VIABILITY AND GENE TRANSCRIPTION OF *Campylobacter jejuni* IN INFANT MILK FORMULAS

VIABILIDADE E TRANSCRIÇÃO GENÉTICA DE Campylobacter jejuni *EM FÓRMULAS LÁCTEAS INFANTIS*

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ABSTRACT: This study simulated the contamination of two varieties of infant milk formulas (homemade and commercial) with 10^3 and 10^4 CFU/mL of *Campylobacter jejuni*, that were kept under refrigeration (4-7ºC) for up to 48 hours. The aim of this study was to verify the maintenance of the viability and ability of *Campylobacter jejuni* to produce transcripts of virulence and resistance to stress conditions during periods of 0 (after preparation), 24 and 48 hours. *C. jejuni* remained viable during all analyzed stages and the presence of coliforms was not detected. In general, the counts reduced 1 log cycle after 48 hours for all samples, except the 10^4 CFU/mL inoculum of commercial formula, which reduced 2 log cycles, indicating greater injury of *C. jejuni* in this food matrix. *C. jejuni* showed to be more adapted to homemade matrix, due to high transcription of the gene related to cell invasion, *ciaB*, and more susceptible in the commercial matrix, due to the high transcription of genes related to conditions of stress tolerance (*dnaJ*, *p19*, *sodB*). The low infective dose of *C. jejuni* coupled with greater vulnerability of children less than five years indicate the need for care in the preparation and maintenance of infant formulas, to prevent the use of contaminated raw material and cross-contamination, especially in homemade formulations.

KEYWORDS: Campylobacteriosis. Infants. Gastroenteritis.

INTRODUCTION

The control of food quality is primordial to reduce the risks of population to get infected through food consumption. Among foodborne pathogens, stands out the bacteria of *Campylobacter* genus (GODOI et al., 2010)

Campylobacter jejuni is the most commonly species associated with human cases of foodborne infections (EFSA, 2013). This is the most isolated microorganism in patients with foodborne infection, exceeding about three to four times other enteropathogens such as *Salmonella* or *Escherichia coli* (WHO, 2012). Campylobacteriosis can cause severe diarrhea with mucus or blood, fever, abdominal pain, nausea, vomiting, headache and muscle aches. Although, in most cases, the infection is limited to the signs and symptoms of gastroenteric infection, there may be complications such as meningitis, recurrent colitis, acute cholecystitis and Guillain Barré syndrome (ESTEVES et al., 2011).

Campylobacter can easily contaminate food, including meat, milk and milk products. Cases of campylobacteriosis in humans are usually associated with contact with domestic birds and pets, beyond the consumption of poultry meat and untreated water. Cross-contamination during food preparation in homes has also been described as an important route of transmission (EFSA, 2013).

In Brazil, the occurrence of *Campylobacter* in foods is still underestimated. Microbiological analysis for this microorganism is not routinely performed in cases of food poisoning (CARDOSO, 2009) and is not covered by existing legislation for both milk-based formulas as for other foods (BRASIL, 2001). The resolution nº 12 of January 2nd, 2001, of ANVISA (Agência Nacional de Vigilância Sanitária) only cites this microorganism as a pathogen (BRASIL, 2001).

Cross-contamination in homes is particularly important, since *Campylobacter* can be transferred between food by the hands or utensils (EFSA, 2013). Thus, the preparation of infant formula in places where there is presence of animal products may favor contamination by the handler. Moreover, the refrigerated storage with other raw foods or unprotected also features a procedure that

can facilitate cross-contamination of infant formulas (LINHARES, 2012).

Infants have high immunological vulnerability and an immature digestive system (MAHAN and ESCOTT-STUMP, 2010). For these reasons, the feed of these individuals requires greater hygiene and health care, as in the manufacturing as in the handling and storage of food, particularly in the preparation of infant milk formula served in nursing bottles or cups.

Whereas children, especially those under five years old are more susceptible to campylobacteriosis (SILVA et al., 2007) and the risk of *Campylobacter* contamination during preparation and storage of the formulations, the present study aims to investigate the viability of *C. jejuni* and also to determine the ability to produce transcripts of virulence and resistance to stress conditions in infant milk formulas.

MATERIAL AND METHODS

Study design

We used two varieties of milk formula (homemade – originated from popular recipe based on corn starch – and commercial) prepared in domestic conditions, by boiling. The samples were experimentally contaminated with 10^3 and 10^4 CFU/mL of *Campylobacter jejuni* and stored under refrigeration at a temperature 4-7ºC for up to 48 hours. As a negative control we used uninoculated formulas stored under the same conditions.

Immediately after preparation (0 hours), 24 and 48 hours of storage, the samples were evaluated for the viability and capacity of transcripts production by microorganism.

Three replicates were performed on different days, with 18 analyzes for each formula, totaling 36 samples evaluated. All procedures were performed in triplicate.

Preparation of infant milk formulas

Two kinds of formulations were tested: one industrialized powder and other homemade recipe. The preparation of them was similar to a real situation of preparation/handling in domestic environment. For the present study it was necessary validation in laboratory.

The commercial formula used in the study is a product in powder, classified as a formula for specific follow-up to the infant in tenth month of life, containing prebiotics in concentration of 0.8 g/100mL, DHA (omega 3), lactose, vegetable oils (palm oil, canola oil, coconut oil, sunflower oil), maltodextrin, whey protein from milk,

galactooligosaccharides, fructo-oligosaccharide, glucose syrup, calcium carbonate, fish oil, vitamin C, iron sulphate, caseinate calcium, inositol, zinc sulfate, tocopherol, dibasic potassium phosphate, L-carnitine, cupric gluconate, calcium phosphate, calcium d-pantothenate, nicotinamide, ascorbyl palmitate, vitamin A, vitamin E, vitamin B1, vitamin D6, manganese sulfate, vitamin B2, potassium iodide, folic acid, vitamin K, sodium selenite, vitamin D3, biotin, lecithin emulsifiers, mono and diglycerides, flavorings.

The method to prepare the commercial formula was in accordance with the information stated on the label by the manufacturer. First, the potable and filtered water was boiled for five minutes and cooled for 30 minutes at room temperature. The standard dilution of 15% reported by the manufacturer indicates the addition of a measuring spoon for powder formula for each 30 mL of warm boiled water. Therefore, to 100mL of warm water were used 15.4 grams of infant milk formula.

In order to prepare the homemade formula, also called infant pap or porridge, we proceeded to standardize the following ingredients: per 100mL of pasteurized milk, boiled and cooled at room temperature, was used three grams of corn starch and five grams of granulated sugar. This formula was cooked to boiling point for more 10 minutes to get a creamy consistency, to simulate the domestic conditions.

Experimental inoculation

The study was conducted at the Laboratório de Biotecnologia Animal Aplicada, Universidade Federal de Uberlândia. For experimental inoculation a standard strain of *C. jejuni* NCTC 11351 (MicroBioLogics®) was used, in first peal after the acquisition.

The reactivation of the standard strain was performed as described by the supplier, which was plated on agar *Campylobacter* Blood- Free Selective Medium (m-CCDA) (Oxoid®). The pure and isolated colonies on agar were transferred to tubes containing 10mL of 0.85% NaCl sterile solution (Synth®) and submitted to serial dilutions to determine the concentration of the inoculum.

Aliquots of 1mL of saline containing *C. jejuni* were inoculated in infant milk formulas, to standardize the contamination of 10^3 and 10^4 CFU/mL. After inoculation, the formulations were immediately analyzed (0 h) and stored in a domestic refrigerator at a temperature of 4 to 7 °C, and then evaluated after 24 and 48 hours.

The control groups were inoculated with 1mL of 0.85 % NaCl sterile solution (Synth®) and after they were kept under the same conditions of the tested groups, and evaluated at the same periods.

Microbiological analysis

The counting of *Campylobacter* was carried out using the technique recommended by ISO 10272-18 (2006), using the *Campylobacter* selective agar, Blood-Free Selective Agar Base - m-CCDA (OXOID ®).

Quantification of mesophilic heterotrophic bacteria was performed by the pour plate method, using Plate Count Agar (PCA) (Difco™). For quantification of total and thermotolerant coliforms the most probable number (MPN) technique was used with pre-enrichment in Sodium Lauryl Sulfate broth (Difco™) and confirmation in Brilliant Green Bile Lactose and EC broths (Difco™). The procedures were performed as recommended by Silva et al. (2007).

Confirmation and rna extraction

The confirmation of isolates as *C. jejuni* species was performed by PCR-multiplex, according to the technique described by Harmon et al. (1997).

RNA extraction was performed according to Li et al. (2008), with modifications proposed by Melo, 2013. The isolates from each sample were peaked to four other CCDA agar plates in order to obtain enough bacteria to do the RNA extraction, and then all of this cultivation was transferred to microcentrifuge tubes containing 2mL of 0.85% NaCl solution (Synth®). The mixture was centrifuged at 12,000 g for ten minutes at 4 °C. To the formed pellet was added 1 mL of Trizol (Invitrogen®) and homogenized by vortexing (Phoenix®). Then, 200μL of chloroform was added (Isofar®), and the procedure of homogenization was repeated by vortexing followed by centrifugation at 12,000g for 15 minutes at 4 °C.

The aqueous portion was transferred to a new microtube, which was added with 500μL of isopropanol (Sigma Aldrich®), again homogenized and centrifuged at 12,000g for 10 minutes at 4 °C. At the formed pellet was added 1mL of ethanol 75% (Sigma-Aldrich®) and after homogenization and centrifugation at 7,500g for 5 minutes at 4 °C, the supernatant was discarded. RNA pellets were dried at room temperature to be diluted in 20μL of DEPC water (Invitrogen®). The quantification of RNA was performed in Nanodrop spectrophotometer (Thermo Scientific®).

Reverse transcription

The reverse transcription polymerase chain reaction (RT-PCR) was used to evaluate the production of transcripts, as recommended by Li et al. (2008). For each sample it was used the following steps: 1μg of total RNA (200ng/uL), 10 U of RNase inhibitor, 40 U of MMLV-RT (Amersham Biosciences®), 1X of MMLV-RT buffer (Amersham Biosciences®), 200μM of dNTP (dGTP, dATP, dCTP and dTTP), 126 pmoles of hexamer oligonucleotides as random primers (Invitrogen®) and 20μL of DEPC water (Invitrogen®). The solution was placed in microfuge at 37 °C for one hour to obtain the cDNA.

Subsequently 3μL of cDNA was used for amplification reaction of 25μL, composed of 0.625U of Taq DNA polymerase, 5 mM of MgCl$_2$, 200mM of dNTPs and 4 pmoles of each primer (Invitrogen®). The positive control, *C. jejuni* NCTC 11351, and the negative control, consisting of sterile ultrapure water, were used in all amplification reactions.

The primers used to determine gene transcription were: *ciaB* (invasion), *dnaJ* (thermotolerance), *p19* (iron transport during stress) and *sodB* (defense to oxidative stress), as shown in Table 1. The *C. jejuni* strain NCTC 11351 shows all these genes in its genome (MELO et al., 2013).

Table 1. Primers used to verify the production of transcripts *ciaB*, *dnaJ*, *p19* and *sodB* gene for *C. jejuni.*

Genes	Sequence 5'→3'	Molecular weight (bp)	Reference
ciaB	ATATTTGCTAGCAGCGAAGAG GATGTCCCACTTGTAAAGGTG	157	Li et al. (2008)
dnaJ	AGTGTCGAGCTTAATATCCC GGCGATGATCTTAACATACA	117	Li et al. (2008)
p19	GATGATGGTCCTCACTATGG CATTTTGGCGTGCCTGTGTA	206	Birk et al. (2012)
sodB	TATCAAAACTTCAAATGGGG TTTTCTAAAGATCCAAATTCT	170	Birk et al. (2012)

Amplification was performed in thermocycler, according to cycles: 1 initial cycle at 94 °C for 3 minutes; 45 cycles of amplification in three stages: denaturation at 94 °C for 15 seconds, annealing at 51 °C for 20 seconds and extension at 72 °C for 20 seconds; completing with one more final cycle of extension at 72 °C for 3 minutes.

The separation of the amplified products was performed by electrophoresis on 1.5% agarose gel (Affymetrix®), using the TBE 0.5X buffer (Invitrogen®) and as a molecular weight standard the 50bp marker (Invitrogen®).

Statistical analysis

The results obtained in triplicate analyzes were tabulated and submitted to descriptive statistics to calculate the percentages for the viability of *C. jejuni* and production of transcripts by isolates recovered from formulas in the three periods of cold storage and in three replications. Student's T and Mann-Whitney tests were used to evaluate differences in counts obtained over time, and Paired T test and Wilcoxon test to identify differences in transcript production, using the Graph Pad Prism 7.0 program.

RESULTS AND DISCUSSION

Bioindicators

In both formulas, in all analyzed samples, no total and thermotolerant coliform were detected.

The mesophilic heterotrophic bacteria had counts less than 1.0×10^1 CFU.mL^{-1}. These results show compliance with the standards established by the Codex Alimentarius, equivalent to a limit of 3 MPN of coliforms and mesophilic bacteria equal to 1.0×10^3 CFU.mL^{-1} (BRASIL, 2001; WHO, 2004). The low scores found for biomarkers microorganisms are related to the use of method of cooking in preparation of formulations. Similar results were found by Linhares (2012), who obtained counts less than 1.0×10^2 CFU.mL^{-1} for mesophilic in two infant milk formulas prepared in lacteries in Belo Horizonte, Minas Gerais, Brazil. The author also associated the lower counts with appropriate handling procedures.

Trindade (2009), found, in an analysis of formulas prepared in lactary, counts higher than that obtained in this work. Assunção (2008) also found samples with counts up to 1.0×10^6 CFU.mL^{-1} in Lisboa, Portugal. Both authors noted that formulas that require more handling time also had higher initial aerobic mesophilic bacteria counts, and after 24 hours of cooling, there is an increase in the count of these bacteria.

Viability of *Campylobacter jejuni*

The results show that *C. jejuni* remained viable and culturable during all stages of the experiment (Table 2).

Table 2. Mean counts of *C. jejuni* (CFU.mL^{-1}) in infant milk formulas experimentally contaminated after storage at 4-7 °C for 0, 24 and 48 hours.

Milk Formula	Hour	Counts (CFU/mL)*					
		Control	Inoculum 10^3			Inoculum 10^4	
			Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3
Homemade	0	0	$1,3\times10^3$	$9,8\times10^2$	$1,8\times10^3$	$2,4\times10^3$	$2,2\times10^3$	$6,1\times10^3$
	24	0	$8,0\times10^1$	$9,0\times10^1$	$7,0\times10^1$	$4,6\times10^2$	$1,5\times10^2$	$1,8\times10^2$
	48	0	$1,0\times10^1$	$2,0\times10^1$	$9,0\times10^1$	$1,7\times10^2$	$1,9\times10^2$	$3,9\times10^2$
Commercial	0	0	$9,6\times10^2$	$8,8\times10^2$	$8,4\times10^2$	$2,1\times10^3$	$3,4\times10^3$	$2,0\times10^3$
	24	0	$6,0\times10^1$	$1,0\times10^2$	$1,1\times10^2$	$8,8\times10^2$	$1,2\times10^2$	$1,4\times10^2$
	48	0	$3,0\times10^1$	$3,0\times10^1$	$5,0\times10^1$	$1,0\times10^2$	$4,0\times10^1$	$1,0\times10^1$

Rep.: repetition; * The average results of triplicate analysis.

The low counts of biomarkers, which could compete with *C. jejuni* for nutrients, may have contributed to the agent survival, since, according Muraoka and Zhang (2011), the mechanisms of competitive exclusion influences directly in the presence of *Campylobacter*, which is considered a fragile and bad competitor.

Moreover, it can be inferred that the bacterium has developed devices that aided in their

survival under injury conditions, in that case, the cold stress. Garénaux et al. (2008) have shown that *Campylobacter* provides an effective response to adapt to stress situations, which can allow its survival in adverse conditions, such as variations in temperature and other harsh environments.

Due to the low number of biomarkers in formulas, it was not possible to assess their influence on survival or reduction in *Campylobacter*

counts. The influence of the microbiota naturally presented in foods should be better investigated in other studies, since that despite Muraoka and Zhang (2011) claims that *Campylobacter* veiculated by raw milk consumption is involved in several outbreaks in American states where the commercialization is allowed. Probably the unpasteurized milk has an endogenous microbiota and some level of subsequent contamination, regardless of hygiene practices applied during milking and packaging, and even under these conditions, *Campylobacter* can survive and infect consumers.

The counts of *C. jejuni* decresed in both formulations, with a mean reduction of 1 log cycle in time zero to 24 hours (p=0,002 – 10^3 inoculum; p<0,0001 – 10^4 inoculum), keeping constant after this period (p=0,171 – 10^3 inoculum, p=0,151 – 10^4 inoculum) (Figure 1).
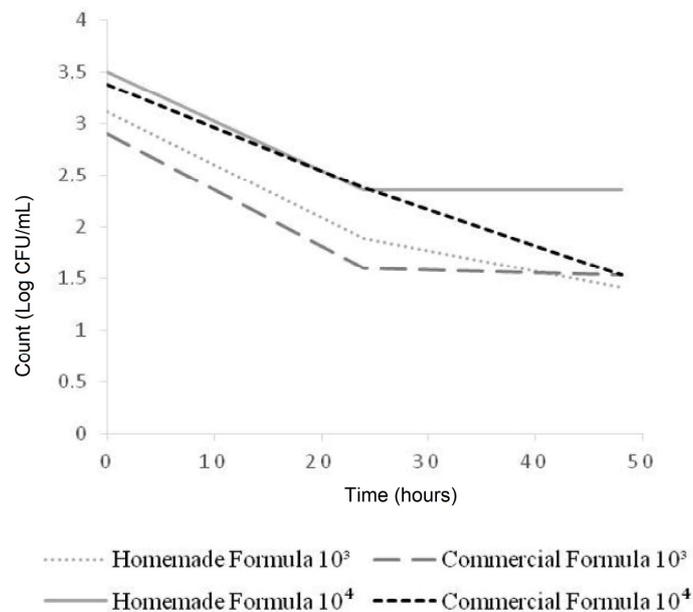
Figure 1. Mean counts of *C. jejuni* during storage at 4-7°C in homemade and commercial infant milk formulas artificially contaminated with 10^3 and 10^4 CFU.mL^{-1}.

It is important to reinforce that for the commercial formula inoculated with 10^4 CFU/mL of *C. jejuni* had a mean reduction of 2 log cycles during the 48 hour period. These findings are in agreement with several previous studies showing that the number of viable *C. jejuni* cells is reduced when kept at 4 °C (EIDEH and AL-QADIRI, 2011; EL-SHIBINY et al., 2009; HADDAD et al., 2009). This decrease is associated with the fact that several biological activities are compromised in these conditions, including protein synthesis, oxygen consumption, catalase activity, ATP generation and motility (HADDAD et al., 2009).

Despite the reduction in cell number of *C. jejuni*, we can infer that the consumption of formulas stored under refrigeration for up to 48 hours can be a risk of infection to children, since the minimum infectious dose is very low, being less than 5.0 x 10^2 CFU/mL (IVANOVIC, 2012).

These results are similar to those obtained by Monteiro (2015), which examined the viability of *Campylobacter* in different types of milk and concluded that the microorganism is able to survive in this food matrix in sufficient numbers to cause infection.

The permanence of the agent in infant milk formulas shows that cross-contamination during the preparation of these foods in home can be an important way of *Campylobacter* transmission. Thus, handling, improper storage, the risk of cross-contamination in the home environment and the fact of the milk (used in the preparation of formulations) is a nutrient medium for the maintenance of pathogens such as *Campylobacter* spp. create conditions that allow the presence of this microorganism in milk formulas (CIDCN, 2014; EFSA, 2013; LINHARES, 2012; MADALOZZO et al., 2007). Allied to this, it is important to emphasize the greater susceptibility to campylobacteriosis in children less than five years, in which the infection can cause complications, including serious diseases such as Guillain Barré Syndrome (CIDCN, 2014; ESTEVES et al., 2011).

Therefore, it becomes essential to adopt measures of good practice in order to reduce the risks during the process of pre and post formulas preparation. Proper hand hygiene in pre preparation,

proper asepsis of household items, which must remain distant from other raw foods or without protection, and storage under refrigeration, are some simple, effective and necessary measures to prevent contamination by this agent.

Gene transcription

The transcriptome analysis is very important to understanding the behavior of *Campylobacter* spp. under several environmental conditions. The number of studies that addresses the determination of environmental effects on virulence and transcription of genes in foodborne pathogens has increased over recent years (BERGHOLZ et al., 2009; OLESEN et al., 2009; OLESEN et al., 2010; RIEU et al., 2010), however little is known about the specific parameters for *Campylobacter* (LIGOWSKA et al., 2011; MA et al., 2009; MELO, 2013; PHONGSISAY et al., 2007; XIE et al., 2011).

The production of transcripts was detected for all the studied genes. The results of RT-PCR performed found a variation in ability to produce transcripts in *ciaB*, *dnaJ*, *sodB* and *p19* genes in *C. jejuni* over time in refrigerated storage (Table 3).

Table 3. Production of transcripts (%) of *C. jejuni* recovered from two infant formulations artificially contaminated with 10^3 and 10^4 CFU.mL^{-1}.

Genes	Hour	Homemade formula		Commercial formula	
		10^3	10^4	10^3	10^4
ciaB	0	-	-	-	-
	24	33,3	33,3	-	66,6
	48	33,3	100	33,3	33,3
dnaJ	0	-	-	33,3	66,6
	24	33,3	33,3	66,6	66,6
	48	33,3	66,6	100	100
p19	0	-	-	-	-
	24	-	-	-	33,3
	48	-	-	33,3	33,3
sodB	0	-	-	-	-
	24	-	-	-	33,3
	48	-	-	-	33,3

(-) No production of transcripts.

In general, gene transcription increased with cold storage. The low production of transcripts at time 0 (immediately after inoculation) may be related to a period of the agent adaptation to the new environment.

The ability of *Campylobacter* to change their transcriptome is associated with the development of a specific mechanisms repertoire that allows rapid metabolic adaptation, as well as changes in growth and behavior in different environments that ensures their survival under stress conditions (LODGE, 2007). Thus, the bacteria regulates gene expression in response to cold shock, heat, and their invasive potential, in order to adapt, survive and, eventually, replicate in these diverse temperatures (STINTZI; WHITWORTH, 2003).

The transcription was most evident in samples inoculated with 10^4 CFU/mL of *C. jejuni* (p=0,0052 – *Test T paired*). Thus, it is likely that a higher number of the microorganism cells favors in the transcription process. According to Guerry (2007) and Lodge (2007), larger quantities of *Campylobacter* inoculum can favor the proteins codification that assist in the maintenance and expression of virulence characteristics, as is the case of FlaA protein (motility) and CadF (colonization).

Regarding the formulations, the transcription of genes for adaptation to stress conditions (*dnaJ*, *p19* and *sodB*) was more evident in strains recovered from commercial formula, but this difference was not significant (p=0,250 W*ilcoxon Test*). Furthermore, the reduction in large number proportions of viable *C. jejuni* cells in these formulations (Table 2) indicates that in this food matrix, the bacteria had become more injured. Therefore, the different conditions that regulates bacterial transcriptome, signaled to the need to transcribe genes that allows their better adaptation to injury and, hence, their survival. Poli et al. (2012) stated that *C. jejuni* can take various properties to modulate its transcriptome, depending on the situation that is submitted, having many alternatives to deal with stress.

Mackichan et al. (2004) identified in *C. jejuni* the existence of a serie of signal transduction which may contribute to bacterial adaptation systems. Several profiles of transcription in *C. jejuni* maintained under a variety of growing conditions, indicates considerable variation in gene expression (HENDRIXSON, 2006; MOURIK, 2011; WÖSTEN et al., 2006; WÖSTEN et al., 2010), supporting the idea that the regulation of genes is essential for maintaining the survival of *C. jejuni*.

The presence of prebiotics and vegetable oils in commercial formulas may have contributed indirectly to the injury of *C. jejuni*. According to Winslow (2005), prebiotics are non-digestible food components that benefits the host by stimulating selectively the growth or activity of desirable bacteria population in the colon. Additionally, the prebiotic after probiotic fermentation process results in the production of metabolites, such as organic acids, which may inhibit the growth of some pathogens, providing additional benefits to the host health. Moreover, vegetable oils, present in the composition of commercial formula are recognized for their antimicrobial properties against spoilage and/or pathogenic bacteria (PIRES; PICCOLI, 2012), as the action of carnosic acid, quercetin and allicin to inhibit the growth of *Campylobacter* (GUTIÉRREZ-DEL-RÍO et al., 2018).

As for homemade formula, no transcription for genes related to environmental adaptation (*p19* and *sodB*) by *C. jejuni* and can be observed smaller percentages of *dnaJ* transcripts compared to the commercial formula. Thus, it is inferred that the conditions of injury (thermal and oxidative stress) were lower in this food matrix and does not need greater energy expenditure for the transcription of these genes. It is possible that the semisolid consistency of homemade formula may have provided microaerophilic conditions, similar to what happens when the microorganisms are inoculated in thioglycolate, favoring its viability. Mourik (2011) stated that in *Campylobacter* gene transcription is regulated to prevent the unnecessary production. Thus, the bacteria did not need to use adaptive mechanisms to ensure its survival.

Furthermore, the use of pasteurized milk in the preparation of homemade formula may also have contributed to the survival and lower transcription of genes adaption, since this nutrient plays a role in cell protection. The protective effect of milk may be related to its action in stabilizing the cell membrane fluidity and the contribution of calcium in cellular enzyme stability (CARVALHO et al., 2001).

Conversely, the production of transcripts associated with invasion process (*ciaB*) was higher in *C. jejuni* maintained in homemade formula. This genetic information shows that, probably, the agent priority in this case was to adapt itself to colonize and cause disease, and no more, to ensure their survival. Therefore, *C. jejuni* showed a higher pathogenic potential when kept in this array.

The *ciaB* is an essential gene for encoding CiaB protein, that is important in the invasion process of epithelial cells (POLI; GUERRY, 2008) and intestinal mucosa (KONKEL et al., 1999; ZIPRIN et al., 2001) The *dnaJ* gene encodes a heat shock protein, which guarantees thermotolerance, and thus the microorganism is able to overcome the abrupt temperature variations, being able to survive due to the adaptation (STINTZI, 2003).

The *sodB* and *p19* genes also have direct correlation with the adaption mechanisms of *C. jejuni*. The translation of *sodB* protein triggers a defense mechanism of the cell against oxidativestress, due to cold shock. Moreover, this gene specifically protects the cellular components, including various cytoplasmic enzymes, DNA, and factors of membrane, against damage caused by oxygen free radicals (STINTZI; WHITWORTH, 2003). The *p19* gene encodes an iron-dependent periplasmic protein whose function is to transport iron (PALYADA et al., 2004) and the regulation of this protein indicates a way to control the level of intracellular iron during stress (BIRK et al., 2012).

CONCLUSIONS

The viability of *C. jejuni* in two infant formulas for up to 48 hours, even under refrigeration, which is a condition of injury, demonstrates that this agent uses devices that ensure their survival in this milk matrix. However, the cold storage was able to reduce the counts of *Campylobacter jejuni* in the studied infant milk formulas.

The highest production of virulence transcripts (*ciaB*) in homemade formulas and the adaptation to stress (*dnaJ*, *p19* and *sodB*) in commercial formulations indicates that transcription by *C. jejuni* is regulated by different mechanisms related priority for their survival. The homemade formula provides the best condition for the agent survival, and thus, allows transcription of the gene related to virulence and has greater importance to public health.

CONFLICTS OF INTEREST
No conflicts exist.

RESUMO: Este estudo simulou a contaminação de duas variedades de fórmulas de leite infantil com *Campylobacter jejuni* (caseiras e comerciais), que foram mantidas sob refrigeração (4-7ºC) por até 48 horas. O objetivo do estudo foi verificar a manutenção da viabilidade e capacidade de produzir transcritos de virulência e resistência a condições de estresse durante os períodos de 0 (após preparação), 24 e 48 horas. *C. jejuni* permaneceu viável durante todas as etapas analisadas e a presença de coliformes não foi detectada. Em geral, as contagens reduziram 1 ciclo log após 48 horas para todas as amostras, exceto o inóculo de 10^4 CFU/mL na fórmula comercial, que reduziu 2 ciclos logarítmicos, indicando maior lesão de *C. jejuni* nesta matriz alimentar. *C. jejuni* mostrou-se mais adaptado à matriz popular, devido à alta transcrição do gene relacionado à invasão celular, *ciaB*, e mais suscetível na matriz comercial, devido à alta transcrição de genes relacionados a tolerância a condições de estresse (*dnaJ, p19, sodB*). A baixa dose infectante de *C. jejuni*, juntamente com maior vulnerabilidade de crianças menores de cinco anos, indicam a necessidade de cuidados na preparação e manutenção de fórmulas infantis, para prevenir o uso de matéria-prima contaminada e contaminação cruzada, especialmente em formulações caseiras.

PALAVRAS-CHAVE: Campilobacteriose. Lactentes. Gastroenterite.

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