Of energy and survival incognito: a relationship between viable but non-culturable cells formation and inorganic polyphosphate and formate metabolism in Campylobacter jejuni

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Abstract

Campylobacter jejuni is a Gram-negative food-borne bacterium that can cause mild to serious diseases in humans. A variety of stress conditions including exposure to formic acid, a weak organic acid, can cause C. jejuni to form viable but non-culturable cells (VBNC), which was proposed as a potential survival mechanism. The inability to detect C. jejuni VBNC using standard culturing techniques may increase the risk of exposure to foods contaminated with this pathogen. However, little is known about the cellular mechanisms and triggers governing VBNC formation. Here, we discuss novel mechanisms that potentially affect VBNC formation in C. jejuni and emphasize the impact of formic acid on this process. Specifically, we highlight findings that show that impairing inorganic polyphosphate (poly-P) metabolism reduces the ability of C. jejuni to form VBNC in a medium containing formic acid. We also discuss the potential effect of poly-P and formate metabolism on energy homeostasis and cognate VBNC formation. The relationship between poly-P metabolism and VBNC formation under acid stress has only recently been identified and may represent a breakthrough in understanding this phenomenon and its impact on food safety.

Keywords: viable but non-culturable cells, Campylobacter jejuni, inorganic polyphosphate, polyphosphate kinase, formate metabolism, formate dehydrogenase, acid stress, energy

INTRODUCTION

Since the initial discovery, there have been hundreds of publications documenting VBNC formation in a variety of bacterial species, including important pathogens such as Helicobacter pylori, Vibrio cholerae, Campylobacter jejuni, and others (Oliver, 2005). For example, in Enterococcus faecalis, the proteomic profiles of starved cells were observed to be different from those of the VBNC (Heim et al., 2002), which potentially indicated that the latter was triggered only in response to certain stresses.

With increasing knowledge about VBNC, their significance as a potential risk for public health became evident. A major concern is the inability to detect pathogens in the VBNC state using standard culture-based techniques. This is significant, because VBNC can potentially retain virulence and can be resuscitated back to "normal"/culturable physiological state under favorable conditions, including those available within hosts (Oliver, 2005, 2010). Subsequently, this may increase the potential for undetectable contamination and the spread of infectious agents to susceptible hosts. Although this is a contentious issue with arguments and research either supporting or disproving the ability of VBNC to cause disease in hosts, it is important to note that the possibility for infection should not be merely disregarded (Oliver, 2005, 2010). The lack

Enter VBNC: A Brief History, Significance, and Controversy

Researchers in 1982 observed that two bacterial species, Escherichia coli and Vibrio cholerae, could not be retrieved from saltwater microcosms using a medium that previously sustained their growth (Xu et al., 1982). Although these bacterial species lost cultural viability in response to stress, they still maintained detectable metabolic activity, which suggested that these cells were still viable (Xu et al., 1982). Based on these observations, researchers suggested that stressed bacterial cells might exist in a viable but non-culturable (VBNC) state in response to stress, while maintaining a non-culturable physiological state under favorable conditions, including exposure to formic acid, a weak organic acid, can cause C. jejuni to form viable but non-culturable cells (VBNC), which was proposed as a potential survival mechanism. The inability to detect C. jejuni VBNC using standard culturing techniques may increase the risk of exposure to foods contaminated with this pathogen. However, little is known about the cellular mechanisms and triggers governing VBNC formation. Here, we discuss novel mechanisms that potentially affect VBNC formation in C. jejuni and emphasize the impact of formic acid on this process. Specifically, we highlight findings that show that impairing inorganic polyphosphate (poly-P) metabolism reduces the ability of C. jejuni to form VBNC in a medium containing formic acid. We also discuss the potential effect of poly-P and formate metabolism on energy homeostasis and cognate VBNC formation. The relationship between poly-P metabolism and VBNC formation under acid stress has only recently been identified and may represent a breakthrough in understanding this phenomenon and its impact on food safety.

Keywords: viable but non-culturable cells, Campylobacter jejuni, inorganic polyphosphate, polyphosphate kinase, formate metabolism, formate dehydrogenase, acid stress, energy

Methodology

Campylobacter jejuni is a Gram-negative food-borne bacterium that can cause mild to serious diseases in humans. A variety of stress conditions including exposure to formic acid, a weak organic acid, can cause C. jejuni to form viable but non-culturable cells (VBNC), which was proposed as a potential survival mechanism. The inability to detect C. jejuni VBNC using standard culturing techniques may increase the risk of exposure to foods contaminated with this pathogen. However, little is known about the cellular mechanisms and triggers governing VBNC formation. Here, we discuss novel mechanisms that potentially affect VBNC formation in C. jejuni and emphasize the impact of formic acid on this process. Specifically, we highlight findings that show that impairing inorganic polyphosphate (poly-P) metabolism reduces the ability of C. jejuni to form VBNC in a medium containing formic acid. We also discuss the potential effect of poly-P and formate metabolism on energy homeostasis and cognate VBNC formation. The relationship between poly-P metabolism and VBNC formation under acid stress has only recently been identified and may represent a breakthrough in understanding this phenomenon and its impact on food safety.

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of complete knowledge in regards to VBNC's virulence, factors influencing their resuscitation, and cognate public health risks and ramifications might at the very least provide supportive impetus for prudence when considering virulence associated with VBNC. In fact, under certain scenarios, potential risks may weigh heavily and can pose a severe threat to public welfare. For example, VBNC contamination of food products (Brown, 2004; Dunn and Bach, 2011) and medical equipment (Zandi et al., 2012) can go undetected, impacting consumers, jeopardizing the safety of food, and threatening the lives of susceptible patients.

Of particular interest for food safety is C. jejuni, a food-borne pathogen that can form VBNC under stress (Rollins and Colwell, 1986; Jackson et al., 2007). C. jejuni is a Gram-negative bacterium that can cause disease in humans, including gastroenteritis and debilitating and life-threatening neuropsychiatries (Vandamme and De Ley, 1991; Ono, 1996). The control of C. jejuni in poultry and other food animals and products (e.g., beef, turkey, and milk) has proved to be challenging, due in part to the atypical pathobiology of this bacterium, which lacks many of the classi-
cal stress response factors associated with other enteric pathogens (Parkhill et al., 2008). This singularity of C. jejuni necessitates a closer consideration of all its possible survival strategies, including VBNC formation, in order to enhance on-going efforts to reduce this pathogen in foods. This viewpoint is supported by research showing that C. jejuni VBNC can adhere to the skin of chicken carcasses (Iang et al., 2007), while a recent study reported that C. jejuni VBNC can still express a protein (CadF) that facilitates its attachment to host cells (Patrone et al., 2013). Further, it was also shown that C. jejuni VBNC can colonize suckling mice (Jones et al., 1991). Therefore, in this minireview, we will briefly discuss some of the molecular factors involved in VBNC formation in bacteria and focus in more detail on C. jejuni, highlighting recent research that associates specific metabolic pathways with VBNC formation in this important pathogen.

UNTHREADING THE MYSTERY: GENETIC FACTORS INVOLVED IN VBNC FORMATION AND VIRULENCE

To date, many of the cellular triggers and genetic factors involved in VBNC formation are not well understood. However, increasing research into this phenomenon has revealed glimpses of potential factors that are likely involved in the persistence and expression of virulence in VBNC. Notably, it has been reported that gene expression in VBNC can continue for extended periods of time; for instance, the cytotoxin–hemolysin (vvhA) transcripts were detected in VBNC of V. vulnificus for up to 4.5 months (Saux et al., 2002). Although it is not clear if these expressed genes are directly involved in VBNC formation, the latter example highlights the possibility for maintaining virulence in the VBNC state. Further, many studies reported the expression of virulence-associated genes in VBNC of other pathogens. For example, in a recent study the expression of cadF, a gene that encodes an outer membrane protein that facilitates binding to fibronectin in host cells, was detected at high levels in C. jejuni VBNC for 3 weeks (Patrone et al., 2013). In parallel to these observations, the authors also reported that C. jejuni VBNC were capable of adhering to intestinal cells in vitro, at levels that were lower than that of the cultivable strain (Patrone et al., 2013). In another study, virulence-associated genes, including those encoding flagellin proteins, the cytotoxin distend-
ing toxin, and a Campylobacter invasion antigen that are involved in invading, interacting with the host's intestinal cells were found to maintain a low level of expression in C. jejuni VBNC (Chaisow- wong et al., 2012). Similarly, the mRNA of the Shiga toxin encoding gene (stx1) was detected in VBNC of E. coli O157:H7 (Yaron and Matthews, 2002), which is a notable finding because these toxins are associated with hemolytic colitis, hemolytic uremic syn-
drome, thrombocytopenia, hemolytic anemia, and renal failure (Karmali, 1989). Coccolid-shaped cells of V. cholerae entering a VBNC state were found to express the toxin co-regulated pilus (TCP), a virulence factor that is important for colonization of the small intestine in humans, and were able to colonize infant mice (Knudt and Taylor, 2011). The authors also noted that in a previous study TCP-like appendages could be seen in micrographs of 1 year old V. cholerae VBNC (Chaiyanan et al., 2007). Another inves-
tigation detected viable non-culturable and coccolid-shaped cells of H. pylori in biopsies collected from 12 dyspeptic patients, and these cells expressed vacA, a gene associated with quorum sensing and bacterial virulence (Cellini et al., 2008). Collectively, the afore-
mentioned studies and other published research (Table 1) support the ability of VBNC to maintain some aspects of virulence and/or regain them after resuscitation.

It was shown that two regulatory genes (algU and pact) that code for the alternative sigma factor (σE) and a response regulator, respectively, may be involved in VBNC formation in Pseudomonas fluorescens CHAO, which is used as a biocontrol agent against black root rot (Mascher et al., 2002). Additionally, it was suggested that resuscitation-promoting factor (Rpf)-like proteins might be involved in the reactivation of non-culturable cells of the human pathogen Mycobacterium tuberculosis (Shihveta et al., 2002). A delay in VBNC formation in an S. Typhimurium LT2 mutant was associated with a 99-bp in-frame deletion in the tcpP gene, which is known to be involved in forming a protease complex that degrades the general stress sigma factor RpoS (Kasumoto et al., 2012). Sub-
sequently, the authors suggested that this ClpXP-RpoS relationship might have affected entry into the VBNC state (Kasumoto et al., 2013). Further, RpoS expression was detected for up to 14 days in VBNC of V. vulnificus (Smith and Oliver, 2006), while this stress factor was implicated in the persistence of E. coli in a VBNC state (Boaretto et al., 2003). In another study, the inactivation of OxyR, an oxygen stress regulator, and the cognate catalase enzyme impacted VBNC formation in V. vulnificus (Kong et al., 2004). Collectively, these are very interesting findings and can potentially shed light on the VBNC state of important pathogens and beneficial bacteria; however, this also raises several important questions. For example, the atypical pathogen, C. jejuni, has sub-
stantially documented VBNC state but lacks RpoS, OxyR, and a σE response (van Vliet et al., 1999; Parkhill et al., 2000), while inves-
tigations of a potential C. jejuni resuscitation factor (Cj0645) in strain NCTC11168 showed that the target was not an Rpf ortholog (Morgan, 2010). These observations suggest that the aforemen-
tioned genes may not necessarily be a factor in all VBNC-forming pathogens, which raises the following question: could there be a ubiquitously distributed cellular mechanism that might affect VBNC formation across many species? This question can perhaps be partially addressed by recent findings (detailed below) that
Table 1 | Example of studies that investigated the factors that trigger VBNC formation in *C. jejuni* and possible approaches to resuscitate these cells.

| Reference       | VBNC inducing factor(s)                                                                 | Resuscitation                      | Expression of virulence genes                  | Other                                                                 |
|-----------------|---------------------------------------------------------------------------------------|------------------------------------|------------------------------------------------|----------------------------------------------------------------------|
| Patrone et al. (2013) | Incubation in freshwater microcosms at 4°C                                             | NA                                 | cadF (mediates binding to fibronectin)          | Adherence to human intestinal cells (Caco-2) in vitro                |
| Chaisowwong et al. (2012) | Cold stress (4°C) in a nutrient rich medium (Bolton broth)                              | Co-incubation with Caco-2 in same experiments | Flagellar genes ( flaA, flaB, flaC), cadF, Campylobacter invasion antigen gene (ciaB), cyclophilin dipeptidyl aminopeptidase (CcpA) | Invasion of Caco-2 cells                                             |
| Gangaiah et al. (2010) | Formic acid in Mueller-Hinton broth at 42°C                                            | NA                                 | NA                                             | NA                                                                  |
| Klancnik et al. (2009) | Short-term starvation (6 h incubation in a low nutrient medium)                         | NA1                                | NA                                             | In vivo systemic campylobacteriosis in mice1. Adhesion, invasion, and survival in Caco-2 for up to 4 days1. Heat-stress resistance (55°C for 3 min)1 |
| Gangaiah et al. (2009) | Formic acid in Mueller-Hinton broth at 42°C                                            | NA                                 | NA                                             | NA                                                                  |
| Guilhu et al. (2008) | Storage in bottled water at 4°C in the dark                                            | Incubation into chicken embryonated eggs | NA                                             | NA                                                                  |
| Jang et al. (2007) | Aerobic conditions at 4, 25, and 37°C                                                  | NA                                 | NA                                             | Found after rinsing on artificially inoculated crevices and feather follicles of chicken skin2 |
| Tangwatcharin et al. (2006) | Cold and heat-stress (60°C in brain heart infusion broth)                                | NA                                 | NA                                             | Some loss in the outer membranes of aging cell suspensions            |
| Baffone et al. (2006) | Artificial sea water at 4°C                                                            | In vivo passage in the mouse intestine (dependent on the titer of respiring bacteria in the VBNC state; >10^8 cell/ml) | NA                                             | Colonization of mice                                                 |
| Ziprin and Harvey (2004) | Sterile water at room temperature                                                      | Failure to resuscitate in day-old hatch leghorn and broiler chicks with experimentally introduced normal gut microflora | NA                                             | No colonization of the chicken oocysts 7 days post-VBNC inoculation   |

(Continued)
| Reference               | VBNC inducing factor(s)                          | Resuscitation                                                                 | Expression of virulence genes | Other                                                                 |
|------------------------|-------------------------------------------------|--------------------------------------------------------------------------------|-------------------------------|----------------------------------------------------------------------|
| Ziprin et al. (2003)   | Sterile water at room temperature               | Failure to resuscitate in day-of-hatch leghorn chickens 1 and 2 weeks after inoculation | NA                           | No colonization of the chicken ceca                                   |
| Chaveerach et al. (2003)| Mueller-Hinton broth with formic acid (pH = 4.0) | Inoculation into specific-free-pathogen fertilized chicken eggs                  | NA                           | Colonization of embryonated eggs                                     |
| Thomas et al. (2002)   | Simulated aquatic conditions at 10°C             | NA                                                                              | NA                           | NA                                                                    |
| Cappelleri et al. (1999)| Starvation in sterilized surface water (pH = 6.0 at 4°C) | Inoculation into yolk sacs of embryonated eggs                                    | NA                           | Ability to adhere to HeLa cells after resuscitation                  |
| Tholozan et al. (1998) | Starvation in sterilized surface water (pH = 6.0 at 4°C) | NA                                                                              | NA                           | Increase in VBNC cell volume, decrease in internal potassium content and the membrane potential. Only AMP was detected after 30 days of incubation |
| Lazaro et al. (1999)   | Suspension in phosphate-buffered saline (pH = 7.3) in the dark at 4 or 20°C | NA                                                                              | NA                           | Up to 7 months of viability. Intact chromosomal DNA after 116 and 61 days at 4 and 20°C, respectively. Bleblike membrane vesicles around cells at 4°C |
| van de Giessen et al. (1996)| Suspension in sterilized surface water and potassium phosphate buffer at 4°C | Failure to resuscitate in chickens and mice                                      | NA                           | No colonization of the ceca and intestines of the chickens and mice |
| Stern et al. (1994)    | Suspension in phosphate-buffered saline (pH = 7.2) at 4°C | Resuscitation in 2 out of 39 one-day-old chickens                              | NA                           | Colonization of the ceca of some chickens                             |
| Medema et al. (1992)   | Starvation in filter-sterilized and pasteurized surface water | Failure to resuscitate in 1-day-old chickens and the allantoic fluid of embryonated eggs | NA                           | No colonization of the chicken ceca                                   |
| Jones et al. (1991)    | Sterilized pond water at 4°C                    | In vivo passage in suckling mice (only two strains out of four were retrieved) | NA                           | NA                                                                    |

Information about virulence properties and gene expression in the VBNC is also highlighted. It was not clear if the authors in these studies used a culture/suspension that only contained VBNC cells. NA, not applicable.
link VBNC formation in C. jejuni to the metabolism of inorganic polyphosphate (poly-P), an ancient molecule that is ubiquitous in bacteria and plays a role in energy storage and production (Kornberg et al., 1999, Rao et al., 2009).

Despite the current gaps in knowledge, the studies highlighted previously present a convincing case for researching the virulence of VBNC and their potential impact on public health. This might be of critical relevance when considering the survival mechanisms of atypical pathogens such as C. jejuni and cognate ramifications to public health, including food safety.

**OF ENERGY AND VBNC: A RELATIONSHIP BETWEEN C. JEJUNI VBNC FORMATION AND INORGANIC POLYPHOSPHATE METABOLISM**

Most of the past work that focused on C. jejuni VBNC mainly described the physical, chemical, and environmental triggers that induce this state such as exposure to oxygen, persistence in aquatic microcosms, changes in temperature and pH, and starvation (Jackson et al., 2009; Table 1). In addition, there was an emphasis on strategies aiming at resuscitation of C. jejuni VBNC in vitro or in vivo (Bovill and Mackey, 1997; Cappelleri et al., 1999; Charverach et al., 2003; Baffone et al., 2006; Table 1). Like in many VBNC-forming bacteria, the genetic mechanisms that are associated with the VBNC state of C. jejuni are largely unknown. However, it was recently shown that VBNC formation in C. jejuni might be impacted by proteins involved in the metabolism of inorganic poly-P. Specifically, poly-P is a linear polymer of orthophosphate residues that plays a vital role in C. jejuni and other bacteria as a source of ATP for approximately 500 cellular reactions and as a modulator of stress and survival phenotypes (Kornberg et al., 1999, Rao et al., 2009, Kassem and Rajashankara, 2011). Since (1) maintaining cellular respiration and relatively high ATP levels are two major features of the VBNC state, (2) conserving energy is a basic survival strategy under stress, and (3) C. jejuni, a bacterium with relatively small genome that lacks typical stress responses, has invested in retaining a network of enzymes associated with poly-P metabolism, a link between the poly-P molecule and C. jejuni VBNC appeared to be plausible. The latter mandated a closer look at C. jejuni that revealed that this pathogen possessed two major enzymes, namely polyphosphate kinase 1 (PPK1) and polyphosphate kinase 2 (PPK2), which are principally involved in the synthesis/accumulation of poly-P and associated GTP production, respectively (Gangaiah et al., 2009, 2010). The inactivation of these enzymes leads to pleiotropic effects, influencing different survival phenotypes in C. jejuni (Gangaiah et al., 2009, 2010). Notably, live/dead cell staining analysis showed that the C. jejuni deletion mutants, Appk1 and Appk2, possessed a significantly reduced ability to form VBNC after challenge with formic acid (Gangaiah et al., 2009, 2010). This was confirmed using flow cytometry analysis that revealed a significant change in the cell size and granularity of the Appk1 mutant as compared to the parental strain (Gangaiah et al., 2009), possibly indicating an increase in dead cells in the acid-stressed mutants (Kusters et al., 1997). While investigating the expression of a number of genes that were believed to contribute to the phenotypes of the Appk1 mutant, it was found that the phosphate regulon genes (phoR, pstS, pstC, and the periplasmic substrate binding protein-encoding gene, CJ01176_0750), the multidrug resistance efflux pump gene (mcrC), the global post-transcriptional regulator (cctA), and the stringent response regulator (spoT) were not affected in the acid-stressed mutant cells (Gangaiah et al., 2009). However, ppk2 was significantly down regulated in the formic acid challenged Δappk1 mutant, but the expression of ppk2 was not affected in similarly treated wild-type cells, which further implicates the ppk1 in C. jejuni’s VBNC formation (Gangaiah et al., 2009). Since the Δppk1 mutant was deficient in the accumulation of poly-P and the ppk2 down regulation would possibly reduce the associated GTP production (Gangaiah et al., 2009), the aforementioned observations suggest that the poly-P-dependent ATP/GTP pools and ratios in the ppk mutants might be deficient as compared to that of the parental strain. This assumption is supported by the lower levels of poly-P-dependent GTP and the higher ATP/GTP ratios that were detected in the Δppk2 cells using thin layer chromatography analysis (Gangaiah et al., 2010). Therefore, it appears that the disruption of the poly-P associated enzymes creates an imbalance in the cellular poly-P-dependent ATP/GTP homeostasis, hence affecting the ability of C. jejuni to enter the VBNC state. Notably, it was shown that a putative ATP synthase was down regulated in the VBNC of Enterococcus faecalis (Heim et al., 2002), which prompted the speculation that the survival of VBNC under unfavorable conditions required alternative metabolic pathways to maintain energy (Heim et al., 2002). This possibly includes using energy that was stored as poly-P, further suggesting that an intact poly-P metabolism is essential for VBNC formation/persistence.

It was reported that poly-P occurs in starved and morphologically altered V. parahaemolyticus (Chen et al., 2009), and this molecule also accumulated in structurally intact coccoïd forms of starved H. pylori (Nilsson et al., 2002). However, no direct mechanistic association between poly-P and the formation of VBNC has been described previously. Although further studies are needed to formulate a comprehensive mechanistic model of the involvement of poly-P and its enzymes in VBNC formation, the advances highlighted above might direct and facilitate future research into the VBNC state.

**THE FORMATE CONNECTION: FORMATE METABOLISM AND VBNC FORMATION IN C. JEJUNI**

The findings linking poly-P to VBNC formation in C. jejuni will generate many extrapolations and questions. For example, the ATP/GTP pools in the cell are not only affected by poly-P and its enzymes; hence, could there be other factors impacting this pool and also contributing to VBNC formation in C. jejuni? Further, the Δppk2 mutant only responded to formic acid with reduced VBNC formation as compared to the parental strain, however, there was no difference in the colony forming units (CFU) counts between the mutant and the wild-type after challenge with other organic acids (acetic acid and propionic acid) and hydrochloric acid (Gangaiah et al., 2010). Could the latter observation suggest some specific relationship between formic acid and VBNC formation in C. jejuni? As a matter of fact, the previous two questions are intimately linked, because C. jejuni possesses a highly branched respiratory chain that serves in energy production.
Kassem et al. Role of poly-P and formate in C. jejuni VBNC (Myers and Kelly, 2004), while one of the major energy sources for this bacterium is formate that is metabolized by the periplasmic respiratory protein, formate dehydrogenase (Hoffman and Goodman, 1982; Weerakoon et al., 2009). This is not surprising because formate is a byproduct of fermentation that occurs in the host’s gut, which is the preferable niche for C. jejuni (Weerakoon et al., 2009). It was also reported that the inactivation of the formate dehydrogenase (fdh) in this bacterium resulted in round shaped cells similar to those associated with the VBNC (Figure 1A); however, the fdh mutant did not lose culturability under normal growth conditions (Kassem et al., 2012). Further, when the fdh mutant was challenged with formic acid as described earlier, it showed a significant decrease in culturability and viability as compared to the wild-type, indicating a severe inability to form VBNC (unpublished data; Figure 1B). Therefore, it can be concluded that formate dehydrogenase and formate metabolism are associated with VBNC formation in C. jejuni, likely via their role in energy production. Along these lines, a question worthy of investigation is whether the energy produced by formate metabolism may be linked to poly-P accumulation/degradation? Regardless, both the formate and poly-P metabolism are associated with energy and VBNC production in C. jejuni, further confirming the role of energy conservation in VBNC formation and subsequent survival.

COVETED POTENTIALS: CONCLUSIONS AND CLOSING REMARKS

It has been reported that poly-P and cognate enzymes occur in many bacterial species, suggesting that poly-P metabolism might be ubiquitous in the prokaryotes (Kornberg et al., 1999; Rao et al., 2009). Further, many of these species have a confirmed ability to enter the VBNC state (Table 2), which increases the appeal of a possible VBNC–poly-P link as a potential universal denominator in the formation of this cellular state. While the latter needs further experimental proof, poly-P appears to be a contributor to VBNC formation in the unconventional C. jejuni. Subsequently, the bearings of the findings listed above on efforts aiming at reducing C. jejuni in the food chain might be important, because it is already known that C. jejuni VBNC can adhere to edible products (e.g., on the skin of chicken carcasses; Jang et al., 2007). Further, phosphate-containing chemicals and weak organic acids (e.g., acetate and formate) have been typically used or investigated as means to process, preserve, or decontaminate foods (Sofos and Smith, 1998; Capita et al., 2002; Hirshfield et al., 2003; Ricke, 2003). Organic acids, including formic acid, have also been used or tested as potential feed additives to reduce food-borne pathogens in animals including chickens, the primary reservoir of C. jejuni.
and (d) conditions that favor their infectivity are important for proper assessment of the impact of VBNC on food safety and public health.

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