitive Development, and Immune Responses in Infants and
984 Children Up to 2 Years of Age in Resource-Poor Environments. Clinical Infectious Diseases.
985 2014;59(suppl_4):S193-S206. doi: 10.1093/cid/ciu653.
986 8. Haque MA, Platts-Mills JA, Mduma E, Bodhidatta L, Bessong P, Shakoor S, et al.
987 Determinants of *Campylobacter* infection and association with growth and enteric inflammation
988 in children under 2 years of age in low-resource settings. Sci Rep. 2019;9(1):17124. Epub
989 20191120. doi: 10.1038/s41598-019-53533-3. PubMed PMID: 31748573; PubMed Central
990 PMCID: PMCPMC6868199.
991 9. Pogreba-Brown K, Baker A, Ernst K, Stewart J, Harris RB, Weiss J. Assessing risk
992 factors of sporadic *Campylobacter* infection: a case-control study in Arizona. Epidemiol Infect.
993 2016;144(4):829-39. doi: 10.1017/s0950268815002162. PubMed PMID: 26828241.
994 10. Igwaran A, Okoh AI. Human campylobacteriosis: A public health concern of global
995 importance. Heliyon. 2019;5(11):e02814. Epub 20191114. doi: 10.1016/j.heliyon.2019.e02814.
996 PubMed PMID: 31763476; PubMed Central PMCID: PMCPMC6861584.
997 11. Harvey P, Battle T, Leach S. Different invasion phenotypes of *Campylobacter* isolates in
998 Caco-2 cell monolayers. J Med Microbiol. 1999;48(5):461-9. doi: 10.1099/00222615-48-5-461.
999 PubMed PMID: 10229543.
1000 12. Ruiz-Palacios G, Torres N, Ruiz-Palacios B, Torres J, Escamilla E, Tamayo J. Cholera-
1001 like enterotoxin produced by *Campylobacter jejuni*: characterisation and clinical significance.
1002 The Lancet. 1983;322(8344):250-3.

1003 13. Johnson WM, Lior H. Toxins produced by *Campylobacter jejuni* and *Campylobacter*
1004 *coli*. Lancet. 1984;1(8370):229-30. doi: 10.1016/s0140-6736(84)92155-x. PubMed PMID:
1005 6141372.
1006 14. Apel D, Ellermeier J, Pryjma M, Dirita VJ, Gaynor EC. Characterization of
1007 *Campylobacter jejuni* RacRS reveals roles in the heat shock response, motility, and maintenance
1008 of cell length homogeneity. J Bacteriol. 2012;194(9):2342-54. Epub 20120217. doi:
1009 10.1128/JB.06041-11. PubMed PMID: 22343300; PubMed Central PMCID: PMCPMC3347078.
1010 15. Turonova H, Briandet R, Rodrigues R, Hernould M, Hayek N, Stintzi A, et al. Biofilm
1011 spatial organization by the emerging pathogen *Campylobacter jejuni*: comparison between
1012 NCTC 11168 and 81-176 strains under microaerobic and oxygen-enriched conditions. Front
1013 Microbiol. 2015;6:709. Epub 20150713. doi: 10.3389/fmicb.2015.00709. PubMed PMID:
1014 26217332; PubMed Central PMCID: PMCPMC4499754.
1015 16. Fearnley C, Manning G, Bagnall M, Javed MA, Wassenaar TM, Newell DG.
1016 Identification of hyperinvasive *Campylobacter jejuni* strains isolated from poultry and human
1017 clinical sources. J Med Microbiol. 2008;57(Pt 5):570-80. doi: 10.1099/jmm.0.47803-0. PubMed
1018 PMID: 18436589.
1019 17. Hofreuter D, Novik V, Galán JE. Metabolic Diversity in *Campylobacter jejuni* Enhances
1020 Specific Tissue Colonization. 2008.
1021 18. Poly F, Ewing C, Goon S, Hickey TE, Rockabrand D, Majam G, et al. Heterogeneity of a
1022 *Campylobacter jejuni* protein that is secreted through the flagellar filament. Infect Immun.
1023 2007;75(8):3859-67. Epub 20070521. doi: 10.1128/iai.00159-07. PubMed PMID: 17517862;
1024 PubMed Central PMCID: PMCPMC1951984.

1025 19. Meurens F, Summerfield A, Nauwynck H, Saif L, Gerdts V. The pig: a model for human
1026 infectious diseases. Trends Microbiol. 2012;20(1):50-7. Epub 20111205. doi:
1027 10.1016/j.tim.2011.11.002. PubMed PMID: 22153753; PubMed Central PMCID:
1028 PMCPMC7173122.
1029 20. Boosinger TR, Powe TA. *Campylobacter jejuni* infections in gnotobiotic pigs. Am J Vet
1030 Res. 1988;49(4):456-8. PubMed PMID: 3377304.
1031 21. Law BF, Adriance SM, Joens LA. Comparison of in vitro virulence factors of
1032 *Campylobacter jejuni* to in vivo lesion production. Foodborne Pathog Dis. 2009;6(3):377-85.
1033 doi: 10.1089/fpd.2008.0183. PubMed PMID: 19278341.
1034 22. Canibe N, O'Dea M, Abraham S. Potential relevance of pig gut content transplantation
1035 for production and research. Journal of Animal Science and Biotechnology. 2019;10(1):55. doi:
1036 10.1186/s40104-019-0363-4.
1037 23. van der Laan JW, Brightwell J, McAnulty P, Ratky J, Stark C. Regulatory acceptability
1038 of the minipig in the development of pharmaceuticals, chemicals and other products. J
1039 Pharmacol Toxicol Methods. 2010;62(3):184-95. Epub 20100616. doi:
1040 10.1016/j.vascn.2010.05.005. PubMed PMID: 20601024.
1041 24. Lodemann U, Amasheh S, Radloff J, Kern M, Bethe A, Wieler LH, et al. Effects of Ex
1042 Vivo Infection with ETEC on Jejunal Barrier Properties and Cytokine Expression in Probiotic-
1043 Supplemented Pigs. Dig Dis Sci. 2017;62(4):922-33. Epub 20161219. doi: 10.1007/s10620-016-
1044 4413-x. PubMed PMID: 27995406.
1045 25. Gefeller EM, Martens H, Aschenbach JR, Klingspor S, Twardziok S, Wrede P, et al.
1046 Effects of age and zinc supplementation on transport properties in the jejunum of piglets. J Anim

1047 Physiol Anim Nutr (Berl). 2015;99(3):542-52. Epub 20140721. doi: 10.1111/jpn.12232. PubMed
1048 PMID: 25039419.
1049 26. Lodemann U, Dillenseger A, Aschenbach JR, Martens H. Effects of age and controlled
1050 oral dosing of Enterococcus faecium on epithelial properties in the piglet small intestine. Benef
1051 Microbes. 2013;4(4):335-44. doi: 10.3920/bm2013.0004. PubMed PMID: 24311317.
1052 27. Harvey RB, Young CR, Ziprin RL, Hume ME, Genovese KJ, Anderson RC, et al.
1053 Prevalence of *Campylobacter* spp isolated from the intestinal tract of pigs raised in an integrated
1054 swine production system. J Am Vet Med Assoc. 1999;215(11):1601-4. PubMed PMID:
1055 14567422.
1056 28. Taylor D, Olubunmi P. A re-examination of the role of Campylobacter fetus subspecies
1057 coli in enteric disease of the pig. The Veterinary Record. 1981;109(6):112-5.
1058 29. Vítovec J, Koudela B, Štěrba J, Tomancová I, Matyáš Z, Vladík P. The gnotobiotic piglet
1059 as a model for the pathogenesis of *Campylobacter jejuni* infection. Zentralblatt für Bakteriologie.
1060 1989;271(1):91-103. doi: 10.1016/s0934-8840(89)80058-1.
1061 30. Abuoun M, Manning G, Cawthraw SA, Ridley A, Ahmed IH, Wassenaar TM, et al.
1062 Cytolethal distending toxin (CDT)-negative Campylobacter jejuni strains and anti-CDT
1063 neutralizing antibodies are induced during human infection but not during colonization in
1064 chickens. Infect Immun. 2005;73(5):3053-62. doi: 10.1128/IAI.73.5.3053-3062.2005. PubMed
1065 PMID: 15845513; PubMed Central PMCID: PMCPMC1087314.
1066 31. Watson RO, Galán JE. *Campylobacter jejuni* survives within epithelial cells by avoiding
1067 delivery to lysosomes. PLoS Pathog. 2008;4(1):e14. doi: 10.1371/journal.ppat.0040014. PubMed
1068 PMID: 18225954; PubMed Central PMCID: PMCPMC2323279.

1069 32. Cooper KK, Cooper MA, Zuccolo A, Joens LA. Re-sequencing of a virulent strain of
1070 *Campylobacter jejuni* NCTC11168 reveals potential virulence factors. Res Microbiol.
1071 2013;164(1):6-11. Epub 20121006. doi: 10.1016/j.resmic.2012.10.002. PubMed PMID:
1072 23046762.
1073 33. Stamps BW, Kuroiwa J, Isidean SD, Schilling MA, Harro C, Talaat KR, et al. Exploring
1074 Changes in the Host Gut Microbiota During a Controlled Human Infection Model for
1075 *Campylobacter jejuni*. Frontiers in Cellular and Infection Microbiology. 2021;11. doi:
1076 10.3389/fcimb.2021.702047.
1077 34. Cody AJ, McCarthy NM, Wimalarathna HL, Colles FM, Clark L, Bowler IC, et al. A
1078 longitudinal 6-year study of the molecular epidemiology of clinical *Campylobacter* isolates in
1079 Oxfordshire, United kingdom. J Clin Microbiol. 2012;50(10):3193-201. Epub 20120718. doi:
1080 10.1128/JCM.01086-12. PubMed PMID: 22814466; PubMed Central PMCID:
1081 PMCPMC3457434.
1082 35. Babakhani FK, Bradley GA, Joens LA. Newborn piglet model for campylobacteriosis.
1083 Infect Immun. 1993;61(8):3466-75. doi: 10.1128/iai.61.8.3466-3475.1993. PubMed PMID:
1084 8335377; PubMed Central PMCID: PMCPMC281024.
1085 36. Rubinchik S, Seddon A, Karlyshev AV. Molecular mechanisms and biological role of
1086 *Campylobacter jejuni* attachment to host cells. Eur J Microbiol Immunol (Bp). 2012;2(1):32-40.
1087 Epub 20120317. doi: 10.1556/EuJMI.2.2012.1.6. PubMed PMID: 24611119; PubMed Central
1088 PMCID: PMCPMC3933988.
1089 37. Monteville MR, Yoon JE, Konkel ME. Maximal adherence and invasion of INT 407 cells
1090 by *Campylobacter jejuni* requires the CadF outer-membrane protein and microfilament

1091 reorganization. Microbiology (Reading). 2003;149(Pt 1):153-65. doi: 10.1099/mic.0.25820-0.
1092 PubMed PMID: 12576589.
1093 38. Konkel ME, Hayes SF, Joens LA, Cieplak W, Jr. Characteristics of the internalization
1094 and intracellular survival of *Campylobacter jejuni* in human epithelial cell cultures. Microb
1095 Pathog. 1992;13(5):357-70. doi: 10.1016/0882-4010(92)90079-4. PubMed PMID: 1297914.
1096 39. Konkel ME, Larson CL, Flanagan RC. *Campylobacter jejuni* FlpA binds fibronectin and
1097 is required for maximal host cell adherence. J Bacteriol. 2010;192(1):68-76. doi:
1098 10.1128/jb.00969-09. PubMed PMID: 19880595; PubMed Central PMCID: PMCPMC2798237.
1099 40. Fauchere JL, Rosenau A, Veron M, Moyen EN, Richard S, Pfister A. Association with
1100 HeLa cells of *Campylobacter jejuni* and *Campylobacter coli* isolated from human feces. Infect
1101 Immun. 1986;54(2):283-7. doi: 10.1128/iai.54.2.283-287.1986. PubMed PMID: 3770943;
1102 PubMed Central PMCID: PMCPMC260156.
1103 41. Newell DG, McBride H, Saunders F, Dehele Y, Pearson AD. The virulence of clinical
1104 and environmental isolates of *Campylobacter jejuni*. J Hyg (Lond). 1985;94(1):45-54. doi:
1105 10.1017/s0022172400061118. PubMed PMID: 3973380; PubMed Central PMCID:
1106 PMCPMC2129391.
1107 42. Peterson MD, Mooseker MS. Characterization of the enterocyte-like brush border
1108 cytoskeleton of the C2BBe clones of the human intestinal cell line, Caco-2. J Cell Sci. 1992;102
1109 (Pt 3):581-600. doi: 10.1242/jcs.102.3.581. PubMed PMID: 1506435.
1110 43. Hughson EJ, Cutler DF, Hopkins CR. Basolateral secretion of kappa light chain in the
1111 polarised epithelial cell line, Caco-2. J Cell Sci. 1989;94 (Pt 2):327-32. doi:
1112 10.1242/jcs.94.2.327. PubMed PMID: 2516089.

1113 44. Lea T. Caco-2 Cell Line. In: Verhoeckx K, Cotter P, López-Expósito I, Kleiveland C,
1114 Lea T, Mackie A, et al., editors. The Impact of Food Bioactives on Health: in vitro and ex vivo
1115 models. Cham (CH): Springer

1116 Copyright 2015, The Author(s). 2015. p. 103-11.

1117 45. Sambuy Y, De Angelis I, Ranaldi G, Scarino M, Stammati A, Zucco F. The Caco-2 cell
1118 line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2
1119 cell functional characteristics. Cell biology and toxicology. 2005;21:1-26.

1120 46. Peterson MD, Bement WM, Mooseker MS. An in vitro model for the analysis of
1121 intestinal brush border assembly. II. Changes in expression and localization of brush border
1122 proteins during cell contact-induced brush border assembly in Caco-2BBe cells. J Cell Sci.
1123 1993;105 (Pt 2):461-72. doi: 10.1242/jcs.105.2.461. PubMed PMID: 8408277.

1124 47. Dharmsathaphorn K, McRoberts JA, Mandel KG, Tisdale LD, Masui H. A human
1125 colonic tumor cell line that maintains vectorial electrolyte transport. Am J Physiol. 1984;246(2
1126 Pt 1):G204-8. doi: 10.1152/ajpgi.1984.246.2.G204. PubMed PMID: 6141741.

1127 48. Devriese S, Van den Bossche L, Van Welden S, Holvoet T, Pinheiro I, Hindryckx P, et
1128 al. T84 monolayers are superior to Caco-2 as a model system of colonocytes. Histochemistry and
1129 Cell Biology. 2017;148(1):85-93. doi: 10.1007/s00418-017-1539-7.

1130 49. Konkel ME, Joens LA. Adhesion to and invasion of HEp-2 cells by *Campylobacter* spp.
1131 Infect Immun. 1989;57(10):2984-90. doi: 10.1128/iai.57.10.2984-2990.1989. PubMed PMID:
1132 2550368; PubMed Central PMCID: PMCPMC260759.

1133 50. Rivera-Amill V, Kim BJ, Seshu J, Konkel ME. Secretion of the virulence-associated
1134 *Campylobacter* invasion antigens from *Campylobacter jejuni* requires a stimulatory signal. J

1135 Infect Dis. 2001;183(11):1607-16. Epub 20010501. doi: 10.1086/320704. PubMed PMID:
1136 11343209.
1137 51. Russell RG, Blake DC, Jr. Cell association and invasion of Caco-2 cells by
1138 *Campylobacter jejuni*. Infect Immun. 1994;62(9):3773-9. doi: 10.1128/iai.62.9.3773-3779.1994.
1139 PubMed PMID: 8063393; PubMed Central PMCID: PMCPMC303030.
1140 52. Konkel ME, Talukdar PK, Negretti NM, Klappenbach CM. Taking Control:
1141 Campylobacter jejuni Binding to Fibronectin Sets the Stage for Cellular Adherence and Invasion.
1142 Frontiers in Microbiology. 2020;11. doi: 10.3389/fmicb.2020.00564.
1143 53. Everest PH, Goossens H, Butzler JP, Lloyd D, Knutton S, Ketley JM, et al. Differentiated
1144 Caco-2 cells as a model for enteric invasion by *Campylobacter jejuni* and *C. coli*. J Med
1145 Microbiol. 1992;37(5):319-25. doi: 10.1099/00222615-37-5-319. PubMed PMID: 1433253.
1146 54. Yao R, Burr DH, Doig P, Trust TJ, Niu H, Guerry P. Isolation of motile and non-motile
1147 insertional mutants of Campylobacter jejuni: the role of motility in adherence and invasion of
1148 eukaryotic cells. Mol Microbiol. 1994;14(5):883-93. doi: 10.1111/j.1365-2958.1994.tb01324.x.
1149 PubMed PMID: 7715450.
1150 55. Doig P, Yao R, Burr DH, Guerry P, Trust TJ. An environmentally regulated pilus-like
1151 appendage involved in *Campylobacter* pathogenesis. Molecular Microbiology. 1996;20(4):885-
1152 94.
1153 56. Yao R, Burr DH, Guerry P. CheY-mediated modulation of *Campylobacter jejuni*
1154 virulence. Mol Microbiol. 1997;23(5):1021-31. doi: 10.1046/j.1365-2958.1997.2861650.x.
1155 PubMed PMID: 9076738.
1156 57. Gaynor Erin C, Cawthraw S, Manning G, MacKichan Joanna K, Falkow S, Newell Diane
1157 G. The Genome-Sequenced Variant of *Campylobacter jejuni* NCTC 11168 and the Original

1158 Clonal Clinical Isolate Differ Markedly in Colonization, Gene Expression, and Virulence-
1159 Associated Phenotypes. Journal of Bacteriology. 2004;186(2):503-17. doi: 10.1128/jb.186.2.503-
1160 517.2004.
1161 58. Novik V, Hofreuter D, Galán JE. Identification of Campylobacter jejuni genes involved
1162 in its interaction with epithelial cells. Infect Immun. 2010;78(8):3540-53. Epub 20100601. doi:
1163 10.1128/iai.00109-10. PubMed PMID: 20515930; PubMed Central PMCID: PMCPMC2916286.
1164 59. Day WA, Jr., Sajecki JL, Pitts TM, Joens LA. Role of catalase in *Campylobacter jejuni*
1165 intracellular survival. Infect Immun. 2000;68(11):6337-45. doi: 10.1128/iai.68.11.6337-
1166 6345.2000. PubMed PMID: 11035743; PubMed Central PMCID: PMCPMC97717.
1167 60. De Melo MA, Gabbiani G, Pechère JC. Cellular events and intracellular survival of
1168 *Campylobacter jejuni* during infection of HEp-2 cells. Infect Immun. 1989;57(7):2214-22. doi:
1169 10.1128/iai.57.7.2214-2222.1989. PubMed PMID: 2731988; PubMed Central PMCID:
1170 PMCPMC313863.
1171 61. Kiehlbauch JA, Albach RA, Baum LL, Chang KP. Phagocytosis of *Campylobacter jejuni*
1172 and its intracellular survival in mononuclear phagocytes. Infect Immun. 1985;48(2):446-51. doi:
1173 10.1128/iai.48.2.446-451.1985. PubMed PMID: 3988342; PubMed Central PMCID:
1174 PMCPMC261341.
1175 62. Gundogdu O, Mills DC, Elmi A, Martin MJ, Wren BW, Dorrell N. The *Campylobacter*
1176 *jejuni* Transcriptional Regulator Cj1556 Plays a Role in the Oxidative and Aerobic Stress
1177 Response and Is Important for Bacterial Survival In Vivo. Journal of Bacteriology.
1178 2011;193(16):4238-49. doi: doi:10.1128/jb.05189-11.
1179 63. Gundogdu O, da Silva DT, Mohammad B, Elmi A, Mills DC, Wren BW, et al. The
1180 *Campylobacter jejuni* MarR-like transcriptional regulators RrpA and RrpB both influence

1181 bacterial responses to oxidative and aerobic stresses. Frontiers in Microbiology. 2015;Volume 6 -
1182 2015. doi: 10.3389/fmicb.2015.00724.
1183 64. Srinivasan B, Kolli AR, Esch MB, Abaci HE, Shuler ML, Hickman JJ. TEER
1184 measurement techniques for in vitro barrier model systems. J Lab Autom. 2015;20(2):107-26.
1185 Epub 20150113. doi: 10.1177/2211068214561025. PubMed PMID: 25586998; PubMed Central
1186 PMCID: PMCPMC4652793.
1187 65. Gerkins C, Hajjar R, Oliero M, Santos MM. Assessment of Gut Barrier Integrity in Mice
1188 Using Fluorescein-Isothiocyanate-Labeled Dextran. J Vis Exp. 2022;(189). Epub 20221118. doi:
1189 10.3791/64710. PubMed PMID: 36468715.
1190 66. MacCallum A, Hardy SP, Everest PH. *Campylobacter jejuni* inhibits the absorptive
1191 transport functions of Caco-2 cells and disrupts cellular tight junctions. Microbiology (Reading).
1192 2005;151(Pt 7):2451-8. doi: 10.1099/mic.0.27950-0. PubMed PMID: 16000735.
1193 67. Hofreuter D, Tsai J, Watson RO, Novik V, Altman B, Benitez M, et al. Unique features
1194 of a highly pathogenic Campylobacter jejuni strain. Infect Immun. 2006;74(8):4694-707. doi:
1195 10.1128/IAI.00210-06. PubMed PMID: 16861657; PubMed Central PMCID:
1196 PMCPMC1539605.
1197 68. Mills DC, Gundogdu O, Elmi A, Bajaj-Elliott M, Taylor PW, Wren BW, et al. Increase in
1198 *Campylobacter jejuni* invasion of intestinal epithelial cells under low-oxygen coculture
1199 conditions that reflect the in vivo environment. Infect Immun. 2012;80(5):1690-8. Epub
1200 20120221. doi: 10.1128/iai.06176-11. PubMed PMID: 22354027; PubMed Central PMCID:
1201 PMCPMC3347453.

1202 69. Brás AM, Ketley JM. Transcellular translocation of *Campylobacter jejuni* across human
1203 polarised epithelial monolayers. FEMS Microbiology Letters. 1999;179(2):209-15. doi:
1204 10.1111/j.1574-6968.1999.tb08729.x.
1205 70. Konkel ME, Mead DJ, Hayes SF, Cieplak W, Jr. Translocation of *Campylobacter jejuni*
1206 across human polarized epithelial cell monolayer cultures. J Infect Dis. 1992;166(2):308-15. doi:
1207 10.1093/infdis/166.2.308. PubMed PMID: 1634802.
1208 71. Kalischuk LD, Inglis GD, Buret AG. *Campylobacter jejuni* induces transcellular
1209 translocation of commensal bacteria via lipid rafts. Gut Pathog. 2009;1(1):2. Epub 20090203.
1210 doi: 10.1186/1757-4749-1-2. PubMed PMID: 19338680; PubMed Central PMCID:
1211 PMCPMC2653720.
1212 72. Kalischuk LD, Leggett F, Inglis GD. *Campylobacter jejuni* induces transcytosis of
1213 commensal bacteria across the intestinal epithelium through M-like cells. Gut Pathogens.
1214 2010;2(1):14. doi: 10.1186/1757-4749-2-14.
1215 73. Lara-Tejero M, Galán JE. A bacterial toxin that controls cell cycle progression as a
1216 deoxyribonuclease I-like protein. Science. 2000;290(5490):354-7. doi:
1217 10.1126/science.290.5490.354. PubMed PMID: 11030657.
1218 74. De Rycke J, Oswald E. Cytolethal distending toxin (CDT): a bacterial weapon to control
1219 host cell proliferation? FEMS Microbiol Lett. 2001;203(2):141-8. doi: 10.1111/j.1574-
1220 6968.2001.tb10832.x. PubMed PMID: 11583839.
1221 75. Gu J, Lin Y, Wang Z, Pan Q, Cai G, He Q, et al. *Campylobacter jejuni* Cytolethal
1222 Distending Toxin Induces GSDME-Dependent Pyroptosis in Colonic Epithelial Cells. Frontiers
1223 in Cellular and Infection Microbiology. 2022;12. doi: 10.3389/fcimb.2022.853204.

1224 76. Fox James G, Rogers Arlin B, Whary Mark T, Ge Z, Taylor Nancy S, Xu S, et al.
1225 Gastroenteritis in NF-κB-Deficient Mice Is Produced with Wild-Type *Camplyobacter jejuni* but
1226 Not with C. jejuni Lacking Cytolethal Distending Toxin despite Persistent Colonization with
1227 Both Strains. Infection and Immunity. 2004;72(2):1116-25. doi: 10.1128/iai.72.2.1116-
1228 1125.2004.
1229 77. Jain D, Prasad KN, Sinha S, Husain N. Differences in virulence attributes between
1230 cytolethal distending toxin positive and negative *Campylobacter jejuni* strains. Journal of
1231 Medical Microbiology. 2008;57(3):267-72. doi: https://doi.org/10.1099/jmm.0.47317-0.
1232 78. Pickett CL, Pesci EC, Cottle DL, Russell G, Erdem AN, Zeytin H. Prevalence of
1233 cytolethal distending toxin production in *Campylobacter jejuni* and relatedness of
1234 *Campylobacter* sp. cdtB gene. Infect Immun. 1996;64(6):2070-8. doi: 10.1128/iai.64.6.2070-
1235 2078.1996. PubMed PMID: 8675309; PubMed Central PMCID: PMCPMC174038.
1236 79. Talukder KA, Aslam M, Islam Z, Azmi IJ, Dutta DK, Hossain S, et al. Prevalence of
1237 virulence genes and cytolethal distending toxin production in *Campylobacter jejuni* isolates from
1238 diarrheal patients in Bangladesh. J Clin Microbiol. 2008;46(4):1485-8. Epub 20080220. doi:
1239 10.1128/jcm.01912-07. PubMed PMID: 18287317; PubMed Central PMCID:
1240 PMCPMC2292937.
1241 80. Eyigor A, Dawson KA, Langlois BE, Pickett CL. Cytolethal distending toxin genes in
1242 *Campylobacter jejuni* and *Campylobacter coli* isolates: detection and analysis by PCR. J Clin
1243 Microbiol. 1999;37(5):1646-50. doi: 10.1128/jcm.37.5.1646-1650.1999. PubMed PMID:
1244 10203548; PubMed Central PMCID: PMCPMC84865.
1245 81. Hickey TE, Baqar S, Bourgeois AL, Ewing CP, Guerry P. Campylobacter jejuni-
1246 stimulated secretion of interleukin-8 by INT407 cells. Infect Immun. 1999;67(1):88-93. doi:

1247 10.1128/IAI.67.1.88-93.1999. PubMed PMID: 9864200; PubMed Central PMCID:
1248 PMCPMC96281.
1249 82. Zheng J, Meng J, Zhao S, Singh R, Song W. *Campylobacter*-induced interleukin-8
1250 secretion in polarized human intestinal epithelial cells requires *Campylobacter*-secreted
1251 cytolethal distending toxin- and Toll-like receptor-mediated activation of NF-kappaB. Infect
1252 Immun. 2008;76(10):4498-508. Epub 20080721. doi: 10.1128/IAI.01317-07. PubMed PMID:
1253 18644884; PubMed Central PMCID: PMCPMC2546826.
1254 83. Hickey TE, McVeigh AL, Scott DA, Michielutti RE, Bixby A, Carroll SA, et al.
1255 *Campylobacter jejuni* cytolethal distending toxin mediates release of interleukin-8 from
1256 intestinal epithelial cells. Infect Immun. 2000;68(12):6535-41. doi: 10.1128/IAI.68.12.6535-
1257 6541.2000. PubMed PMID: 11083762; PubMed Central PMCID: PMCPMC97747.
1258 84. MacCallum AJ, Harris D, Haddock G, Everest PH. *Campylobacter jejuni*-infected human
1259 epithelial cell lines vary in their ability to secrete interleukin-8 compared to in vitro-infected
1260 primary human intestinal tissue. Microbiology (Reading). 2006;152(Pt 12):3661-5. doi:
1261 10.1099/mic.0.29234-0. PubMed PMID: 17159219.
1262 85. Van Deun K, Haesebrouck F, Heyndrickx M, Favoreel H, Dewulf J, Ceelen L, et al.
1263 Virulence properties of *Campylobacter jejuni* isolates of poultry and human origin. J Med
1264 Microbiol. 2007;56(Pt 10):1284-9. doi: 10.1099/jmm.0.47342-0. PubMed PMID: 17893162.
1265 86. Malik-Kale P, Raphael BH, Parker CT, Joens LA, Klena JD, Quiñones B, et al.
1266 Characterization of genetically matched isolates of *Campylobacter jejuni* reveals that mutations
1267 in genes involved in flagellar biosynthesis alter the organism's virulence potential. Appl Environ
1268 Microbiol. 2007;73(10):3123-36. Epub 20070316. doi: 10.1128/aem.01399-06. PubMed PMID:
1269 17369342; PubMed Central PMCID: PMCPMC1907099.

1270 87. Korlath JA, Osterholm MT, Judy LA, Forfang JC, Robinson RA. A point-source
1271 outbreak of campylobacteriosis associated with consumption of raw milk. J Infect Dis.
1272 1985;152(3):592-6. doi: 10.1093/infdis/152.3.592. PubMed PMID: 4031557.
1273 88. Bacon DJ, Alm RA, Burr DH, Hu L, Kopecko DJ, Ewing CP, et al. Involvement of a
1274 plasmid in virulence of *Campylobacter jejuni* 81-176. Infect Immun. 2000;68(8):4384-90. doi:
1275 10.1128/iai.68.8.4384-4390.2000. PubMed PMID: 10899834; PubMed Central PMCID:
1276 PMCPMC98329.
1277 89. Pei Z, Burucoa C, Grignon B, Baqar S, Huang XZ, Kopecko DJ, et al. Mutation in the
1278 peb1A locus of *Campylobacter jejuni* reduces interactions with epithelial cells and intestinal
1279 colonization of mice. Infect Immun. 1998;66(3):938-43. doi: 10.1128/iai.66.3.938-943.1998.
1280 PubMed PMID: 9488379; PubMed Central PMCID: PMCPMC107999.
1281 90. Black RE, Levine MM, Clements ML, Hughes TP, Blaser MJ. Experimental
1282 *Campylobacter jejuni* infection in humans. J Infect Dis. 1988;157(3):472-9. doi:
1283 10.1093/infdis/157.3.472. PubMed PMID: 3343522.
1284 91. Corcionivoschi N, Clyne M, Lyons A, Elmi A, Gundogdu O, Wren BW, et al.
1285 Campylobacter jejuni Cocultured with Epithelial Cells Reduces Surface Capsular
1286 Polysaccharide Expression. Infection and Immunity. 2009;77(5):1959-67. doi:
1287 doi:10.1128/iai.01239-08.
1288 92. Corcionivoschi N, Clyne M, Lyons A, Elmi A, Gundogdu O, Wren BW, et al.
1289 *Campylobacter jejuni* cocultured with epithelial cells reduces surface capsular polysaccharide
1290 expression. Infect Immun. 2009;77(5):1959-67. Epub 20090309. doi: 10.1128/iai.01239-08.
1291 PubMed PMID: 19273563; PubMed Central PMCID: PMCPMC2681765.

1292 93. Taboada EN, Acedillo RR, Carrillo CD, Findlay WA, Medeiros DT, Mykytczuk OL, et
1293 al. Large-scale comparative genomics meta-analysis of *Campylobacter jejuni* isolates reveals
1294 low level of genome plasticity. J Clin Microbiol. 2004;42(10):4566-76. doi:
1295 10.1128/jcm.42.10.4566-4576.2004. PubMed PMID: 15472310; PubMed Central PMCID:
1296 PMCPMC522315.
1297 94. Woodcock DJ, Krusche P, Strachan NJC, Forbes KJ, Cohan FM, Méric G, et al. Genomic
1298 plasticity and rapid host switching can promote the evolution of generalism: a case study in the
1299 zoonotic pathogen *Campylobacter*. Scientific Reports. 2017;7(1):9650. doi: 10.1038/s41598-
1300 017-09483-9.
1301 95. Hofreuter D, Tsai J, Watson Robert O, Novik V, Altman B, Benitez M, et al. Unique
1302 Features of a Highly Pathogenic *Campylobacter jejuni* Strain. Infection and Immunity.
1303 2006;74(8):4694-707. doi: 10.1128/iai.00210-06.
1304 96. Leonard EE, 2nd, Tompkins LS, Falkow S, Nachamkin I. Comparison of *Campylobacter*
1305 *jejuni* isolates implicated in Guillain-Barré syndrome and strains that cause enteritis by a DNA
1306 microarray. Infect Immun. 2004;72(2):1199-203. doi: 10.1128/iai.72.2.1199-1203.2004. PubMed
1307 PMID: 14742576; PubMed Central PMCID: PMCPMC321608.
1308 97. Parker Craig T, Quiñones B, Miller William G, Horn Sharon T, Mandrell Robert E.
1309 Comparative Genomic Analysis of *Campylobacter jejuni* Strains Reveals Diversity Due to
1310 Genomic Elements Similar to Those Present in C. jejuni Strain RM1221. Journal of Clinical
1311 Microbiology. 2006;44(11):4125-35. doi: 10.1128/jcm.01231-06.

1312