



UDC 636.09:616.98  
DOI: 10.31548/ujvs.13(3).2022.34-41

## Immunosuppressive Activity of *Campylobacter Jejuni* Isolates in Relation to the Cellular Link of the Body's Immunoprotection

Tetiana Mazur<sup>1\*</sup>, Nataliia Shchur<sup>2</sup>, Serhii Boianovskiy<sup>3</sup>

<sup>1</sup>National University of Life and Environmental Sciences of Ukraine  
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<sup>2</sup>State Scientific and Research Institute of Laboratory Diagnostics  
and Veterinary and Sanitary Expertise  
03151, 30 Donetska Str., Kyiv, Ukraine

<sup>3</sup>State Scientific Control Institute of Biotechnology and Strains of Microorganisms  
03151, 30 Donetska Str., Kyiv, Ukraine

**Abstract.** Global environmental changes have caused transformations in the biology of microorganisms, especially among campylobacter, which are currently associated with food toxic infections. The means of influence of these bacteria on susceptible organisms, namely toxins, have not been finally clarified. The purpose of this study was to investigate the genetic conditionality of toxin formation in isolates of *Campylobacter jejuni* and determination of the degree of inhibition of the body's protective reactions by toxic fractions of *Campylobacter* protein compounds. The methodology of this study was based on the polymerase chain reaction using primers to indicate the nucleotide sequences of the *Campylobacter jejuni* genome that encode the synthesis of toxins. Samples from 4 *Campylobacter* isolates were examined for the content of protein fractions according to the Lowry assay. The analysis of the electropherogram of the results of DNA amplification in a comparative aspect with the data of standard samples allowed establishing the presence of genome elements that indicate the potential ability to produce toxins in *Campylobacter jejuni* isolates sampled from the material under study. Toxic fractions separated from the supernatant of *Campylobacter jejuni* broth culture are represented by protein-carbohydrate substances. The obtained peak toxigenic fractions of the dialysate of the bacterial culture sediment contained protein within 9.5-17 µg/ml. In the dialysate of the broth culture supernatant, where 5 groups of toxigenic fractions were distinguished, their protein content ranged within 10-85 µg/ml. By reproducing the opsono-phagocytic reaction involving toxigenic fractions of *Campylobacter jejuni*, a sufficiently pronounced immunosuppressive effect of these complexes on the body of warm-blooded animals was established with an opsonic index of  $2.6 \pm 0.03$ . The obtained results allow clarifying the connection between toxin formation in *Campylobacter jejuni* and their immunosuppressive effect on the body of warm-blooded animals and humans, which in the future will positively affect the improvement of measures for the prevention and treatment of animals with this pathology

**Keywords:** campylobacter isolates, DNA amplification, protein fraction, opsono-phagocytic reaction

### Suggested Citation:

Mazur, T., Shchur, N., & Boianovskiy, S. (2022). Immunosuppressive activity of *Campylobacter jejuni* isolates in relation to the cellular link of the body's immunoprotection. *Ukrainian Journal of Veterinary Sciences*, 13(3), 34-41.

\*Corresponding author

## Introduction

Campylobacteriosis is the most common foodborne bacterial zoonosis in the world, caused by *Campylobacter fetus*, *jejuni*, and *coli*. EU countries carry out annual monitoring of the spread of campylobacter among animals and poultry. This infectious pathology is caused by *Campylobacter fetus* and *jejuni*. During infection, campylobacter act in many ways, namely through toxins. As a food toxic infection, campylobacteriosis is registered in many regions of the world [1; 2].

The national medical associations of the countries of the world, as well as the World Health Organization (WHO), emphasize the special role of *Campylobacter* in the aetiology of food toxic infections and attribute them to a single series of classical enteropathogenic genera of bacteria, such as *Salmonella*, *Shigella*, and *Yersinia* [3; 4]. This is caused by the extraordinary geographical distribution of *Campylobacter*, high rates of morbidity and intensive circulation of the pathogen among animals and certain social groups of the population [5; 6]. Separate studies indicate a high degree of carriage of campylobacteriosis in cattle, pigs, dogs, and poultry against the background of a significant spread of campylobacterial diarrhoea in humans [6; 7]. The most pathogenic are *Campylobacter* of the *fetus* and *jejuni* subspecies, as well as the *coli* species [8; 9].

*Campylobacter* is included in group No. 2, which combines aerobic (microaerophilic, motile, spiral) and gram-negative bacteria [10]. The genus *Campylobacter* includes 15 species of bacteria: *fetus*, *hyointestinales*, *jejuni*, *cinaedi*, *coli*, *concisus*, *cryaerophila*, *fennelie*, *lari*, *mucosalis*, *nitrofigillis*, *sputorum*, *upsaliens*, as well as *Weilonella curva* and *Weilonella succinogenes* species added to this genus. In turn, some species combine subspecies and biovars. Thus, the *fetus* species unites the *fetus* and *venerealis* subspecies, the *jejuni* species – *jejuni* and *doylei* subspecies, the *sputorum* species – *sputorum*, *bubulus*, and *fecalis* biovars [11].

*Campylobacter* bacteria are divided into catalase-positive (*C. fetus* with subspecies: *spp. venerialis*, *spp. fetus*; *C. Jejuni* with subspecies: *spp. jejuni*, *spp. doylei*; *C. coli*; *C. lari*; *C. hyointestinalis*) and catalase-negative (*C. sputorum* with two biovars: *sputorum* and *bubulus*, *C. mucosalis*) [5]. Some species that previously belonged to the genus *Campylobacter* are now separated into a new genus. Thus, *C. piloridis*, associated with gastric ulcer disease, is separated into an independent genus of *Helicobacter*. The range of microbial species that are candidates for inclusion in this taxon is also constantly increasing [10; 12]. The antigenic composition of *Campylobacter* is quite complex and contains H-, O-, and K-antigens [13].

Important surface antigens are lipopolysaccharide and acid-soluble protein fraction. These antigens play a leading role during serotyping of *C. hepaticus* and *C. jejuni* and serodiagnosis of campylobacteriosis. The question of the existence of a “universal” *Campylobacter* antigen, i.e., such an antigen, the antibodies to which can be detected in the serum of all patients in sufficiently high titres, stays open. But all *Campylobacter* serotypes have a common protein antigen with a molecular weight of about 62 kDa. This protein fraction is optimal for identification of campylobacteriosis during serological diagnosis [14].

Antigenic differences between bacteria of different serotypes relate to the carbohydrate composition of the

internal lipopolysaccharide of *Campylobacter* [15]. The chemical composition of lipopolysaccharide of *Campylobacter jejuni* is similar to analogous antigens of other enterobacteria. Apart from the similarity of the chemical structure, the existence of a significant immunological affinity between lipopolysaccharides of *Campylobacter* and other pathogens of intestinal infections – *Salmonella*, *Yersinia*, *Brucella*, *Shigella* [16] has been proven.

*Campylobacter* is endowed with a powerful list of virulence factors that cause intoxication of the body and predetermine the development of pathological processes with distinct clinical manifestations [17]. The issue of *Campylobacter* toxin production is of important practical and theoretical importance [18].

Like all Gram-negative bacteria, *Campylobacter* contains endotoxins. It is known that the endotoxin of Gram-negative bacteria is a lipopolysaccharide of the cell wall [15]. Even though, in experiments, the thermostable endotoxin of *Campylobacter* can cause haemorrhagic and necrotic changes in the skin at the places of introduction of the bacterial suspension and even the death of animals, its role in the pathogenesis of campylobacter enteritis is not considered leading [19].

The ability of various species of *Campylobacter* to produce a thermolabile enterotoxin, which has antigenic affinity with cholera toxin and enterotoxin of *E. coli*, is generally recognized [18]. Bacterial toxins can cause an inflammatory reaction of the skin, spleen hyperaemia, necrosis of lymphoid elements, and in blood vessels – thrombosis and intravascular coagulation, collapse, and haemoglobin enrichment of their walls [20].

In addition to endotoxins and enterotoxins, *Campylobacter* synthesizes cytotoxins. A considerable number of such compounds were found among highly cytopathogenic “chicken” strains (44.1%), which determined the leading role of chickens as a potential source of pathogens of campylobacteriosis [7; 21].

The susceptibility of the macroorganism to campylobacteriosis causes immunological disorders, including various immunodeficiency states [22]. Although the study of the issue of toxin formation among microorganisms is quite popular, the specific features of toxin formation in *Campylobacter* depending on the virulence of the pathogen, the nature of the manifestation of the toxic effect of these objects *in vivo* are still unclear, which forms the relevance of this area of research.

The purpose of this study was to identify the degree of inhibition of the body's defence reactions by toxic fractions of *Campylobacter* protein compounds and to investigate the genetic conditionality of toxin formation in *Campylobacter jejuni* isolates.

## Materials and Methods

The study was conducted at the State Research Institute of Laboratory Diagnostics and Veterinary-Sanitary Examination (Kyiv) during 2021-2022 per ISO 10272-1:2017 (Microbiology of the food chain – Horizontal method of detection and counting of *Campylobacter spp.* – Part 1: Detection method) [23].

From 2,120 samples of faeces and droppings, caecum (caecal processes) with contents from cattle, pigs,

and poultry, obtained from farms of various forms of ownership in Ukraine during 2021, 448 isolates – pathogens of zoonoses and commensal microorganisms – were isolated and identified, which is 21.1% of the total number of samples. 33 isolates of *Campylobacter jejuni* were typed according to general biological properties. According to the source of isolation: from poultry – 26, cattle – 5, pigs – 2, from which 4 isolates of *Campylobacter jejuni* were selected for work.

During the study, certified nutrient media and selective additives (horse blood serum) were used. Antibiotic susceptibility of isolates was investigated using the disk diffusion method, according to EUCAST recommendations [24].

The sample preparation sequence for obtaining DNA from campylobacter field isolates included several steps. 100 µl of a 48-hour broth culture of bacteria washed with buffered physiological solution (BPS) was placed in microcentrifuge tubes (Eppendorf type) with a capacity of 1.5 cm<sup>3</sup>. Then 300 µl of lysis buffer containing 6M guanidine thiocyanate was added to each sample and mixed with a vortex. The test tubes with the mixture were heated at 65°C for 5 minutes and re-mixed using a vortex. The sample material was precipitated by centrifugation at  $5 \times 10^3$  rpm for 5 seconds. Subsequently, 30 µl of silica gel was added to each sample. The mixture was vortexed for 10-15 seconds and left at rest at room temperature for 2 min until the sorbent turned into sediment. The samples were then re-mixed and settled for 5 minutes.

To separate the sorbent fractions with the material under study and solvents, the test tubes were centrifuged at 5,000 rpm for 30 seconds. Each sample was washed first with 300 µl of lysis solution, and then twice with 500 µl. The mixture consisted of 70% ethanol, 100 mM NaCl, and 10 mM Tris-HCl (pH 8.0). The supernatant was removed. The precipitated substance was dried for 5-7 minutes under a thermostat at 65°C. Subsequently, 50 µl of TE buffer (pH 8.0) was added to each Eppendorf for DNA elution and placed in a thermostat at 65°C for 5-6 min. During elution, the samples were vortexed every minute. The substance was again precipitated by centrifugation at 12,000 rpm for 2 minutes. The supernatant containing the DNA obtained for further research was collected in pre-prepared test tubes.

Before setting up the polymerase chain reaction (PCR), two reaction mixtures were prepared – “upper” and “lower”. The “lower” reaction mixture contained 2.5 µl of primer and nucleotides, mixed on a vortex, with a final concentration of each primer of 25 rMol/sample. The primers used in the study were manufactured by Wizard® (USA). 5 µl of each obtained mixture of the corresponding sample was placed in 0.5 cm<sup>3</sup> microtubes, followed by the application of 10 µl of wax melted at 95°C to the surface until the entire surface was completely covered. The composition of the “upper” reaction mixture included, considering controls, 10.0 µl of 5% PCR buffer, 3.0 µl of 50 mM MgSO<sub>4</sub>, 6.0 µl of H<sub>2</sub>O, 1.0 µl of Taq polymerase. In test tubes with the “lower” reaction mixture, the surface of the wax layer was covered with 10 µl of the “upper” reaction mixture and, according to the sample labelling, 10 µl of experimental or control DNA and two drops of petroleum jelly were applied. Mixtures of DNA of somatic cells of agricultural animals (cattle, pigs, sheep) and DNA of *Escherichia coli* and *Salmonella typhimurium* were used as negative control

during amplification. The polymerase chain reaction was performed according to the program using the thermal cycler “Tertsik” in the active control mode.

Determination of the nature of the toxic component of *Campylobacter jejuni* isolates was based on the establishment of its biochemical nature. To obtain the exotoxin, the protein fractions of the culture liquid of *Campylobacter*, grown for 48 h at 42°C in Preston’s broth, were extracted. After precipitation of bacterial cells by centrifugation at 3,000 rpm for 30 minutes, the supernatant obtained from the samples was transferred to clean test tubes. Protein fractions of each sample were isolated by adding up to 60% saturation of dry ammonium sulphate salt to each of the test tubes with simultaneous pH control. Denatured proteins of the samples were obtained as a result of settling the mixtures for 12 h at 4°C, centrifuged at 3,000 rpm for 30 min and dissolved in 3 M ammonium sulphate in a ratio of 1:3.

The quantitative content of the isolated proteins was investigated using the Lowry protein assay [25]. Samples from each of the 4 isolates of *Campylobacter* included 5 samples: culture fluid, supernatant after protein precipitation of culture fluid (4.14 mg/ml), supernatant dialysate (1.015 mg/ml), sediment (0.397 mg/ml), and sediment dialysate (0.23 mg/ml). The constituents of the sediment and supernatant were detected using ion exchange chromatography on TSK gels.

Ion exchange chromatography of the sample (e.g., sediment) was performed on a column (2 × 35 cm) Fractogel DEAE-650-s “Merck” (Germany), equilibrated with 0.01 M Tris-HCl buffer (pH 7.0). The sample (20 ml, 50 mg of protein) was applied to the column, eluted with a linear gradient of NaCl (0-1 M, 150 ml each) at 24 ml/h. Substances identical to the fractions of the obtained peaks were tested for toxic activity. The harmful effect of *Campylobacter jejuni* toxin on the body was studied by determining the opsonic index.

Under sterile conditions, a mixture of 0.1 ml of rabbit blood and 0.1 ml of 2% sodium citrate solution was introduced into centrifuge tubes. The test tubes were labelled according to the campylobacter isolate under study, and 0.1 ml of 2 billion suspensions of the washed agar culture of *Campylobacter jejuni* bacteria were added to the citrated rabbit blood in a certain test tube. In another test tube with similar components, 0.1 ml of a certain fraction of toxin-containing substances obtained from the supernatant of *Campylobacter jejuni* broth culture was added. The reagent tubes were kept for 30 minutes in a thermostat at 37°C, then centrifuged at 3,000 rpm and the supernatant with white blood cells was selected with a pipette. Smears were prepared from the leukocyte-containing substance and stained according to the Romanowsky-Giemsa method [4] to count phagocytic bacteria in toxin-containing and toxin-free fractions.

Ion exchange chromatography of the sample (supernatant) was performed using a column (3.0 × 40 cm) Fractogel DEAE-650-m “Merck” (Germany), equilibrated with 0.01 M Tris-HCl buffer (pH 7.0). The sample (10 ml, 40 mg of protein) was applied to the column and eluted using a linear NaCl gradient (0-1 M, 150 ml each) at 30 ml/h. Substances identical to the fractions of the obtained peaks were tested for toxic activity. Statistical analysis was performed according to the program R: A Language [26].

## Results and Discussion

All 33 typed field isolates of *Campylobacter* compared to the reference strain *Campylobacter jejuni* ATCC 33291 demonstrated similar biochemical properties – they

produced oxidase, catalase, and urease, decomposed hippurate, and showed growth at +37°C and +42°C (Table 1). This is consistent with the results of studies by E. Lucio, I. Sakaridis, and L. García-Sánchez [5; 6; 10].

**Table 1.** Differential features of pathogenic isolates of *Campylobacter jejuni*

Isolate	Origin	Oxidase	Catalase	Urease	Growth at +25°C	Growth at +37°C	Growth at +42°C	Hippurate Na	Cephalothin
C. jejuni No. 1	Broiler chicken	+	+	–	–	+	+	+	sensitive
C. jejuni No. 2	Broiler chicken	+	+	–	–	+	+	+	sensitive
C. jejuni No. 3	Broiler chicken	+	+	–	–	+	+	+	sensitive
C. jejuni No. 4	Broiler chicken	+	+	–	–	+	+	+	sensitive
C. jejuni ATCC 33291	Reference strain	+	+	–	–	+	+	+	sensitive

Careful verification of the basic cultural, morphological, and biochemical properties of field isolates is extremely necessary since these data are essential when working with experimental material. They confirm the work with a pure bacterial culture.

Under unfavourable conditions, *Campylobacter* species are transformed from the classic mobile spiral form into a ball-shaped one. Therewith, they stay workable, but transition into an uncultivated state. The use of media with elements of animal blood during the study helped speed up the adaptation time of the isolates to artificial cultivation conditions. In the presence of organic iron compounds in the nutrient medium, the cell membranes of the bacterial wall become more resistant to the previously mentioned stresses.

As the experience of many scientists proves [1], the use of PCR testing in the search for agents and products of their metabolism is more effective compared to cultural methods, especially if it concerns *Campylobacter spp.* To identify the toxin-synthesizing ability in a comparative aspect, the order of the DNA nucleotide sequences of each of the 4 selected isolates of *Campylobacter jejuni* was analysed. To identify specific oligonucleotide primers, data on DNA sequences of campylobacter were used according to the MLST principle. They are registered in the international GenBank database and implemented using Pub MLST [27] and Genome Profiler software. The search resulted in primers with the following nucleotide sequences: F (ATGAAAAATATTTAGTTTTTGCA) and R (ATTTTATTATTTGTAGCAGCG) (Table 2).

**Table 2.** Temperature regimes of the amplifier for PCR reproduction using forward and reverse primers

Primers F: ATGAAAAATATTTAGTTTTTGCA R: ATTTTATTATTTGTAGCAGCG		
Stage	Mode	Number of cycles
1	t 95°C – 5 min	1
2	t 94°C – 1 min	–
	t 56°C – 1 min	4
	t 72°C – 2 min	–
3	t 94°C – 30 s	–
	t 55°C – 30 s	28
	t 72°C – 30 s	–
4	t 75°C – 5 min	1
5	t 10°C	1

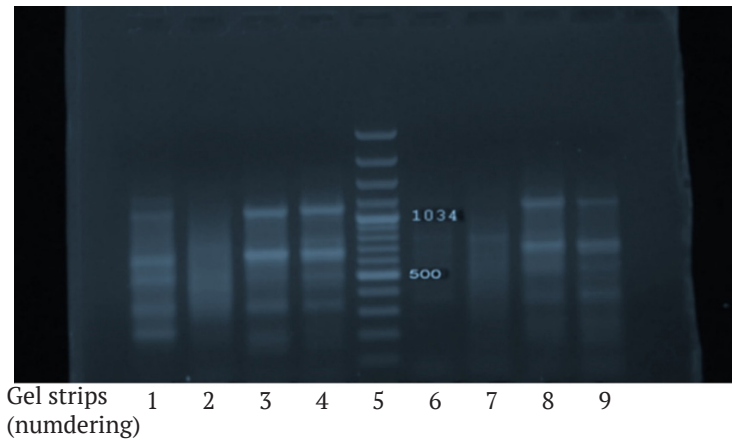
Such a nucleotide sequence corresponds to one of the genes (cdtA), as a result of which the synthesis of the so-called cytolethal stretching toxin takes place [22]. This gene is included in the triad of genes (cdtA, cdtB, and cdtC) necessary for the synthesis of an active ternary holotoxin, which causes full cellular toxicity.

Genomic features of field isolates using the primer responsible for the synthesis of the toxic protein fraction of *Campylobacter jejuni* were examined for the presence of potential genes in each isolate and the reference strain [14].

The specific feature of the PCR reproduction in this case concerned the installation of Eppendorfs and the program launch, starting with recording of the “hot star” conditions in the thermal cycler (temperature +93°C). The study and analysis of compounds obtained as a result of

amplification began after separating a section of DNA in 1.5% agarose gel. An indispensable condition for using agar gel is its enrichment with a solution of ethidium bromide at 25 µL per plate with a thickness of 6 mm.

At the stage when the reaction mixture for PCR already contained glycerol, and for marker staining it contained xylenecyanol, the samples under study were introduced directly into the wells. In the electrophoretic chamber, the gel strips were placed in wells towards the anode. 10.0 µl of amplified samples were placed in the central wells, and 3 µl of marker was placed in the extreme wells. After the dye (xylenecyanol) covered about half the length of the gel, electrophoresis was performed in a voltage gradient of 10 V/cm. The calculation of the results of DNA amplification on electropherograms using a transilluminator in ultraviolet light is presented in Figure 1.



**Figure 1.** Results of electrophoresis of amplification of toxicity genes in the DNA of *Campylobacter jejuni* isolates

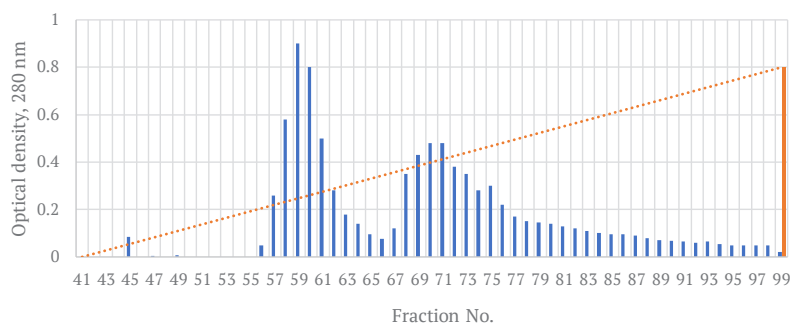
Comparative analysis of the electropherogram of DNA amplification with the data of standard samples as a result of three repetitions helped establish the presence of genome elements ( $P < 0.01$ ) that indicate the potential ability to produce toxins in *Campylobacter jejuni* isolates sampled from the material under study (lanes 1 to 4). The fifth lane coincided with the sample of the reference strain *Campylobacter jejuni* ATCC 33291. Empty tracks 6, 7, 8, and 9 belong to samples containing control samples (without the presence of *Campylobacter* and somatic cell cultures in poultry).

Therefore, the gene of interest (*cdtA*) was identified as specific for *Campylobacter jejuni*. It was present in all 100% of isolates (four isolates) and was not detected in the control negative samples. These are samples with epithelial

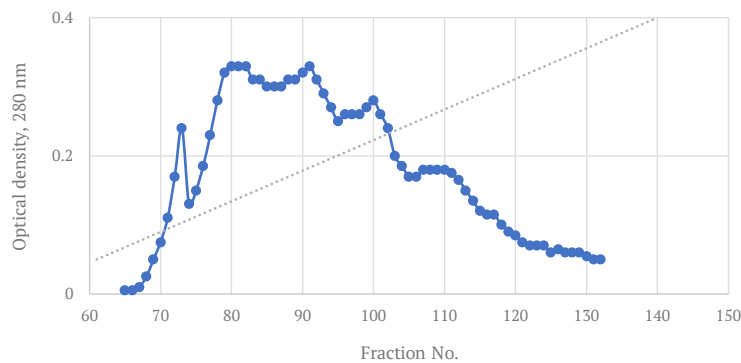
cells and samples containing cultures of *Escherichia coli* and *Salmonella typhimurium*, which is quite natural.

Since, according to statistics, among the three most common intestinal infections associated with the presence of toxin-producing bacteria (*Shigella sonnei*, *Campylobacter spp.*, *Salmonella spp.*), *Campylobacter* is ahead of salmonella; some scientists point to the need to investigate toxin production by *Campylobacter* [4]. *Campylobacter* came under the close attention of specialists because they are included in the top-5 group of pathogens of intestinal zoonoses, which can spread through animal faeces [5].

The study of the nature of the toxic products of the sediment and the supernatant of the broth culture of selected *Campylobacter* strains is presented in Figure 2 and 3, respectively.



**Figure 2.** Distribution of protein fractions of the precipitate of *Campylobacter jejuni* isolate No. 1 (ion exchange chromatography)



**Figure 3.** Distribution of protein fractions of the supernatant of *Campylobacter jejuni* isolate No. 1 (ion exchange chromatography)

The two peak values of the protein components of the sediment coincide with the 59<sup>th</sup> and 70<sup>th</sup> fractions.

The results of studies of the supernatant of a *Campylobacter jejuni* isolate with potential toxic properties allowed identifying 5 protein fractions, which constitute a

considerable advantage over other substances of this class. These are fractions No. 73, 80, 91, 100, and 105. Data on the quantitative and qualitative biochemical composition of samples of elements of the supernatant of *Campylobacter* isolates, which were subject to verification, are presented in Table 3.

**Table 3.** Protein content in sediment and supernatant fractions of *Campylobacter jejuni* No. 1 ( $M \pm m$ ,  $n = 6$ ,  $P < 0.01$ )

Sample	Protein content, µg/ml
Sediment (dialysed)	238
Fraction 59	17
Fraction 70	9.5
Supernatant (dialysed)	242.5
Fraction 73	24.5
Fraction 80	85
Fraction 91	55
Fraction 100	20
Fraction 105	10

The secretion of a protein substance by *Campylobacter* is activated due to the contact of the bacteria with the host cells. These are the so-called Cia proteins, which are necessary for activation of chemotaxis and mass invasion of intestinal epithelial cells by *Campylobacter jejuni* bacteria. This is consistent with literature data on the system that implements the synthesis of Cia proteins and is in the flagellar apparatus of these microorganisms. It is associated with the activation of the diguanylate cyclase enzyme [15]. Intensification of the activity of the latter helps alter the biological state of the *Campylobacter* culture. There is a transition from the swarming phase to the phase with the development of morphologically stem-like cells. This phase involves the removal of flagella from bacterial cells and symbolizes the beginning of cell division [13].

Apart from enzyme proteins, a crucial factor in the pathogenic effect on the body in *Campylobacter jejuni* is cytolethal distension toxin (CdtA, CdtB, CdtC). It causes apoptosis of host cells, which is accompanied by a similar phenomenon—elongation of affected cells [22]. The use of isolated toxic fractions of protein compounds of *Campylobacter jejuni* isolates in the suspension of the leukocyte fraction of rabbit blood led to the occurrence of a similar effect – apoptosis with cell elongation. This can explain the principle of the immunosuppressive effect of cytolethal stretching toxin on the activity of white blood cells that lose their phagocytic function.

The degree of such changes can be estimated by the result of an opsonophagocytic reaction. The number of bacterial cells captured by phagocytes (phagocytic number) for  $n = 6$  in the sample with sediment of *Campylobacter jejuni* bacterial culture (isolate No. 1) was  $530 \pm 12$  microbial cells per 100 leukocytes, which is equal to  $5.3 \pm 0.12$ .

In the presence of the supernatant of the *Campylobacter jejuni* bacterial culture (isolate No. 1), the phagocytic number in the sample for  $n = 6$  did not exceed  $202 \pm 4$  microbial cells per 100 leukocytes, which is equal to  $2.0 \pm 0.04$ .

The analysis of the obtained data indicates that outside the body, rabbit blood leukocytes treated with the protein fraction of the dialysed sediment of *Campylobacter*

*jejuni* No. 1 with a protein content of 238 µg/ml showed little functional activity. Their ability to absorb *Campylobacter jejuni* bacterial cells by phagocytosis was over 2.6 times less than that of leukocytes treated with the supernatant of the same isolate with a protein content of 242.5 µg/ml. This indicates a high cytotoxic effect of protein compounds of the dialysed supernatant, which consists of 5 fractions.

Such a difference in the immunosuppressive action of protein fractions of *Campylobacter jejuni* bacterial cultures of different origins was confirmed by the calculation of the opsonic index ( $I_o$ ) based on the quantitative indicators of phagocytic numbers of the 1st and 2nd groups of the experiment. It was as follows:  $I_o = (5.3 \pm 0.12)/(2.0 \pm 0.04) = 2.6 \pm 0.03$ .

Thus, the obtained quantitative characteristics of the interaction of various components of the bacterial culture of *Campylobacter jejuni* with a suspension of rabbit blood leukocytes indicate the manifestation of the cytotoxic effect of the *Campylobacter* supernatant, which contains toxigenic protein fractions. Since such an effect is observed when interacting with immunocompetent cells, the toxic compounds obtained in the experiment can be qualified as factors with an immunosuppressive cellular effect. The obtained results are fully consistent with the data of other scientists [14; 19], which confirm the acquisition of plasmids in the genome by *Campylobacter*, which cause the synthesis of protein compounds with pronounced cytotoxic properties.

## Conclusions

As a result of the study of 33 isolates of *Campylobacter jejuni* from poultry (faeces and caecal processes of the intestine), 4 cultures were selected to establish the presence of the *cdtA* gene. Only these cultures were similar in biochemical properties to the reference strain *Campylobacter jejuni* ATCC 33291.

A positive result was obtained for the PCR study to detect the *cdtA* gene in 4 isolates of *Campylobacter jejuni* in a comparative aspect, along with samples of somatic cells and cultures of *Escherichia coli* and *Salmonella typhimurium*. The presence of this gene confirms the presence of toxin-forming ability in *Campylobacter*.

The effect of *Campylobacter jejuni* waste products on the body of warm-blooded animals was studied by detecting changes in the phagocytic activity of rabbit blood leukocytes due to their susceptibility to the protein fraction of the dialysed supernatant and the dialysed *Campylobacter* sediment (isolate No. 1). The cytotoxic activity of the dialysed supernatant with a protein content of 242.5 µg/ml exceeded the intensity of inhibition of the phagocytic function of blood cells treated with the

dialysed sediment of the same broth culture with its content of 238 µg/ml.

The calculated level of potential blocking of the opsono-phagocytic reaction by *Campylobacter jejuni* culture exchange products corresponded to the opsonic index value of  $2.6 \pm 0.03$ . For the successful treatment and prevention of infections caused by *Campylobacter jejuni*, it is necessary to constantly monitor the presence of toxin-producing strains in sensitive organisms.

## References

- [1] Stevens, E., Carleton, H., Beal, J., Tillman, G., Lindsey, R., Lauer, A., Pightling, A., Jarvis, K., Ottesen, A., Ramachandran, P., Hintz, L., Katz, L., Folster, J., Whichard, J., Trees, E., Timme, R., McDermott, P., Wolpert, B., Bazaco, M., Zhao, S., Lindley, S., Bruce, B., Griffin, P., Brown, E., Allard, M., Tallent, S., Irvin, K., Hoffmann, M., Wise, M., Tauxe, R., Gerner-Smidt, P., Simmons, M., Kissler, B., Defibaugh-Chavez, S., Klimke, W., Agarwala, R., Lindsay, J., Cook, K., Austerman, S.R., Goldman, D., McGarry, S., Hale, K.R., Dessai, U., Musser, S.M., & Braden, C. (2022). Use of whole genome sequencing by the Federal Interagency Collaboration for Genomics for Food and Feed Safety in the United States. *Journal of Food Protection*, 85(5), 755-772. doi: 10.4315/JFP-21-437.
- [2] Walter, S., Crim, M., Bruce, B., & Griffin, M. (2020). Incidence of *Campylobacter*-associated Guillain-Barré syndrome estimated from health insurance data. *Foodborne Pathogens and Disease*, 17(1), 23-28. doi: 10.1089/fpd.2019.2652.
- [3] Bian, X., Garber, J.M., Cooper, K.K., Huynh, S., Jones, J., Mills, M.K., Rafala, D., Nasrin, D., Kotloff, K.L., Parker, C., Tennan, S., Miller, W., & Szymanski, K. (2020). *Campylobacter* abundance in breastfed infants and identification of a new species in the global enterics multicenter study. *American Society for Microbiology Journals*, 5(1), article number e00735-19. doi: 10.1128/mSphere.00735-19.
- [4] Metreveli, M., Bulia, S., Shalamberidze, I., Tevzadze, L., Tsanova, S., Goenaga, J., Stingl, K., & Imnadze, P. (2022). *Campylobacteriosis*, shigellosis and salmonellosis in hospitalized children with acute inflammatory diarrhea in Georgia. *Pathogens*, 11(2), article number 232. doi: 10.3390/pathogens11020232.
- [5] Lúcio, É., Barros, M., Mota, R., de Cássia, C., & Pinheiro, J. (2019). Identification of *Campylobacter fetus* subsp. *venerealis* virulence genes in cervical mucus from cows. *Brazil Journal Microbiology*, 50(4), 1133-1137. doi: 10.1007/s42770-019-00127-w.
- [6] Sakaridis, I., Ellis, J., Cawthraw, S., van Vlie, M., Stekel, D., Penell, J., Chambers, M., La Ragione, R., & Cook, A. (2018). Investigating the association between the caecal microbiomes of broilers and *Campylobacter* burden. *Frontiers in Microbiology*, 9, article number 927. doi: 10.3389/fmicb.2018.00927.
- [7] Sicun, F., Derek, F., Shaohua, Z., Sampa, M., Yesha, S., Cameron, P., & Sophia, K. (2022). Genomic analysis reveals that isolation temperature on selective media introduces genetic variation in *Campylobacter jejuni* from bovine feces. *Pathogens*, 11(6), article number 678. doi: 10.3390/pathogens11060678.
- [8] Halimeh, F., Rafei, R., Diene, S., Osman, M., Kassem, I., Jamal, A., Moudani, W., Hamze, M., & Rolain, J. (2022). Genome sequence of a multidrug-resistant *Campylobacter coli* strain isolated from a newborn with severe diarrhea in Lebanon. *Folia Microbiologica*, 67(2), 319-328. doi: 10.1007/s12223-021-00921-w.
- [9] Patrick, M., Henao, O., Robinson, T., Geissler, A., Cronquist, A., Hanna, S., Hurd, S., Medalla, F., Pruckler, J., & Mahon, B. (2018). Features of illnesses caused by five species of *Campylobacter*, Foodborne Diseases Active Surveillance Network (FoodNet) – 2010-2015. *Epidemiology & Infection*, 146(1), 1-10. doi: 10.1017/S0950268817002370.
- [10] García-Sánchez, L., Melero, B., & Rovira, J. (2018). *Campylobacter* in the food chain. *Advances in Food and Nutrition Research*, 86, 215-252. doi: 10.1016/bs.afnr.2018.04.005.
- [11] Icen, H., Corbo, M., Sinigaglia, M., Korkmaz, B., & Bevilacqua, A. (2022). Using microbial responses viewer and a regression approach to assess the effect of pH, activity of water and temperature on the survival of *Campylobacter* spp. *Foods*, 11(5), article number 637. doi: 10.3390/foods11050637.
- [12] Larsen, S., Orsted, I., Tarpgaard, I., & Nielsen, H. (2021). *Helicobacter cinaedi* bacteraemia secondary to enterocolitis in an immunocompetent patient. *Gut Pathogens*, 13, article number 26. doi: 10.1186/s13099-021-00422-8.
- [13] Cox, C., Bogacz, M., Abbar, F., Browning, D., Hsueh, B., Waters, C., Lee, V., & Thompson, S. (2021). The *Campylobacter jejuni* response regulator and cyclic-di-GMP binding CbrR is a novel regulator of flagellar motility. *Microorganisms*, 10(1), article number 86. doi: 10.3390/microorganisms10010086.
- [14] Muralidharan, C., Anwar, A., Wilson, T., Scott, P., Moore, R., & Van, T. (2020). Development of an enzyme-linked immunosorbent assay for detecting *Campylobacter hepaticus* specific antibodies in chicken sera – a key tool in Spotty Liver Disease screening and vaccine development. *Avian Pathology*, 49(6), 658-665. doi: 10.1080/03079457.2020.1813252.
- [15] Huddleston, J., Anderson, T., Girardi, N., Thoden, J., Taylor, Z., Holden, H., & Raushel, F. (2021). Biosynthesis of d-glycero-l- gluco-heptose in the capsular polysaccharides of *Campylobacter jejuni*. *Biochemistry*, 60(19), 1552-1563. doi: 10.1021/acs.biochem.1c00183.
- [16] Bereswill, S., Ekmekci, I., Escher, U., Fiebiger, U., Stingl, K., & Heimesaat, M. (2017). *Lactobacillus johnsonii* ameliorates intestinal, extra-intestinal and systemic pro-inflammatory immune responses following murine *Campylobacter jejuni* infection. *Scientific Reports*, 7(1), article number 2138. doi: 10.1038/s41598-017-02436-2.
- [17] Heimesaat, M., Mrazek, K., & Bereswill, S. (2019). Murine fecal microbiota transplantation alleviates intestinal and systemic immune responses in *Campylobacter jejuni* infected mice harboring a human gut microbiota. *Frontiers in Immunology*, 10, article number 2272. doi: 10.3389/fimmu.2019.02272.

- [18] Béjaoui, A., Gharbi, M., Bitri, S., Nasraoui, D., Ben Aziza, W., Ghedira, K., Rfaik, M., Marzougui, L., Ghram, A., & Maaroufi, A. (2022). Virulence profiling, multidrug resistance and molecular mechanisms of *Campylobacter* strains from chicken carcasses in Tunisia. *Antibiotics*, 11(7), article number 830. doi: 10.3390/antibiotics11070830.
- [19] Heimesaat, M., Genger, C., Klove, S., Weschka, D., Mousavi, S., & Bereswill, S. (2020). The host-specific intestinal microbiota composition impacts *Campylobacter coli* infection in a clinical mouse model of campylobacteriosis. *Pathogens*, 9(10), article number 804. doi: 10.3390/pathogens9100804.
- [20] Ankit, M., Brudvig, J., Gadsden, B., Ethridge, A., & Mansfield, L. (2022). *Campylobacter jejuni* induces autoimmune peripheral neuropathy via Sialoadhesin and Interleukin-4 axes. *Gut Microbes*, 14(1), article number 2064706. doi: 10.1080/19490976.2022.2064706.
- [21] Pearson, A., Greenwood, M., Donaldson, J., Healing, T., Jones, D., Shahamat, M., Feltham, K., & Colwell, R. (2000). Continuous source outbreak of campylobacteriosis traced to chicken. *Journal of Food Protection*, 63(3), 309-314. doi: 10.4315/0362-028X-63.3.309.
- [22] Mousavi, S., Bereswill, S., & Heimesaat, M. (2021). Murine models for the investigation of colonization resistance and innate immune responses in *Campylobacter jejuni* infections. In *Fighting campylobacter infections* (pp. 233-263). Cham: Springer. doi: 10.1007/978-3-030-65481-8\_9.
- [23] ISO 10272-1:2017/Amd 1 “Microbiology of the food chain – Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1: Detection method”. (2017). Retrieved from <https://www.iso.org/standard/77639.html>.
- [24] EUCAST. (2013). *EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance*. Retrieved from [http://www.infectioncontrol.org.ua/wp-content/uploads/2016/03/2-EUCAST\\_detection\\_of\\_resistance\\_ua.pdf](http://www.infectioncontrol.org.ua/wp-content/uploads/2016/03/2-EUCAST_detection_of_resistance_ua.pdf).
- [25] Kresqe, N., Simoni, R., & Hill, R. (2005). The most highly cited paper in publishing history: Protein determination by Oliver H. Lowry. *Journal of Biological Chemistry*, 280(28), 25-28. doi: 10.1016/S0021-9258(20)56859-2.
- [26] R Core Team. (2018). *R: A language and environment for statistical computing*. Retrieved from <https://www.R-project.org>.
- [27] Official website of the Pub MLST. (n.d.). *Primers for MLST of Campylobacter jejuni/coli*. Retrieved from <https://pubmlst.org/organisms/campylobacter-jejunicoli/primers>.

## Імуносупресивна активність ізолятів *Campylobacter jejuni* щодо клітинної ланки імунопротекції організму

Тетяна Василівна Мазур<sup>1</sup>, Наталія Володимирівна Щур<sup>2</sup>,  
Сергій Олександрович Бояновський<sup>3</sup>

<sup>1</sup>Національний університет біоресурсів і природокористування України  
03041, вул. Героїв Оборони, 15, м. Київ, Україна

<sup>2</sup>Державний науково-дослідний інститут з лабораторної діагностики  
та ветеринарно-санітарної експертизи  
03151, вул. Донецька, 30, м. Київ, Україна

<sup>3</sup>Державний науково-контрольний інститут біотехнології і штамів мікроорганізмів  
03151, вул. Донецька, 30, м. Київ, Україна

**Анотація.** Глобальні зміни довкілля викликали трансформації в біології мікроорганізмів, зокрема й серед кампілобактерій, котрі асоціюються нині з харчовими токсикоінфекціями. Інструменти впливу цих бактерій на сприйнятливі організми, зокрема токсини, остаточно не з'ясовані. Метою дослідження стало вивчення генетичної обумовленості токсинування в ізолятів *Campylobacter jejuni* та визначення ступеня пригнічення захисних реакцій організму токсичними фракціями білкових сполук кампілобактерій. Основою методології досліджень була полімеразна ланцюгова реакція з використанням праймерів для індикації нуклеотидних послідовностей геному *Campylobacter jejuni*, що кодують синтез токсинів. Зразки проб від 4-х ізолятів кампілобактеру досліджували на вміст білкових фракцій за методом Лоурі. Аналіз електрофореграми результатів ампліфікування ДНК в порівняльному аспекті з даними стандартних зразків дозволив встановити наявність елементів геному, які вказують на потенційну здатність до токсинування у виділених з досліджуваного матеріалу ізолятів *Campylobacter jejuni*. Токсичні фракції, відокремлені з супернатанту бульйонної культури *Campylobacter jejuni*, представлені речовинами протеїново-вуглеводної природи. Отримані пікові токсигенні фракції діалізату осаду бактерійної культури містили в собі білок у межах 9,5–17 мкг/мл. У діалізаті супернатанту бульйонної культури, де було визначено 5 груп токсигенних фракцій, уміст в них білка коливався в межах від 10 до 85 мкг/мл. За відтворення опсоно-фагоцитарної реакції із залученням токсигенних фракцій *Campylobacter jejuni* встановлено достатньо виражену імуносупресивну дію цих комплексів на організм теплокровних тварин при опсонічному індексі  $2,6 \pm 0,03$ . Отримані результати дають змогу з'ясувати зв'язок між токсинуванням у *Campylobacter jejuni* та їх імуносупресивною дією на організм теплокровних тварин і людини, що в перспективі позитивно впливатиме на удосконалення заходів щодо профілактики та лікування тварин за цієї патології

**Ключові слова:** ізоляти кампілобактерій, ампліфікація ДНК, білкова фракція, опсоно-фагоцитарна реакція