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Title: Role of environmental survival in transmission of *Campylobacter jejuni*

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Running title: *Campylobacter* survival in the environment

Abstract

Campylobacter species are the most common cause of bacterial gastroenteritis, with *C. jejuni* responsible for the majority of these cases. Although it is clear that livestock, and particularly poultry, are the most common source, it is likely that the natural environment (soil, water) plays a key role in transmission, either directly to humans or indirectly via farm animals. It has been shown using multilocus sequence typing that some clonal complexes (such as ST-45) are more frequently isolated from environmental sources such as water, suggesting that strains vary in their ability to survive in the environment. Although *C. jejuni* are fastidious microaerophiles generally unable to grow in atmospheric levels of oxygen, *C. jejuni* can adapt to survival in the environment, exhibiting aerotolerance and starvation survival. Biofilm formation, the viable but non-culturable state, and interactions with other microorganisms can all contribute to survival outside the host. By exploiting high throughput technologies such as genome sequencing and RNA Seq, we are well placed to decipher the mechanisms underlying the variations in survival between strains in environments such as soil and water, and to better understand the role of environmental persistence in the transmission of *C. jejuni* directly or indirectly to humans.

Introduction

Campylobacter is the most common cause of acute bacterial gastroenteritis worldwide. In the UK alone it causes an estimated 700,000 infections each year (Tam *et al.*, 2012) and presents an economic burden of over £1 billion per annum (Humphrey *et al.*, 2007). Campylobacteriosis, typically lasting for about a week, is characterised by often bloody diarrhoea, cramping, abdominal pain and fever, and may be accompanied by nausea and vomiting. Occasionally, in immunocompromised patients, the pathogen can spread systemically, leading to more severe sequelae, and it is also a major predisposing cause of the peripheral nervous system disorder, Guillain-Barré Syndrome (Nachamkin *et al.*, 1998).

Campylobacter are spiral members of the Epsilonproteobacteria with small, AT-rich genomes (typically 1.5 – 2 Mb). They are often considered fragile because of the difficulty in growing and maintaining the bacteria in laboratory culture. *Campylobacter* grow optimally at 37-42°C but cannot tolerate drying and are unable to grow in atmospheric levels of oxygen, requiring instead conditions with reduced oxygen levels (5-10% v/v) but raised carbon dioxide levels (5-10% v/v).

Although most human infections (approximately 90%) are associated with *Campylobacter jejuni*, around 10% are caused by *C. coli*, with other species also occasionally causing disease. However, for the purposes of this review, we focus on the most common pathogenic species, *C. jejuni*.

Here, we review the potential role of environments such as soil or water in the transmission of *C. jejuni*, outlining current knowledge about the strategies adopted by *C. jejuni* to persist in such environments, and discussing the evidence that such environments contribute directly or indirectly to the burden of human disease. We use the term "environment" throughout to refer to natural and farmland environments such as soil or

water. We further highlight the key issue of inter-strain variability, emphasising the need to use multiple strains before drawing species-wide conclusions about *C. jejuni*.

Genotyping of *Campylobacter*

There have been a number of genetic approaches used to sub-divide species of *Campylobacter*, especially *C. jejuni* and *C. coli*, including pulsed-field gel electrophoresis (PFGE) (Wassenaar & Newell, 2000), flagellin genotyping (Clark *et al.*, 2005), random amplified polymorphic DNA (RAPD) typing (Nielsen *et al.*, 2000) and ribotyping (Ahmed *et al.*, 2012). However, the development of a multilocus sequence typing (MLST) scheme for *Campylobacter* was a significant step forward in the study of diversity amongst *Campylobacter* populations and the relationships between species within the genus (Dingle *et al.*, 2001). MLST enables unequivocal data to be compared between laboratories world-wide through the use of a readily accessible database (pubmlst.org/campylobacter) containing data for > 28000 isolates (last accessed May 2014) (Jolley & Maiden, 2010).

The initial MLST scheme was based on the analysis of sequences from seven housekeeping genes (*aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkt* and *uncA*) and allows the assignment of isolates to clonal complexes (clusters of closely-related sequence types). Using this approach, it was possible to identify the most abundant common clonal complexes (such as ST-21), though it is also evident that the *C. jejuni* population overall is highly diverse (Dingle *et al.*, 2001, Dingle *et al.*, 2005). Others have extended the MLST scheme for improved applicability to other *Campylobacter* species (Dingle *et al.*, 2008) (Miller *et al.*, 2005). However, the advent of affordable whole genome sequencing (WGS) technologies means that a scheme based on much wider genomic comparisons is likely to supersede MLST. Since the first genome sequence (of strain NCTC11168) was published in 2000 (Parkhill *et al.*, 2000), numerous other *Campylobacter* genomes have been sequenced, revealing extensive within-

94 species diversity (Fouts *et al.*, 2005, Hofreuter *et al.*, 2006, Hepworth *et al.*, 2011). Since
95 MLST profiles can be readily extracted from WGS data, the widespread adoption of WGS
96 would not preclude comparison with previous datasets.
97
98 **Use of genotyping to attribute routes of infection**
99 Most cases of campylobacteriosis occur as isolated, sporadic cases, rather than as part of
100 larger outbreaks, as typically seen with other bacterial pathogens associated with diarrhoea.
101 It is believed that zoonotic transmission of *Campylobacter* spp. to humans occurs primarily
102 through the consumption and handling of livestock, with poultry being the most common
103 source. However, it is clear that other infection routes, including the natural environment,
104 may also contribute.
105 *C. jejuni* has been isolated from diverse animal, human and environmental sources
106 and the isolates obtained subjected to genotyping. Although traditional typing schemes have
107 been of limited use with respect to identification of infection sources, using molecular typing
108 coupled with epidemiological analysis, we are now in a better position to identify and track
109 specific strain types of *C. jejuni* and *C. coli.* Several studies have sought to determine the
110 prevalence of specific clones amongst *C. jejuni* isolates from diverse sources by applying
111 MLST (Colles *et al.*, 2003, Manning *et al.*, 2003, Sails *et al.*, 2003, Dingle *et al.*, 2005,
112 French *et al.*, 2005, Karenlampi *et al.*, 2007, McCarthy *et al.*, 2007, Taboada *et al.*, 2008,
113 Wilson *et al.*, 2008, Sheppard *et al.*, 2009). These studies show that whilst some MLST
114 clonal complexes, such as the ST-21 complex, are widespread, others, such as the ST-61
115 complex, have a more restricted distribution. Although generally considered to be poor
116 survivors outside of their animal hosts, some *C. jejuni* appear to be more able to survive and
117 persist in environmental niches (French *et al.*, 2005, Sopwith *et al.*, 2008). For example, a
118 study of *C. jejuni* in a specific area of cattle farmland in the UK found that environmental

119 water isolates clustered within the ST-45 clonal complex much more frequently than other
120 common clonal complexes (Biggs *et al.*, 2011). The prevalence of specific strain types
121 amongst isolates from multiple sources, including animals and the natural environment, can
122 be compared with similar data from isolates associated with infections in humans. This
123 enables us to model the relative contributions of particular sources to transmission (Wilson *et*
124 *al.*, 2008, Sheppard *et al.*, 2009, Strachan *et al.*, 2009).
125
126 **The natural and farmland environment as a reservoir or source of infection**
127 There have been a number of reports implicating environmental water as the source of an
128 outbreak of campylobacteriosis (Lind *et al.*, 1996, Clark *et al.*, 2003, Auld *et al.*, 2004, Kuusi
129 *et al.*, 2004, O'Reilly *et al.*, 2007). Studies in many countries have shown that drinking water
130 can be a direct source of human infection (Abe *et al.*, 2008, Uhlmann *et al.*, 2009,
131 Karagiannis *et al.*, 2010, Gubbels *et al.*, 2012). Perhaps, more importantly, the environment is
132 also an important source for the primary and secondary colonisation of food animals,
133 particularly chickens (Pearson *et al.*, 1993, Ogden *et al.*, 2007, Perez-Boto *et al.*, 2010). It is
134 likely that routes of transmission flowing through the environment, farm animals and wild
135 animals through to humans interact in complex ways (Figure 1). These interactions would be
136 driven by factors such as the defecation of wild birds or farm animals, water flow due to
137 climatic conditions, spread by flies and other complex ecological parameters. An as yet
138 unexplained phenomenon of seasonality has been reported, with *Campylobacter* infection
139 peaks in late spring (McCarthy *et al.*, 2012, Nichols *et al.*, 2012, Spencer *et al.*, 2012, Taylor
140 *et al.*, 2013). It has been postulated that the natural environment plays a role in this
141 reproducible seasonality, though there is much work to be done before this link is fully
142 established and understood.
143

Campylobacter sub-types associated with non-livestock sources

In addition to the reported link between the ST-45 clonal complex and water sources (French *et al.*, 2005, Sopwith *et al.*, 2008), a number of novel MLST types absent from human isolates have been identified from both environmental water and wild-life, such as wild birds and rabbits (French *et al.*, 2005, Levesque *et al.*, 2008, Hepworth *et al.*, 2011). Members of the ST-45 complex have a widespread distribution but are more frequently encountered in environmental samples than some other "generalists" (French *et al.*, 2005). However, these unusual MLST types are rarely identified amongst isolates from human or farm animal sources. One example of this apparent niche specialisation is ST-3704, which has a specific association with the bank vole (Williams *et al.*, 2010, Hepworth *et al.*, 2011). Comparative genome hybridisation and genome sequence analysis has shown that such strains often lack many of the genes previously associated with the ability to colonise chickens and form a novel clade distinct from the *C. jejuni* strains that are commonly associated with human infections (Hepworth *et al.*, 2011).

Although *C. jejuni* has a relatively small genome, it carries significant levels of variation, potentially indicative of evolution leading to niche specialisation. Comparative genome analyses using microarrays indicate high levels of genome diversity but low levels of genome plasticity in *C. jejuni* (Dorrell *et al.*, 2001, Leonard *et al.*, 2003, Pearson *et al.*, 2003, Champion *et al.*, 2005, On *et al.*, 2006)(Dorrell *et al.*, 2005). These studies have identified discrete regions of diversity within the *C. jejuni* pangenome, called plasticity regions PR1-PR7 (Pearson *et al.*, 2003) or hypervariable regions 1-16 (Taboada *et al.*, 2004, Hofreuter *et al.*, 2006, Parker *et al.*, 2006). This approach was used to sub-divide *C. jejuni* into "livestock" and "non-livestock" clades (Champion *et al.*, 2005, Stabler *et al.*, 2013) and has led to the development of multiplex PCR assays as predictive tests for whether human infection cases were attributable to water and wildlife or domesticated sources (Stabler *et al.*,

2013). The development of new sequencing technologies has made it feasible to carry out much larger and more detailed *Campylobacter* comparative genomics in order to better identify genes or genomic regions associated with isolates from particular sources (Sheppard *et al.*, 2013).

Oxygen tolerance and survival in low nutrient environments

In order to survive in natural environments *C. jejuni* must cope with a number of stresses (Figure 2). Despite the absence of many classic stress response mechanisms, *C. jejuni* strains can survive in a wide range of environments (Kassem & Rajashekara, 2011). In particular, the organism needs to defend itself against atmospheric levels of oxygen and reactive oxygen species (ROS). If the cell is unable to neutralise these toxic compounds, they can lead to protein, nucleic acid and membrane damage. Exposure of *Campylobacter* to oxygen induces catalase, not superoxide dismutase (SOD), the major defence against oxidative stress in most bacteria (Garenaux *et al.*, 2008), though basal activity of SOD may be important (Pesci *et al.*, 1994). The best described catalase in *C. jejuni* is encoded for by *katA* (Cj1385 in *C. jejuni* NCTC11168) (Day *et al.*, 2000, Atack & Kelly, 2009). However, recently another protein (Cj1386) implicated in defence against ROS has been described, encoded by a gene located immediately downstream of *katA* . Cj1386 is an ankyrin-containing protein involved in the same detxoxification pathway as catalase (Flint *et al.*, 2012). Unlike most bacteria, which contain two distinct types of SOD, SodA and SodB, only SodB is present in *C. jejuni. sodB* mutants show elevated sensitivity to oxidative stress (Purdy *et al.*, 1999). Alkyl hydroperoxide reductase (Ahp), consisting of an AhpC catalytic and an AhpF flavoprotein subunit, can also play a role in aerotolerance (Baillon *et al.*, 1999, Poole *et al.*, 2000, Atack & Kelly, 2009). *C. jejuni* appear to lack the flavoprotein domain and only contain the *ahpC* gene. The thioredoxin reductase TrxB is a possible candidate for

reducing oxidised AhpC (Parkhill *et al.*, 2000, Palyada *et al.*, 2004).. The methionine sulfoxide reductases MsrA and MsrB counteract the formation of Met-SO in *C. jejuni*, preventing oxidative damage caused by conformational changes and inactivation of proteins (Moskovitz, 2005, Atack & Kelly, 2008). It has been demonstrated that the heat-shock related proteins HtrA and HspR can promote short-term survival in oxygen (Andersen *et al.*, 2005, Brondsted *et al.*, 2005), which may be important in terms of transmission. *C. jejuni* also differs in its choice of regulatory genes from other enteropathogenic bacteria; KatA and AhpC are regulated by PerR and not OxyR, which is lacking (Cabiscol *et al.*, 2000). The OmpR-type response regulator CosR also plays a role in regulation of the oxidative stress response (Hwang *et al.*, 2011). Fur (ferric uptake regulator) controls expression of a range of oxidative stress genes, preventing the build up of toxic levels of iron within the cell (Stintzi *et al.*, 2008). Other regulatory systems important in *C. jejuni* oxidative stress response are the global transcriptional regulator CsrA, and the two-component regulatory systems CprRS and RacRS (Fields & Thompson, 2008, Svensson *et al.*, 2009, Gundogdu *et al.*, 2014). Different strains of *C. jejuni* can vary with respect to the carriage of genes implicated in aerotolerance. For example, Cj1556, encoding a MarR family transcriptional regulator with a role in oxidative stress response (Gundogdu *et al.*, 2011), is found at much higher prevalence amongst livestock-associated strains than non-livestock associated strains (Champion *et al.*, 2005), suggesting subtle variations in aerotolerance that may contribute to the higher prevalence of some strain genotypes in environmental samples.

In nutrient poor environments, such as water, *C. jejuni* must cope with starvation. *C. jejuni*, in contrast to other bacteria, is generally unable to utilize sugars and relies on amino acids (mainly aspartate, glutamate, serine and proline) and organic acids for energy and growth (Velayudhan *et al.*, 2004, Guccione *et al.*, 2008, Hofreuter *et al.*, 2008). It is likely that *in vivo* peptides provide amino acid sources for *C. jejuni.* Cj0917, a homologue of

carbon starvation protein A (CstA) in *E. coli*, is involved in peptide utlisation and is the most upregulated *C. jejuni* gene during starvation (Rasmussen *et al.*, 2013).

C. jejuni lacks the RpoS-mediated stress resistance system associated with the stringent response in many Gram-negative bacteria (Parkhill *et al.*, 2000). Generally Gram-negative bacteria rely on *relA* and *spoT* to control the stringent response, but there are exceptions, including *C. jejuni*, which relies on *spoT* only (Wells & Long, 2002, Gaynor *et al.*, 2005). It has also been shown that Ppk1-dependent increases in poly-P inside the *C. jejuni* cell are important in low-nutrient-stress survival, osmotic stress survival and biofilm formation (Candon *et al.*, 2007).

Biofilm formation

Biofilm formation is another common strategy for bacterial survival in harsh environmental conditions. *C. jejuni* can form biofilms in water systems and on a variety of abiotic surfaces commonly used in such systems as well as in natural aquatic environments (Lehtola *et al.*, 2006, Maal-Bared *et al.*, 2012). It has been demonstrated that low nutrient conditions (Reeser *et al.*, 2007) and aerobic environments (Reuter *et al.*, 2010) can promote *C. jejuni* biofilm formation, and that this species can survive within polymicrobial biofilms (Ica *et al.*, 2012). Molecular understanding of the mechanisms underlying *Campylobacter* biofilm formation is still in its infancy. Mutational studies have revealed that surface proteins, flagella and quorum sensing appear to be required for maximal biofilm formation (Asakura *et al.*, 2007, Reeser *et al.*, 2007). Transcriptomic and proteomic studies indicate that there is a shift in expression levels of proteins synthesized by biofilm-grown cells, towards iron uptake, oxidative stress defence and membrane transport (Kalmokoff *et al.*, 2006, Sampathkumar *et al.*, 2006).

However, it has been noted that different strains of *C. jejuni* can vary in their ability to form biofilms (Buswell *et al.*, 1998, Joshua *et al.*, 2006). Again, this could be due to subtle differences in gene content between different strains of *C. jejuni*, with potential implications for survival in the natural environment and transmission. For example, the quorum sensing system of *C. jejuni* has been implicated in biofilm formation (Plummer, 2012), yet some strains lack *luxS*, including some strains more associated with water/wild-life sources (Hepworth *et al.*, 2011).

The viable but non-culturable (VBNC) state

It has been reported that *C. jejuni* can respond to unfavourable conditions, including low nutrient environments, by entering a viable but non-culturable (VBNC) state (Rollins & Colwell, 1986, Pearson *et al.*, 1993, Murphy *et al.*, 2006), and that oxygen can accelerate this transition to VBNC (Klancnik *et al.*, 2006). In the VBNC state, bacteria lose the ability to form colonies on normal growth media and reduce their metabolic activity but retain viability and the potential to recover, and even cause infections (Barer & Harwood, 1999). Some evidence suggests that VBNC state formation may be impacted by proteins involved in inorganic polyphosphate (poly-P) metabolism, such as Ppk1, Ppk2 and SpoT (Gaynor *et al.*, 2005, Gangaiah *et al.*, 2009, Gangaiah *et al.*, 2010, Kassem & Rajashekara, 2011).

During the VBNC state, gene expression can be detected for extended periods of time; for instance, the gene *cadF*, encoding a fibronectin-binding protein involved in adhesion and invasion, was expressed at high levels for 3 weeks in *C. jejuni* cells that had entered the VBNC state (Patrone *et al.*, 2013). Furthermore, it has been demonstrated that *C. jejuni* in the VBNC state can adhere to chicken carcasses (Jang *et al.*, 2007) as well as intestinal cells *in vivo* (Patrone *et al.*, 2013).

In this dormant state, *C. jejuni* cells often undergo morphological changes, such as a switch to coccoid form and a reduction in size. Despite the presence of flagella, coccoid forms are non-motile; it has been suggested that the cells simply do not have the energy to maintain motility (Moran & Upton, 1986, Moore, 2001). However, similar changes can be observed when the organism is cultured in the laboratory, suggesting that this may merely represent degeneration of the organism (Moran & Upton, 1986, Moran & Upton, 1987). It has been suggested that different types of coccoid cell forms exhibiting different characteristics exist (Hazeleger *et al.*, 1995). Hence, coccoid cells could be either viable or non-viable.

It has been shown that *Campylobacter* can survive for as long as seven months in phosphate buffered saline at 4^{o}C, with cellular integrity and respiratory activity being maintained for much longer than culturability (Lazaro *et al.*, 1999). Interestingly, the ability to enter the VBNC state varies between strains of *C. jejuni* (Medema *et al.*, 1992, Lazaro *et al.*, 1999, Tholozan *et al.*, 1999, Cools *et al.*, 2003), potentially explaining why certain sub-types of *C. jejuni* are more often found associated with environmental sources. The ability to recover from such a state and retain the ability to cause infections can also vary. Some studies suggest that *C. jejuni* cannot revert from a VBNC state to a form capable of colonisation of chicks (Beumer *et al.*, 1992, Medema *et al.*, 1992, Hazeleger *et al.*, 1995, Hald *et al.*, 2001, Ziprin *et al.*, 2003, Ziprin & Harvey, 2004), whereas others report successful reversion after passage through animals (Saha *et al.*, 1991, Talibart *et al.*, 2000, Baffone *et al.*, 2006). Therefore, this area of research remains controversial and inconclusive.

Interactions with other microorganisms in the environment

The relatively small genome of *C. jejuni*, encoding limited biosynthesis pathways (Kelly, 2001) but multiple transport systems (Dorrell & Wren, 2007), suggests the possibility of reliance on uptake of resources produced by surrounding microbiota. Diverse microorganisms within polymicrobial biofilm communities present a wealth of nutrients, secondary metabolites and iron-bound siderophores that *Campylobacter* could exploit (Pickett *et al.*, 1992, Xavier & Foster, 2007). In addition, secretion of viscous exopolymers by other species can contribute to protection from stresses such as desiccation and killing by disinfectants. It has been suggested that *C. jejuni* are secondary colonisers of pre-existing biofilms sampled from poultry farm environments (Hanning *et al.*, 2008).

Pseudomonas species are ubiquitous in the natural environment and commonly isolated from poultry farms (Arnaut-Rollier *et al.*, 1999). These robust bacteria can grow in mono-species biofilms on a wide range of carbon sources and produce viscous exopolymers that not only capture secondary colonisers (Sasahara & Zottola, 1993) but also protect other species in the biofilm from harsh conditions, antimicrobials and predatory bacteriophages (Rainey *et al.*, 2007, Hanning *et al.*, 2008). *Pseudomonas* have been identified in mixed species communities sampled from chickens and poultry farm environments and have been suggested as primary colonisers that recruit food-borne pathogens into stable mixed biofilm communities (Sasahara & Zottola, 1993, Trachoo *et al.*, 2002, Sanders *et al.*, 2007, Ica *et al.*, 2012).

C. jejuni in biofilms exhibited enhanced attachment and survival when co-cultured with *Pseudomonas* isolated from a meat processing plant (Trachoo *et al.*, 2002). In addition, mixed species communities that include *Pseudomonas* promote *C. jejuni* biofilm growth (Sanders *et al.*, 2007, Teh *et al.*, 2010). Live/dead staining shows that *C. jejuni* is able to maintain a culturable physiological state in biofilms formed with *P. aeruginosa* that are significantly more robust than those formed in monoculture (Ica *et al.*, 2012). In addition, co-

316 culture with different *Pseudomonas* spp. isolated from poultry meat prolonged the survival of
317 over 100 *C. jejuni* field isolates at atmospheric O_2 levels for >48 h. Scanning electron
318 microscopy of these co-cultures demonstrated a close proximity between the different species
319 surrounded by fibre-like structures (Hilbert *et al.*, 2010). These observations indicate inter-
320 species interaction on several levels, affecting metabolic, structural and morphological
321 phenotypes. In addition, strain-specific interactions have been observed between a range of
322 *Pseudomonas* and *C. jejuni* isolates (Hilbert *et al.*, 2010). These observations suggest that
323 *Pseudomonas* biofilms could provide an environmental refuge allowing the survival of *C.*
324 *jejuni* outside the host.

325 It has been proposed that survival within water-borne protozoa, such as
326 *Acanthamoeba polyphaga*, may also enable *C. jejuni* to persist in the environment
327 (Axelsson-Olsson *et al.*, 2005, Snelling *et al.*, 2006). However, compelling evidence that
328 protozoa represent a potential reservoir for *C. jejuni* in natural environments is lacking (Bare
329 *et al.*, 2011). In contrast, it has been suggested that predation, such as grazing by the
330 freshwater crustacean *Daphnia carinata*, might control the abundance of *C. jejuni* in natural
331 waters (Schallenberg *et al.*, 2005).

332

333 **Experiments to analyse survival of *Campylobacter* in water**

334 There have been a number of studies aimed at determining the survival of *Campylobacter* in
335 laboratory model systems representing environmental niches. For example, it has been
336 shown that different *Campylobacter* isolates vary in their ability to survive in water
337 microcosms (Buswell *et al.*, 1998). Survival in water was temperature dependent, with
338 *Campylobacter* generally surviving much better at low temperatures (10 to 16^{o}C) compared
339 to room temperature. Similarly, different *C. jejuni* strains from various origins exhibited
340 origin-dependent ability to survive in sterilised drinking water (Cools *et al.*, 2003). *C. jejuni*

strains can also survive for long periods in well water (Gonzalez & Hanninen, 2012). Although these studies did not include any isolate genotyping, they are consistent with the notion that *C. jejuni* can be sub-divided on the basis of survival in water, and this may reflect the observation that some sub-types are more commonly recovered from natural environments. It is certainly clear that some strains of *C. jejuni* survive in aquatic environments sufficiently well to pose a risk to humans directly through the consumption of untreated water, as well as to promote their chances of transmission via alternative routes.

Conclusion

Campylobacter employs a number of strategies enabling it to survive in the environment and genomics and molecular studies are helping us to better understand the mechanisms involved. There have been considerable efforts to employ genotyping, and more recently genome sequencing, in order to characterise the genetic variation within the species *C. jejuni*. In parallel, epidemiological surveys and phenotypic analyses have revealed differences between *C. jejuni* strain types with respect to prevalence in environmental samples or the ability to survive environmental conditions. The challenge now is to make the link between the genotypic and phenotypic data in order to understand better the mechanisms influencing *C. jejuni* persistence in natural environments such as soil and water, and the role that this might play in transmission of this important pathogen. The reported variations between different strain types of *C. jejuni* also emphasise the limitations of drawing species-wide conclusions based on single strain studies. Only by combining these different strands will we be able to fully understand the role played by environmental survival in the transmission of this important pathogen.

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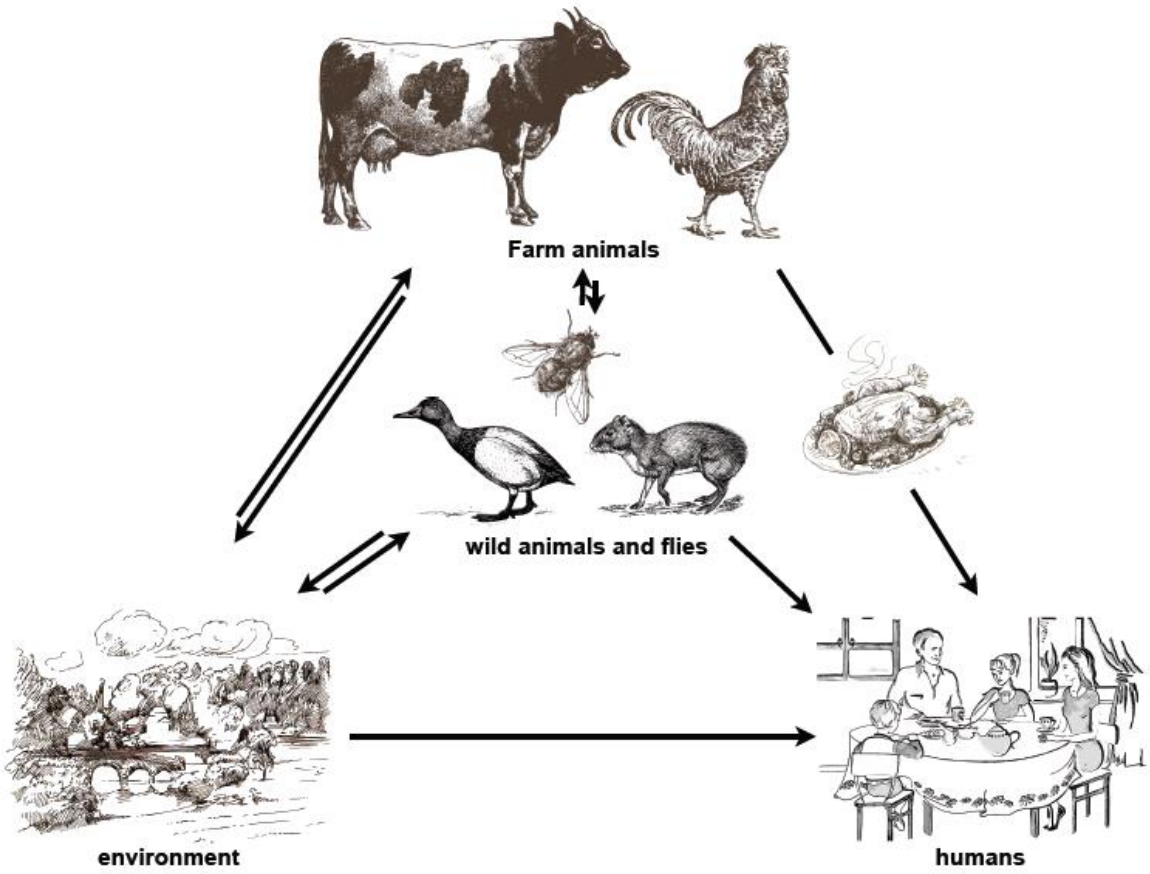

Figure 1. Routes of transmission for *C. jejuni*.

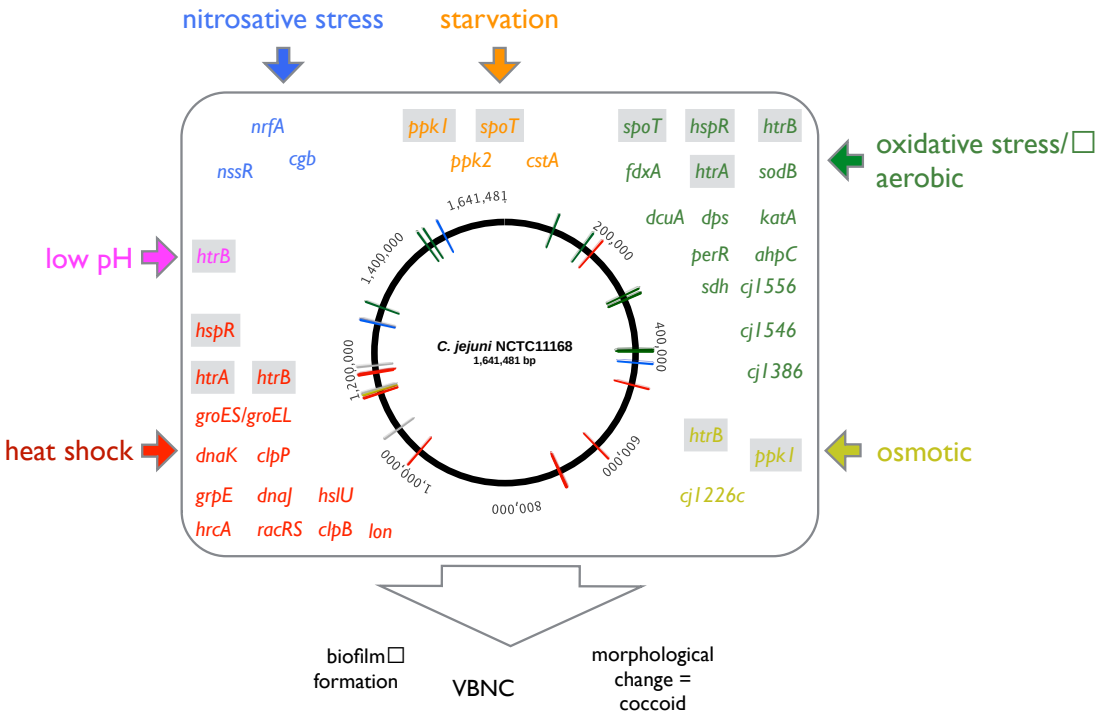

Figure 2. Summary of *C. jejuni* responses to stresses.

The chromosome of *C. jejuni* NCTC11168 is represented by a black circle on which the location of genes, involved in stress responses, are shown as coloured lines. Genes are coloured according to their role; gene names shaded in grey are involved in multiple stress responses. VBNC; viable but non-culturable state.