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Intracellular Survival and Translocation Ability of Human and Avian *Campylobacter jejuni* and *Campylobacter coli* Strains

Raffaella Campana and Wally Baffone

Abstract

Campylobacter acts using complex strategies to establish and promote intestinal infections. After ingestion via contaminated foods, this bacterium invades and can survive within the intestinal cells, also inducing epithelial translocation of non-invasive intestinal bacteria. In this investigation, the ability of human and avian *C. jejuni* and *C. coli* isolates to survive within two different intestinal epithelial cells lines, Caco-2 and INT 407, as well as the intestinal translocation phenomenon, was assessed. Our data demonstrated that both *C. jejuni* and *C. coli* strains survived in Caco-2 (81.8% and 100% respectively) and INT 407 monolayers (72.7% and 100% respectively) within the first 24 h post-infection period, with a progressive reduction in the prolonged period of 48 h and 72 h post-infection. The translocation of the non-invasive *E. coli* 60/06 FB was remarkably increased in *C. jejuni* treated Caco-2 monolayers (2.36 ± 0.42 log cfu/mL) ($P < 0.01$) and less in those treated with *C. coli* (1.2 ± 0.34 log cfu/mL), compared to *E. coli* 60/06 FB alone (0.37 ± 0.14 log cfu/mL). Our results evidenced the ability of both human and avian strains of *C. jejuni* and *C. coli* to efficiently survive within intestinal cells and induce the translocation of a non-invasive pathogen. Overall, these findings stress how this pathogen can interact with host cells and support the hypothesis that defects in the intestinal barrier function induced by *Campylobacter* spp. could have potentially negative implications for human health.

Keywords

Campylobacter spp. · Virulence · Gut health · Intracellular survival · Epithelial translocation

Abbreviations

CJHS	*Campylobacter jejuni* human sample
CJAS	*Campylobacter jejuni* avian sample
CCHS	*Campylobacter coli* human sample
CCAS	*Campylobacter coli* avian sample

R. Campana (✉)
Department of Biomolecular Science, Division of Pharmacology and Hygiene, University of Urbino, Urbino, Italy
e-mail: raffaella.campana@uniurb.it

W. Baffone
Department of Biomolecular Science, University of Urbino, Urbino, Italy

1 Introduction

Campylobacter is considered the most common and important cause of bacterial gastroenteritis in humans in the developed world (Kaakoush et al. 2015; Cody et al. 2019). In the European Union (EU) the number of confirmed cases has continued to escalate over the years (214,000 in 2013 to 246,000 in 2016 and 2017) (European Food Safety Authority 2018), and in the United States, more than 800,000 cases are annually estimated (Scallan et al. 2011). Poultry is considered to be one of the most important reservoirs of human infections (Wilson et al. 2008; Friesema et al. 2012) and consumption, as well as handling of poultry meat, have been identified as important risk factors (Humphrey et al. 2007; Krutkiewicz and Klimuszko 2010; Wysok and Wojtacka 2018).

The molecular genetics of *Campylobacter* pathogenesis has been extensively studied (Krutkiewicz and Klimuszko 2010) and it is well acknowledged that important virulence factors are involved in the pathogenesis of *Campylobacter* infections, such as the flagella-mediated motility, adherence to intestinal epithelial cells, invasion and survival in the host cells, as well as the ability to produce toxins (Bang et al. 2003; Fouts et al. 2005). During the infection process, *Campylobacter* spp. can pass through the mucous layer of the epithelial cells, attach and then penetrate them (Bolton 2015; Igwaran and Okoh 2019). This interaction is possible to the motility of the bacteria, reached by flagellin, a protein of flagella encoded by *fla*A and *fla*B genes (Lertsethtakarn et al. 2011). Among these, *fla*A is the gene expressed at a higher level, thus considered crucial for bacterial motility. The other genes, *cad*F and *rac*R, control *Campylobacter* spp. colonization (de Oliveira et al. 2019), while invasion ability is supported by the products of *virB*11 and *pld*A genes (Talukder et al. 2008; Wysok and Wojtacka 2018). Other important virulence genes and proteins synthesized by *Campylobacter* species including the invasion antigen C protein, involved in the full invasion of INT 407 cells, and the invasion associated protein gene (*iam*A) (Bolton 2015). Once inside the host cell, *Campylobacter* is able to survive intracellularly for a relatively long period of time, both in phagocytes and intestinal epithelial cells (Naikare et al. 2006; Šikić Pogačar et al. 2009; Buelow et al. 2011). Indeed, it is assumed that the invasion factors activate host cell plasma membrane invaginations (Cróinín and Backert 2012), forming an endosomal compartment that is transported from the apical to the basolateral surface, enabling *Campylobacter* translocation across the intestinal epithelial barrier (Konkel et al. 1992; Hu et al. 2008; Louwen et al. 2012; Backert and Hofreuter 2013). The *Campylobacter* translocation phenomenon may also translocate across the intestinal epithelium other commensal pathogens (Kalischuk et al. 2009), leading to important implications in human mucosal inflammatory responses towards intestinal bacteria.

In this research, the intracellular survival ability of different human and avian *C. jejuni* and *C. coli* strains, as well as the *Campylobacter* induced translocation phenomenon of a non-invasive pathogen was investigated.

2 Materials and Methods

2.1 Bacterial Strains and Growth Conditions

A total of 20 *Campylobacter* spp. belonging to our laboratory collection of strains isolated in Central Italy from human and animal were considered in this study. Samples included 11 *C. jejuni* strains, isolated from patients with gastroenteritis (n = 6) and from avian collected from slaughterhouse environment (n = 5), and 9 *C. coli* from human patients with gastrointestinal diseases (n = 5) and from avian collected from slaughterhouse environment (n = 4). All isolates were previously characterized for adhesion and invasion properties, as well as for the presence of several virulence genes (*cad F*, *flaA*, *dnaJ*, *ciaB*, *pldA*, *cdtA*, *cdtB* and *cdtC*) (unpublished data). The strains were grown on Columbia agar base (VWR, Milan, Italy) plates

supplemented with 5% of Laked Horse Blood (Oxoid, Milan, Italy) and Preston Campylobacter Selective Supplement (VWR) h at 37 °C for 48 h under microaerophilic conditions (5% O_2; 10% CO_2; 85% N_2).

2.2 Epithelial Cells

Caco-2 (human colon adenocarcinoma) cells were propagated as monolayers in Dulbecco's Modified Eagle's Medium (D-MEM) (Sigma, Milan, Italy) supplemented with 10% foetal bovine serum (FBS) (Sigma), 1% non-essential amino acids (Sigma) and 1% antibiotics solution (5000 U of streptomycin-penicillin; Sigma). Intestine 407 (INT 407) cells were cultured in Minimal Essential Media (MEM) (Sigma) supplemented with 10% foetal bovine serum (FBS) (Sigma) and 1 mM sodium pyruvate (Sigma). All the cells were maintained at 37 °C in a 5% CO_2 humidified atmosphere. For internalization and survival assays, Caco-2 and INT 407 cells were seeded at 2×10^4 cells per well in 6-well plastic plates (VWR) and incubated for 7 days at 37 ° C in a 5% CO_2 humidified atmosphere. Before the assays, the cell monolayers were washed twice with phosphate-buffered saline (PBS) pH 7.2.

2.3 Intracellular Survival of *C. jejuni* and *C. coli* Strains in Caco-2 and INT 407 Cells

C. jejuni and *C. coli* strains were cultured for 48 h in Mueller Hinton broth (VWR) with 5% of FCS at 37 °C in a shaking incubator under the above-mentioned microaerophilic conditions. Each bacterial suspension was centrifuged at 1.500 × g for 15 min, the pellet was resuspended in D-MEM containing 1% FBS and adjusted spectrophotometrically to approximately 1×10^8 cell/mL (OD_{660}). One mL of this suspension was inoculated in 6-wells plates containing semi-confluent Caco-2 and INT 407 cells monolayers. Infected cells were then incubated at 37 °C for 3 h in an atmosphere of 5% CO_2. After incubation, the infected Caco-2 and INT 407 cells were washed twice in PBS after 250 μg/mL gentamicin killing and further cultivated in D-MEM containing a sub-lethal dose of gentamicin (10 μg/mL) to suppress eventual growth of viable bacteria from cells. Quantification of intracellular bacteria after until 72 h was performed by twice washings in PBS and subsequent lysing with 1% Triton X-100. The number of surviving bacteria was determined by plating serial dilution of the lysates in sterile physiological solution on Columbia Agar base (VWR); the plates were incubated for 48 h at 37 °C under the described microaerophilic conditions for the quantification of the colonies forming units (cfu). Data were expressed as mean ± sd (cfu/mL) of three independent experiments performed in duplicate.

2.4 Selection of Non-invasive *E. coli* Strain

Four human strains of *E. coli* (*E. coli* 21/01, *E. coli* 126/18 FB, *E. coli* 85/27 AP, and *E. coli* 60/06 FB), kindly furnished by Gamma Laboratory (Fano, Pesaro, Italy), were used in this study. The identification, as well as the antibiotic susceptibility, was performed using VITEK 2 Compact (bioMérieux, Vercieux, France). All the strains were maintained in Tryptic Soya Agar (TSA) (VWR) at 37 °C, while stock cultures were kept at −80 °C in Nutrient Broth n°2 (Oxoid) with 15% glycerol.

To select one non-invasive *E. coli*, invasion assay on Caco-2 cell monolayers was carried out. Briefly, *E. coli* strains were grown in 20 mL of Tryptic Soya Broth (TSB) (VWR) at 37 °C for 18 h. At the end of the incubation period, the suspensions were centrifuged at 3500 rpm for 15 min and bacteria were harvested and re-suspended in 10 mL of DMEM at 1% FBS; for each strain, the optical density was spectrophotometrically adjusted to approximately 1×10^8 cell/mL ($OD_{610\ nm}$) and 1 mL was added to 24 wells plate containing Caco-2 cells prepared as described above. After 6 h of incubation at 37 °C in an atmosphere of 5% CO_2, infected cells were washed twice in PBS and

incubated for another 2 h in DMEM containing 250 μg/mL of gentamicin to kill remaining viable extracellular bacteria. At this point, monolayers were lysed with 1% Triton X-100 (Sigma), serially diluted in physiological saline solution and plated onto TSA (VWR) for the enumeration of invasive *E. coli* (cfu/mL) as described above. Data are expressed as the mean values obtained in three independent experiments performed in duplicate (mean ± sd of cfu/mL). The invasion index, in percentage, was then calculated as a mean of cfu invasive bacteria/cfu inoculated bacteria. The *E. coli* strain with the lowest invasion index was identified as non-invasive and then used for the following experiments.

2.5 Caco-2 M-Like Cell Model

Caco-2 cells were grown in DMEM supplemented with 10% (v/v) fetal bovine serum, 200 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and incubated at 37 °C and 5% CO_2. For *E. coli* translocation assay, cells were seeded onto Transwell filters (3 μm pore size, 1.13 cm^2; Costar Corning Inc., Corning, NY) at 1.5 × 10^5 cells per filter and grown for 21 days, with a change of medium every 2 days.

2.6 *E. coli* Translocation Assays

Briefly, transwell-grown monolayers were washed with PBS and antibiotic-free DMEM was added to the apical and basal compartments. *E. coli* inoculum was added to the apical compartment of all monolayers to achieve a multiplicity of infection (MOI) of 100 cfu per enterocyte. Monolayers were then divided into two groups and half were inoculated with the selected strains of *C. jejuni* or *C. coli* at MOI of 100, whereas the other half received an equivalent volume of sterile broth (control treatment). Following incubation, *E. coli* recovered in the basal compartment (indicating translocation) were enumerated by spreading serial dilutions onto McConkey agar (VWR), incubating the cultures aerobically at 37 °C for 24 h, and enumerating at the dilution yielding 30–300 colony forming units (cfu) per culture. Preliminary experiments were performed to verify that 6 h was the optimal incubation time for *E. coli* internalization and/or translocation (data not shown).

2.7 Statistical Analysis

Statistical analysis was performed using Prism 5.0 (GraphPad Software, Inc., La Jolla, USA). All the data are expressed as the mean values obtained in three independent experiments performed in duplicate. The conditions necessary to perform the parametric tests (one-way Anova, Student t-test) were checked before conducting the analysis, otherwise, non-parametric tests (such as Kruskall-Wallis or Mann-Whitney U test) were utilized. The level of significance was always considered $\alpha = 0.05$.

3 Results

3.1 Survival Ability of *Campylobacter* Spp.

A total of 20 *Campylobacter* spp. were examined for their ability to intracellularly survive in Caco-2 and INT 407 cells up to 72 h post-infection. As shown, most of the strains were able to survive within Caco-2 (90.0%) (Fig. 1a) and INT 407 (85.0%) (Fig. 1b) 24 h post-infection period. A progressive reduction in the survival ability of *Campylobacter* spp. was observed in the following 48 h post-infection period (55% and 40% within Caco-2 and INT 407 respectively). Finally, in the last 72 h post-infection period, only 15% of the strains survived within Caco-2 cells and 20.0% within INT 407 (Fig. 1a, b).

3.1.1 *Campylobacter* Spp. Survival Within Caco-2

The survival of *C. jejuni* and *C. coli* strains within cultured Caco-2 cells was analysed over a 72 h period of incubation (Fig. 2). Quite similar survival characteristics were observed for *C. jejuni*

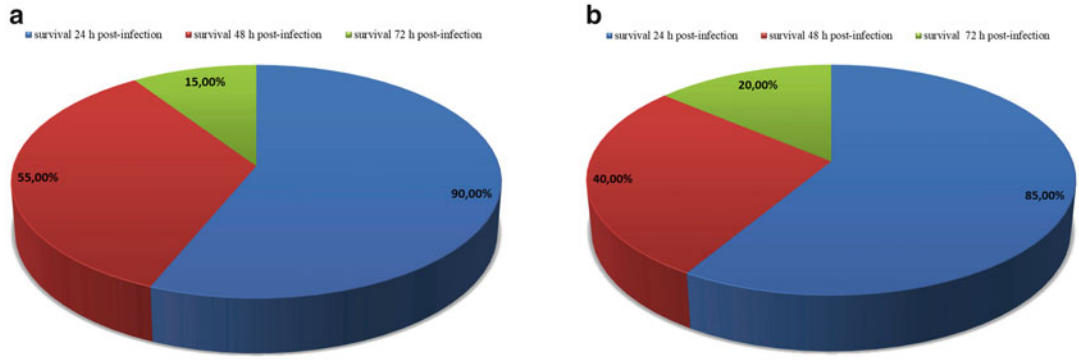

Fig. 1 Percentage of *Campylobacter* spp. survival ability within Caco-2 (**a**) and INT 407 cells (**b**)

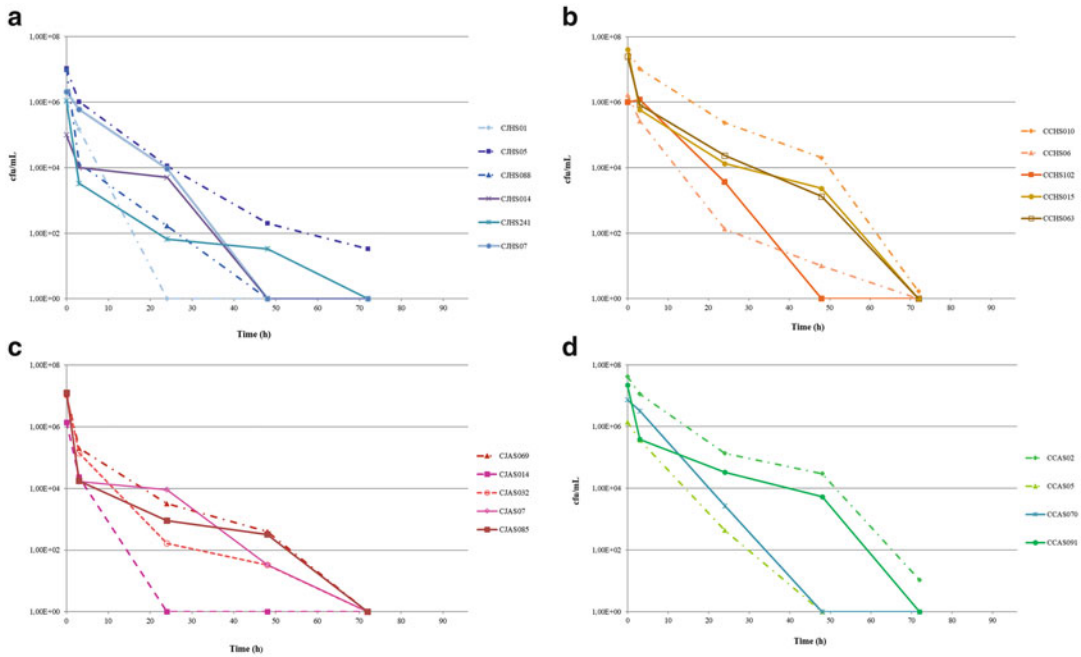

Fig. 2 Survival ability of *Campylobacter* spp. within Caco-2 monolayers up to 72 h post-infection period: (**a, b**) *C. jejuni* and *C. coli* human strains; **(c, d)** *C. jejuni* and *C. coli* avian strains. Data represent mean values of three independent experiments performed in duplicate

and *C. coli* human isolates, though with different cfu/mL values, with a progressive decrease in the number of intracellular bacteria after 24 and 72 h post-infection. In detail, in the case of CJHS05, 1.3×10^4 cfu/mL were recovered 24 h post-infection period higher than the values observed for the other strains (ranging from 6.67×10^1 to 9.33×10^3 cfu/mL); the only strain CJHS01 resulted unable to survive for 24 h within Caco-2 cell. In the following 48 h post-infection, the bacterial survival within Caco-2 cells was remarkably reduced, as showed by the low cfu/mL values recovered for only two *C. jejuni* strains (2×10^2 and 3.3×10^1 cfu/mL for CJHS241 and CJHS05 respectively); moreover, most of the strains did not survive 72 h post-infection (Fig. 2a). Considering *C. coli* human strains, the obtained cfu/mL values after 24 h of incubation were slightly higher compared to *C. jejuni* human isolates, reaching

2.37×10^5 cfu/mL in the case of CCHS010 and ranging from 1.33×10^2 to 2.33×10^4 cfu/mL for the other strains. After 48 h of incubation, CCHS010, CCHS015 and CCHS063 still survived within Caco-2 cells, showing 2×10^4, 2.33×10^3 and 1.33×10^3 cfu/mL respectively. Furthermore, in this case, no strain was able to survive up to 72 h (Fig. 2b).

As regards the survival of *C. jejuni* avian isolates within Caco-2 monolayers, 9×10^3 and 3.3×10^3 cfu/mL were recovered for CJAS07 and CJAS069 respectively 24 h post-infection, while for the other strains about 10^2 cfu/mL were determined; the only strain CJAS014 did not survive. After 48 h of incubation, most of the strains showed low survival ability (about 10^1 cfu/mL) with the only exception of CJAS069 and CJAS1085 that resulted still present within Caco-2 cells (4×10^2 and 3.2×10^2 cfu/mL respectively). No strain survived up to 72 h (Fig. 2c). In the case of *C. coli* avian strains, a higher survival ability compared to *C. jejuni* human isolates was evidenced after 24 h of incubation, with values ranging from 4.3×10^2 to 1.37×10^5 cfu/mL; in the prolonged 48 h post-infection period, only CCAS02 and CCAS091 still survived within Caco-2 cells, showing 5.3×10^3 and 3×10^4 cfu/mL respectively, while no strain was able to survive up to 72 h (Fig. 2d).

3.1.2 *Campylobacter* Spp. Survival Within INT 407

As regards the survival ability of *C. jejuni* and *C. coli* strains within INT 407 cells, data are presented in Fig. 3. Specifically, 24 h post-infection period, most of the human *C. jejuni* strains showed very low survival ability with values ranging from 6.7×10^1 to 3.0×10^2 cfu/mL and, in some cases, no survival was observed (CJHS01, CHJS088 and CJHS24). In the following 48 h post-infection, only the strain CJHS07 still survived with 2.3×10^2 cfu/mL and, as expected, no survival was evidenced 72 h post-infection period (Fig. 3a). Considering the human *C. coli* strains, after 24 h of incubation the

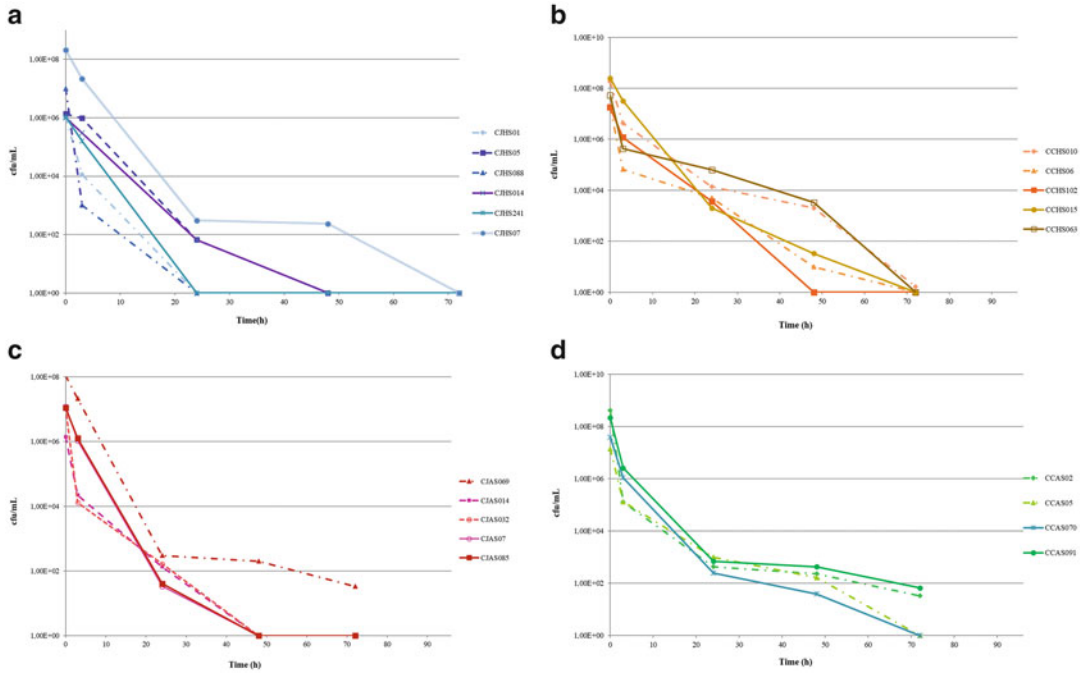

Fig. 3 Survival ability of *Campylobacter* spp. within INT 407 monolayers up to 72 h post-infection period: (**a, b**) *C. jejuni* and *C. coli* human strains; (**c, d**) *C. jejuni* and *C. coli* avian strains. Data represent mean values of three independent experiments performed in duplicate

survival ability resulted higher compared to *C. jejuni* human isolates, with values ranging from to 2×10^3 cfu/mL (CCHS015) to 6.3×10^4 cfu/mL (CCHS063). Most of the strains (CCHS010, CCHS015, CCHS063) were also able to survive 48 h post-infection (values ranging from 1.0×10^1 to 3.3×10^3 cfu/mL) but not up to the prolonged 72 h post-infection period (Fig. 3b).

In the case of the avian *C. jejuni* isolates, all the strains survived only after 24 h post-infection with values ranging from 3.3×10^1 to 1.6×10^2 cfu/mL, while no strains resulted able to survive up to 48 or 72 h post-infection (Fig. 3c). As already noted, all the *C. coli* avian strains showed higher ability to survive after 24 h post-infection in comparison to *C. jejuni* avian isolates, with values ranging from 7.0×10^2 cfu/mL (CCAS091) to 1.1×10^3 cfu/mL (CCAS02). In the following 48 h post-infection period, all the strains resulted still alive within INT 407 (from 3.93×10^1 cfu/mL to 4.33×10^2 cfu/mL), while in the prolonged 72 h post-infection period, only two strains (CCAS02 and CCAS091) still survived within INT 407 cells with low values (about 10^1 cfu/mL) (Fig. 3d).

3.2 Comparison of *C. jejuni* and *C. coli* Survival Ability

In Table 1 are summarized the different survival ability of *C. jejuni* and *C. coli* strains (human and avian source) in both the used cell lines. As shown, in the first 24 h post-infection period, most of *C. jejuni* strains (81.1%) were able to survive within Caco-2 cells regardless of the source (human and avian), as evident by the similar obtained percentages of survival (83.3 and 80% respectively), whilst in the case of *C. coli* 100% of the strains (both for human and avian) resulted surviving. In the second 48 h post-infection period, among the *C. jejuni* isolates, survived only 33.3% of the human strains in comparison to 80% of the avian ones. Similarly, 60% of human and 50% of avian *C. coli* strains were survived within Caco-2 cells. In the last 72 h post-infection period, only a survival percentage of 9.09% was determined for the *C. jejuni* strains, remarkable lower compared to 22.2% of the *C. coli* isolates (20% human strains and 25% avian strains).

As regards the survival ability within INT 407 cells, as already observed for Caco-2 cells, in the first 24 h post-infection period, 72.7% of

Table 1 Survival of *C. jejuni* and *C. coli* strains of different sources (human and avian) within Caco-2 and INT 407 cells after 24 h, 48 h and 72 h post-infection period

	C. jejuni			*C. coli*		
	Human (n = 6)	Avian (n = 5)	Total (n = 11)	Human (n = 5)	Avian (n = 4)	Total (n = 9)
Survival within Caco-2 cells						
24 h post-infection	5 (83.3%)	4 (80%)	**9 (81.8%)**	5 (100%)	4 (100%)	**9 (100%)**
48 h post-infection	2 (33.3%)	4 (80%)	**6 (54.5%)**	3 (60%)	2 (50%)	**5 (55.5%)**
72 h post-infection	1 (16.7%)	0 (0%)	**1 (9.09%)**	1 (20%)	1 (25%)	**2 (22.2%)**
Survival within INT 407 cells						
24 h post-infection	3 (50%)	5 (100%)	**8 (72.7%)**	5 (100%)	4 (100%)	**9 (100%)**
48 h post-infection	1 (16.7%)	1 (20%)	**2 (18.2%)**	4 (80%)	2 (50%)	**6 (66.7%)**
72 h post-infection	0 (0%)	1 (20%)	**1 (9.09%)**	1 (20%)	2 (50%)	**3 (33.3%)**

Data are expressed as number of surviving bacteria (%) recovered after gentamicin protection assay

C. jejuni strains were able to survive (50% human and 100% avian), as well as all the *C. coli* strains (100% human source and 100% avian source). In the second 48 h post-infection period, only 18.2% of *C. jejuni* isolates survived (16.7% human strains and 20% avian strains), in comparison to 66.7% of *C. coli* (80% human strains and 50% avian strains). In the last 72 h post-infection period, only 9.09% of the *C. jejuni* strains survived in opposition to 33.3% of the *C. coli* isolates (20% human strains and 50% avian strains).

3.3 *E. coli* Translocation Induced by *Campylobacter* Spp.

In the last part of this investigation, the *E. coli* translocation induced by *Campylobacter* spp. was examined. As first, we have selected two strains of *Campylobacter*, specifically *C. jejuni* CJHS05 and *C. coli* CCHS06, possessing broad invasive abilities (ID 38.2% and 25.7% respectively, unpublished data), as well as the non-invasive E. coli 60/06 FB resulted to possess the lowest invasive index (ID 0.67%) among the four examined strains (Table 2). In the following experiments, the translocation of *E. coli* 60/06 FB was remarkably increased in CJHS05 treated Caco-2 monolayers (2.36 ± 0.42 log cfu/mL) ($P < 0.01$) and less in CCHS06 treated Caco-2 monolayers (1.2 ± 0.34 log cfu/mL), compared to related control (0.37 ± 0.14 log cfu/mL) (Fig. 4).

4 Discussion

Campylobacteriosis is a significant public health concern worldwide with most human infections caused by *C. jejuni* and *C. coli* (Thakur et al. 2010) through the ingestion of poultry and retail meat products (Igwaran and Okoh 2019). Indeed, these microorganisms can colonise the intestine caecum in extremely high numbers causing severe watery or bloody diarrhoea depending on the individual itself (Scallan et al. 2011). *In vitro* culture assays and molecular methods (Friis et al. 2005; Zheng et al. 2006; Casabonne et al. 2016) have stressed the importance in determining the pathogenicity of *Campylobacter* spp. In addition, the survival of these microorganisms inside various cells has been proposed as a fundamental key in their pathogenesis and infections persistence (Klančnik et al. 2006; Buelow et al. 2011).

Table 2 Invasive index (ID) on Caco-2 cells of the examined *E. coli* strains

Strains	ID
E. coli 21/01	5.67%
E. coli 126/18 FB	3.33%
E. coli 85/27 AP	0.70%
E. coli 60/06 FB	0.67%

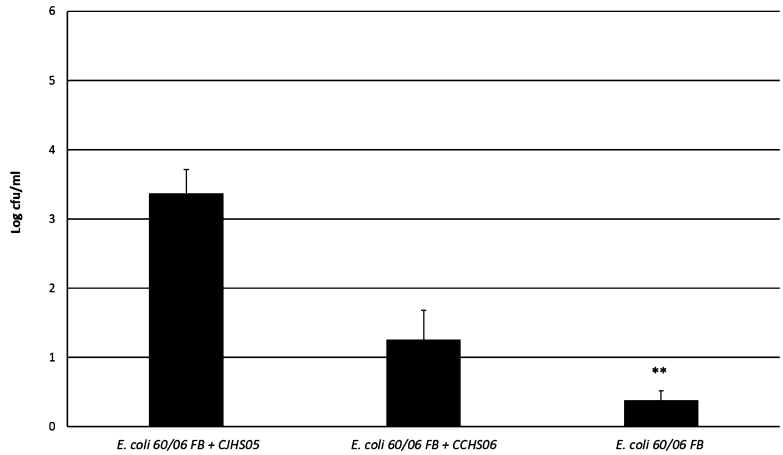

Fig. 4 *E. coli* translocation in Caco-2 monolayers inoculated with *E. coli* 60/06 FB and *C. jejuni* CCHS05 or *C. coli* CCHS06 versus monolayers treated with *E. coli* 60/06 FB alone (control). Data represent mean values of three independent experiments performed in duplicate and asterisks denote values statistically significant compared to the related control (**$P < 0.01$; Student t-test)

In this context, we have examined the survival ability of 20 human and avian *Campylobacter* strains (11 *C. jejuni* and 9 *C. coli*) within two different cell lines, specifically Caco-2 and INT 407, widely used to study *Campylobacter* virulence (Naikare et al. 2006; Buelow et al. 2011; Negretti et al. 2019). The *in vitro* cellular model herein applied was utilized to understand the pathogenic behavior of *C. jejuni* and *C. coli* in a different type of epithelial cell. Indeed, INT 407 cells are considered HeLa derivative because were originally derived from the jejunum and ileum of a 2-month-old Caucasian embryo, later contaminated with HeLa cells, while Caco-2 cells were originally derived from human colon carcinoma. In term of performance, INT 407 cells have the advantage to grow rapidly, we're easy to maintain and manipulate, while Caco-2 cells have a slower grown and are less applicable for some experimental design. Our results showed that both *Campylobacter* spp. were efficiently internalized in Caco-2 and INT 407 monolayers within the first 24 h post-infection period (90% and 85% respectively). Interestingly, 48 h post-infection period, the number of survived bacteria was reduced almost twice within Caco-2 cells (55.5%) and INT 407 (40%), while after 72 h post-infection period, no substantial difference in the survival ability were visible in both the used cell lines (15% and 20% respectively). As regards the relation between *Campylobacter* isolation source (human and avian) and the observed survival ability, was evident that, after 24 and 48 h post-infection periods within Caco-2, the avian strains showed survival percentages higher (from 50% to 100%) compared to those of the human isolates (from 33.3% to 100%). On the contrary, in the case of INT 407 monolayers, in the same post-infection periods, the avian strains showed survival percentages ranging from 20% to 100%, quite similar to those of the human *Campylobacter* isolates (from 16.7% to 100%). All these observations are in line with data of similar researches that reported the ability of *Campylobacter* to survive within various types of non-phagocytic cells for at least 24 h (Backert and Hofreuter 2013) or more prolonged time (Naikare et al. 2006). Nevertheless, while the entry process of *C. jejuni* or *C. coli* into eukaryotic cells has been focused by many studies (Corcionivoschi et al. 2015; Rawat et al. 2018), only a few reports have examined the intracellular fate of these pathogens within eukaryotic cells (Naikare et al. 2006; Watson and Galán 2008; Buelow et al. 2011). Moreover, in the case of *C. coli*, to our knowledge, no reports are available on its ability to survive within intestinal cells, making difficult a comparison of the presented data. In this direction, our data give novel and additional information on the survival ability of *Campylobacter* species, with particular attention to *C. coli* strains that resulted able to highly survive in both the used cell lines.

The functionality of the intact gut barrier is fundamental to gut health, and any impairment of this barrier, including pathogens, toxins or drugs, can increase host susceptibility to various infectious and inflammatory diseases. As a result, certain bacteria could utilize this "active passage" to translocate across the gut epithelium, leading to a different typology of gut-associated diseases (Nagpal and Yadav 2017). This mechanism, defined as bacterial translocation, has been recognized for *Campylobacter* as a phenomenon that may facilitate the translocation of different non-invasive commensal bacteria. In literature Lamb-Rosteski and collaborators (2008) have hypothesized that *C. jejuni* can induce tight junctional disruption, allowing basolateral receptors to migrate to the apical cell surface and promote the following translocation of *E. coli*. In the last part of our research, a Caco-2 M-like cell model, as described by Kalischuk et al. (2010), was developed on transwell filters to study the induced translocation by *C. jejuni* or *C. coli* of a selected non-invasive *E. coli*. In the literature is well documented the translocation of *E. coli* induced by *C. jejuni* 81–176 strain (Kalischuk et al. 2009; Kalischuk et al. 2010) and, to reinforce this observation, here we presented evidence that not only *C. jejuni* but also *C. coli* can cross the intestinal epithelial barrier allowing the translocation of *E. coli*. Therefore, our data support the hypothesis that defects in the intestinal function and/or the disruption of intestinal barrier induced by pathogens, such as *C. jejuni* and

C. coli, can increase the translocation of other bacteria across the intestinal barrier, with possible negative effects on gut and human health. Indeed, this phenomenon can lead to the loss of immunological tolerance (Shen and Turner 2006) and, the failure to downregulate these inflammatory responses in particularly susceptible individuals, can promote the insurgence of chronic inflammations (Resta-Lenert et al. 2005; Kalischuk and Buret 2010).

In conclusion, our investigation showed that both human and avian strains of *C. jejuni* and *C. coli* were efficiently internalized and survived within Caco-2 and INT 407 monolayers for 24–48 h after infection, but not for the prolonged period of 72 h. In addition, we showed that *Campylobacter* spp. can cross the intestinal epithelium thus promoting the translocation of other microorganisms, such as non-invasive E. coli, to extra-intestinal organs. In view of this, further investigations can be addressed to verify whether *Campylobacter* species may favor the translocation of other non-invasive intestinal bacteria, as well as to delineate the consequences of host responses in order to obtain more information on the gut ecosystem equilibrium in human health and diseases.

Conflict of Interest The authors declare that they have no competing interests.

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