Detection and quantification of *Campylobacter* in foods: New analytic approaches to detect and quantify *Campylobacter* spp. in food samples

Maria Francesca Peruzy,[1] Yolande Thérèse Rose Proroga,[2] Federico Capuano,[2] Federica Corrado,[2] Serena Santonicola,[3] Dario De Medici,[4] Elisabetta Delibato,[4] Nicoletta Murru[1]

[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Napoli; [2]Department of Food Microbiology, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici; [3]Department of Medicine and Health Science Vincenzo Tiberio, University of Molise, Campobasso; [4]Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy

Abstract

The aim of the present study was to develop rapid qualitative and quantitative methods based on the use of Real-Time PCR and Droplet Digital PCR (ddPCR), in order to have reliable techniques to detect and quantify *Campylobacter* spp. in food samples. The gene 16S-rRNA was used as specific target for *Campylobacter* spp. Real-Time PCR evaluation assay and a not competitive internal control was ushered in it. To investigate the selectivity of the method, 26 *Campylobacter* strains and 40 non-*Campylobacter* strains were tested and in order to verify the application of Real-Time PCR method, 5 pork meat samples were experimentally inoculated with a *Campylobacter jejuni* strain. Subsequently, dilutions with a bacterial load of *Campylobacter jejuni* within 10-10^6 CFU/mL were chosen for the optimization of the ddPCR assay. Lastly, a total of 54 naturally contaminated foods samples were analyzed through molecular (Real-Time PCR and ddPCR) and traditional methods. The Real-Time PCR protocol demonstrated to amplify only the *Campylobacter* spp. strains and when *Campylobacter jejuni* was experimentally inoculated in meat samples the pathogen was always detected. The ddPCRs assay allowed to quantify a level of contamination of 10 CFU/mL, but it was unable to quantify levels of 10^5 – 10^6 CFU/mL. Lastly, *Campylobacter* spp. was never detected in the 54 samples tested. In conclusion, the novel analytic approach proposed, based on an initial screening of the samples with Real-Time PCR and then on quantification of *Campylobacter* spp. with a ddPCR on those positive, represents a quick monitoring tool and, if used correctly, it would allow the implementation of food safety.

Introduction

Since 2005, *Campylobacter* (*C*) is the most commonly reported gastrointestinal bacterial pathogen in humans in the European Union (EU) (EFSA, 2018). In particular, according to the European Food Safety Authority (EFSA), in 2017 there were 246,158 confirmed cases of human campylobacteriosis in the EU (EFSA, 2018). Globally, the incidence varies among countries, but the current incidence of campylobacteriosis is probably underestimated because of difficulties with diagnosis, differences in reporting systems and in surveillance (Hansson *et al.*, 2018). Among *Campylobacter* species, *C. jejuni* and *C. coli* have the highest rate of foodborne-related clinical campylobacteriosis (Ricke *et al.*, 2019). Humans can acquire the infection through direct contact with infected animal or through the consumption of contaminated foods. The gastrointestinal illness usually is self-limiting, but in a small number of cases complications such as reactive arthritis and neurological disorders may occur (Fabiani *et al.*, 2019). The most commonly identified sources of human infection are represented by broiler meat and milk, but also dairy product and red meat have been responsible for outbreaks in 2017 (EFSA, 2018). Conventional methods for the qualitative and quantitative detection of *Campylobacter* involve the use of selective media and elevated incubation temperature (42°C) in microaerophilic atmosphere (ISO 10272-1 and ISO 10272-2) but although inexpensive and simple, these methods are long and time-consuming, taking up to 6 days (Erdosi *et al.,* 2018; Fabiani *et al.*, 2019). The development of rapid methods is important to conduct a proper control of the contaminated products, to identify quickly outbreak sources and to prevent the spread of illness (Wang and Salazar, 2017.). Over the past few decades, to reduce the detection time from days to hours, molecular methods, such as polymerase chain reaction (PCR), have been introduced in daily laboratory routines to detect pathogenic bacteria in different foods (Peruzy *et al*., 2019; Ricke *et al.*, 2019). According to the EC regulation 1495/2017 a quantitative determination of *Campylobacter* is required, thus rapid, reliable and reproducible methods for the quantification of this pathogen in foods are needed (Yu *et al.*, 2019).

Recently, Droplet digital PCR (ddPCR) emerged as a useful tool for precise quantification of nucleic acids in different samples and it allows the detection of target DNA in complex matrices in presence of abundant sequences. In this technique, target DNA molecules are distributed across thousands of small droplets. Each droplet constitutes a separate reaction compartment and contains 0 or 1 (or more) template copies (Hindson *et al*., 2011). After amplification through a conventional PCR, a Poisson distribution is used to extrapolate the fraction of positive end-point reactions. It has been firstly proposed for clinical and environmental studies but more recently it has been introduced also in food microbiology analysis (Gobert *et al.*, 2018). In fact, this technology has been recently used to quantify *Bacillus cereus* and *Salmonella typhimurium* in milk (Porcellato *et al.,* 2016; Wang *et al.*, 2018) and noroviruses in oysters (Persson *et al.*, 2018). Moreover, compared with Real-Time PCR, ddPCR is more rapid since does not require standard curve for the analysis and it is less sensitive to inhibitions (Gobert *et al.*, 2018; Wang *et al*., 2018).

Correspondence: Yolande Thérèse Rose Proroga, Department of Food Microbiology, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute, 2, Portici, NA, Italy.
Tel.: +39081/7865208
E-mail: yolande.proroga@cert.izsmportici.it

Key words: *Campylobacter* spp.; Real-Time PCR, Droplet digital PCR.

Conflict of interest: The authors declare no potential conflict of interest.

Funding: This study was supported by the Italian Health Ministry-Grant IZS-ME 07/14 RC.

Availability of data and material: All data and materials of the study are available from the corresponding author.

Received for publication: 30 September 2019.
Revision received: 21 December 2019.
Accepted for publication: 7 January 2020.

Italian Journal of Food Safety 2020; 9:8591
doi:10.4081/ijfs.2020.8591

The aim of the present study was the development of rapid qualitative and quantitative methods based on the use of Real-Time PCR and Droplet Digital PCR (ddPCR), respectively, in order to have sensitive and reliable techniques to detect and quantify *Campylobacter* spp. in food samples. Moreover, the occurrence of *Campylobacter* spp. in different pork meat samples and dairy products was evaluated.

Materials and Methods

Campylobacter strains and other organism

Twenty-six *Campylobacter* strains, belonging to *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis* and *Campylobacter fetus fetus* and 40 non-*Campylobacter* microorganisms (Table 1) isolated from foods and human cases were included in this study. The strains were supplied from the collection of the Pathogenic Enterobacteria Unit of Istituto Superiore di Sanità, Rome, Italy. Bacterial strains were cultured in Tryptic Soy Agar (TSA; CM0131, OXOID, Basingstoke,UK) for 48-h at 41,5°C in microaerophilic conditions.

DNA extraction

DNA was extracted using the Chelex-100-resin method (Bio-Rad, Hercules, CA, USA) whereby one colony of each strain was suspended in 300 µL of 6% Chelex 100 by vortexing, and incubated for 20 min at 56°C and again for 8 min at 100°C. The suspension was immediately chilled on ice for 1 min and centrifuged for 5 min at 10,000 ×g at 4°C (Peruzy *et al.*, 2019).

qPCR

The gene 16S-rRNA was used as specific target for *Campylobacter* spp. Real-Time PCR evaluation assay. In particular, 5 µL of DNA extracted were used as template and added to a PCR Mix (Fabiani *et al.*, 2019). The mix contained: 1X Quantitect multiplex PCR NoROX, 440 nM forward primer 5′ CTGCTTAACACAAGTTGAGTAGG 3, 480 nM reverse primer 5′ TTCCTTAGGTACCGTCAGAA 3′, 200 nM 16S-rRNA fluorescent probe 5′ FAM-TGTCATCCTCCACGCGGCGTTGCTGC-BHQ1 3′and water for molecular biology. Moreover, in order to determine the inhibition in the biological samples and to identify false negative results a not competitive internal control (IAC), primer pUC 18-F (5‘–TGT CGT GCC AGC TGC ATT A-3‘), primer pUC 18-R (5‘–GAG CGA GGA AGC GGA AGA g–3‘) and the probe Tm-pUC18 (5‘–HEX– AAT CGG CCA ACG CGC GG –BHQ1–3) were added. In particular, the optimal IAC concentration was 1000 copies per reaction.

The reaction was run online at 95°C for 15 min, followed by 40 cycles at 95°C for 30 s and 58°C for 60 s and an extension phase of 1 cycle at 95°C for 60 s, 60°C for 60 s, and 95°C for 60 s (ramp time, 19.59 min). Amplification reactions were performed with Bio-Rad CFX96 platform, using a 96-well PCR multiplate, from Bio-Rad.

To investigate whether the primer set and the amplification protocol amplify DNA from *Campylobacter* spp. (inclusivity/exclusivity), the DNA extracted with the Chelex-100-resin method of the twenty-six *Campylobacter* strains and of the 40 non-Campylobacter strains (Table 1) were tested as described above.

Inoculation of the food matrix

In order to verify the application of the above described method, five pork meat samples purchased at different supermarkets, were experimentally inoculated with *Campylobacter jejuni* strain (ATCC33291). In particular, *Campylobacter jejuni* was activated twice in Tryptone Soy Broth (TSB; CM0129, OXOID, Basingstoke, UK) and incubated at 41,5 °C for 48-h in a microaerophilic conditions and standardized by count on tryptone soy agar.

Each sample was divided into three aliquots, one was used as negative control and the other two were inoculated with a bacterial load within 1-10 and 10-10^2 CFU/mL, respectively. In particular, 25 grams of each sample were placed in a sterile stomacher bag and homogenized for 3 minutes at 230 rpm using a peristaltic homogenizer (BagMixer®400 P, Interscience, Saint Nom, France) after the addition of 225 mL (1:10, W/W) of sterilized Bolton Selective Enrichment Broth (BSEB), prepared by adding bolton broth selective supplement and laked horse blood in bolton Broth, following technical data sheet. After inoculation, all samples were shaken for 1 min to optimize the distribution of the inoculum and then they were incubated at 37°C for 4-h and 41,5°C for 44-h in microaerophilic conditions.

After the incubation, two mL of each incubated homogenate were subjected to the DNA extraction phase using the procedure above described. Subsequently the Real-Time PCR analysis was performed as above described.

Moreover, in order to compare the

Table 1. Number and species of the *Campylobacter* strains and other organisms included in the study and Real-Time PCR results.

Bacterial strains	N. of strains	Real-Time PCR
Campylobacter strains		
Campylobacter jejuni	10	+
Campylobacter coli	10	+
Campylobacter lari	2	+
Campylobacter upsaliensis	2	+
Campylobacter fetus fetus	2	+
Other microorganisms		
Aeromonas idrofila	1	-
Bacillus subtilis	1	-
Citrobacter freundii	1	-
Enterobacter aerogene	1	-
Enterobacter cloacae	1	-
Escherichia coli	1	-
Listeria innocua	1	-
Listeria monocytogenes	2	-
Proteus hauseri	1	-
Pseudomonas aerugionosa	3	-
Shighella boydii	1	-
Shighella flexeneri	1	-
Shighella sonnei	1	-
Staphyloccocus aureus	6	-
Staphyloccocus xylosus	1	-
Yersinia enterocolitica	5	-
Yersinia intermedia	1	-
Yersinia kristensenii	1	-
S. Enteritidis	2	-
S. Typhimurium	2	-
S. Napoli	2	-
S. Thompson	2	-
S. Veneziana	2	-

results of the molecular method with the microbiological reference method (EN/ISO 10272-1:2017), after 4 and 44 hours of incubation a sterile loop was used to transfer the homogenate broth on the surface of Modified Charcoal Cefoperazone Desoxycholate Agar (mCCD, CM0739 and SR0155, OXOID, Basingstoke, UK) and *Campylobacter* agar base (karmali, CM0935, OXOID, Basingstoke, UK) with the *Campylobacter* selective supplement (karmali supplement, SR0167, OXOID, Basingstoke, UK). Subsequently all the plates were incubated in a microaerophilic conditions at 41,5°C for 44-h. Afterward, *suspected* colonies were picked, subcultured on Columbia blood agar (CM0331, OXOID, Basingstoke, UK) and incubated in a microaerophilic conditions at 41,5°C for 24/48-h for the phenotypic and biochemical confirmation.

Digital droplet PCR reaction

Ten-fold serial dilutions of the culture of *Campylobacter jejuni* were prepared in TSB and the concentration of cells was measured using McFarland turbidity standards. Dilutions with a bacterial load within 10-10^6 CFU/mL were chosen for the optimization of the Droplet Digital PCR (ddPCR). The same DNA extraction protocol, primer set and probe used for the above described Real-Time PCR assay were applied for the optimization of the ddPCR assay. In particular, ddPCR reaction were performed using 1×ddPCR Supermix for probe (Bio-Rad, Pleasanton, CA) and the final volume of the reaction was 22 µL. Twenty microliter of the ddPCR reaction were used to generate the droplet mix in an 8-well cartridge using the QX100 droplet generator (Bio-Rad). The emulsion (40 µL) was then transferred to a 96-well plate and amplified using the same condition of the previously described Real-Time PCR. The PCR products were denatured at 98°C for 10 min and kept at 4°C until the droplets were read. Ramp rate used in the droplet PCR was 2°C s−1. The 96-well plate was then transferred to the QX100 droplet reader (Bio-Rad) and data acquisition and analysis was performed using QuantaSoft software ver 1.7 (Bio-Rad). The fluorescence amplitude threshold used for the discrimination of the positive and negative droplets in QuantaSoft software was set between 2000 and 2200. The concentration values were calculated by QuantaSoft software (in copies µL−1) and multiplied by 22 (the initial PCR volume) to obtain the absolute number of copies added to the PCR reaction.

Campylobacter spp. detection from naturally contaminated samples

In order to evaluate the presence of *Campylobacter* spp. in naturally contaminated food samples, a total of 28 pork meat samples and 26 dairy products were collected in the Campania region in southern Italy (Table 2) and analyzed through molecular methods (Real-Time PCR and ddPCR) as described above and traditional microbiological methods (EN/ISO 10272-2:2017). In particular, for the traditional microbiological method, from each sample 10 grams were placed in a sterile stomacher bag and homogenized for 3 minutes after the addition of 90 mL (1:10, W/W) of sterilized Buffered Peptone Water (BPW; CM0509, OXOID, Basingstoke, UK). All samples were incubated in a microaerophilic conditions at 41,5°C for 1-h and 48-h. After 1 and after 48-h, 10-fold serial dilutions of the incubated homogenate were prepared in BPW and 0,1 mL of the appropriate 10-fold serial dilutions were spread on the surface of Modified Charcoal mCCD. Subsequently all the plates were incubated in a microaerophilic condition at 41,5°C for 24/48-h. After viable counts, five *suspected* colonies were picked up from each agar plate, subcultured on Columbia blood agar (CM0331, OXOID, Basingstoke, UK) and incubated in a microaerophilic conditions at 41,5°C for 24/48-h. Afterward, phenotypic and biochemical test were used for the confirmation of the suspected colonies.

Results

Real-time PCR assay and detection of Campylobacter jejuni in experimentally contaminated foods

The optimal amount of internal

Table 2. Meat samples and dairy products (type and number) collected in Campania region and analyzed through Real-Time PCR, ddPCR and EN/ISO 10272 method for the evaluation of the presence of *Campylobacter* spp.

Matrices	Description	N. of samples
	Meat samples	
Capocollo	dry-cured pork neck	4
Filone stagionato	dry-cured pork meat	4
Guanciale stagionato al pepe	dry-cured pork jowl with pepper	4
Pancetta al pepe e finocchietto	dry-cured rolled pork belly with pepper and fennel	4
Salame	dry-cured pork meat	4
Salsiccia	fresh pork meat	8
	Dairy samples	
Cow's milk		
Caciocavallo	semi-hard pasta filata cheese	6
Mozzarella	semisoft pasta filata cheese	2
Mozzarella nella mortella	semisoft pasta filata cheese wrapped in a myrtus leaf	2
Ricotta	unripened acid-heat coagulated dairy product	2
Scamorza	semi-hard pasta filata cheese	6
Goat's milk		
Caciotta fresca	fresh semi-soft cheese	1
Caciotta stagionata	cured semi-soft cheese	1
Buffalo's milk		
Mozzarella	semisoft pasta filata cheese	2
Ricotta	unripened acid-heat coagulated dairy product	2
Scamorza	semi-hard pasta filata cheese	2

amplification control to allow target detection, even when present with low copy numbers, was estimated as 1000 copies/reaction yielding a 28-30 Ct value. The Real-Time PCR protocol demonstrated to amplify only the *Campylobacter* spp. strains (Table 1) and not the DNA of the 40 non-*Campylobacter* strains tested showing a 100% of inclusivity and exclusivity.

Moreover, when *Campylobacter jejuni* was experimentally inoculated in pork meat samples to verify the application of the molecular method, the pathogen was always detected, both when the lowest (1-10 CFU/mL) and the highest (10-10^2 CFU/mL) concentration were used. Furthermore, the molecular method evaluated showed a concordance rate of 100% with the reference microbiological method (EN/ISO 10272-1:2017).

Digital droplet PCR reaction

Evaluation of the ddPCR sensitivity, was performed using serial dilution of *Campylobacter jejuni* DNA. The lowest level of detection was 10 CFU/mL whereas ddPCR was unable to quantify high concentration of the pathogen (10^5-10^6 CFU/mL) (Figure 1).

Detection of Campylobacter in naturally contaminated samples

Campylobacter spp. was never detected in the 54 samples tested, both with molecular (Real-Time PCR and ddPCR) and traditional microbiological methods (EN/ISO 10272-2:2017).

Discussion

Conventional methods based on the culture on microbiological media and identification through the biochemical test are still used in the daily laboratory routine for the isolation and/or enumeration of *Campylobacter* species in food samples. Although these methods are sensitive and inexpensive, they are long and time-consuming. Over the past few decades, molecular methods, as the nucleic acid-based technology, have been championed as a promising alternative for the fast and accurate detection and quantification of microorganisms from different foods (Peruzy *et al.*, 2017).

In the present work, two molecular platforms (Real-Time PCR and ddPCR), based on nucleic acid amplification, have been developed in order to determine and quantify *Campylobacter* spp. from food samples.

Real-Time PCR, performed on a set of different *Campylobacter* and non-*Campylobacter* species, discriminated successfully the target DNA from the other microorganisms (100% inclusivity and exclusivity). Moreover, when *C. jejuni* was experimentally contaminated in pork samples, this platform resulted to be effective at detecting the pathogen even when present at low concentration (1-10 CFU/mL). Furthermore, since the presence of inhibitor in food matrices may lead to false-negative results, a non-competitive IAC has been away used in the Real-Time PCR assay, in order to have a tool to check on inhibition effect in PCR.

Even though the principal purpose of the Real-Time PCR technology is the quantification of target bacteria, its use in qualitative analysis, as performed in the present work, should not be underestimated. In fact, compared with traditional PCR (end-point PCR), Real-Time PCR is more precise, quicker, as does not require post-PCR processing (es. preparation of gels), and moreover, it is safer, as the ethidium bromide is not needed (Ahmed *et al.*, 2009). Thus, the PCR developed may be a useful tool for initial screening of food samples and only those samples positive for *Campylobacter* would then be subjected to further analysis.

However, according to EU Regulation 2017/1495, a quantitative determination of *Campylobacter* in broiler carcasses is required. Thus, a rapid molecular method for the precise quantification of this pathogen is also needed. Over the past years, quantitative Real-Time PCR has been successfully used to quantify target DNA in different samples. However, the major limitations of the Real-Time PCR are the request of a standard curve for the calculation of the target concentration and the sensitivity of the amplification to inhibitors (Gobert *et al.*, 2018). Recently, droplet digital PCR has been introduced for the absolute quantification of a DNA target; it does not require a standard curve and, moreover, the PCR reaction partitioning in the ddPCR assay reduces the exposure to PCR inhibitors in the droplet (Porcellato *et al.*, 2016). In the present work, ddPCR allowed the quantification of low numbers of target molecules (10 CFU/mL) but it was unable to quantify high concentrations of *Campylobacter jejuni* (≥ 10^5 CFU/ml), probably because the ddPCR droplets became completely saturated (Tang *et al.*, 2016).

The two developed molecular platforms (Real-Time PCR and ddPCR) together with the microbiological reference method (EN/ISO 10272:2017) were used in order to evaluate the presence of *Campylobacter* spp. in different food samples. Although in the present work the number of the samples were limited, our results are in disagreement with the ones of previously published researches where the detection/isolation of *Campylobacter* in different food samples was higher (El-zamkan *et al.*, 2016;Karki, *et al.*, 2018; Zhao *et al.*, 2001).

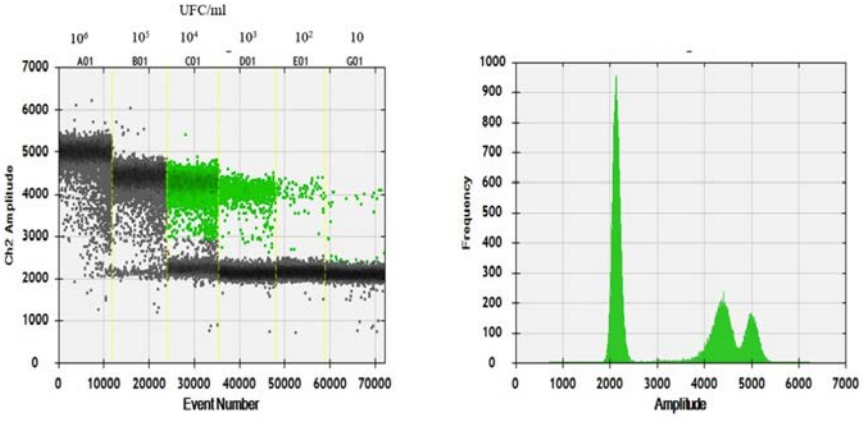

Figure 1. Droplet distribution obtained from the serial dilution of *Campylobacter jejuni* genomic DNA.

Conclusions

The novel analytic approach proposed, based on an initial screening of the samples with Real-Time PCR and then on quantification of *Campylobacter* spp. with a ddPCR on those samples resulted positive, represent a quick monitoring tool and, if used, would allow the implementation of food safety. Moreover, by using these methods could be possible to gain more data for a timely and accurate risk assessment. Furthermore, this proposed strategy, developed in the present study for *Campylobacter*, could be used, also, for a rapid detection and enumeration of other

microorganisms important both in food and in clinical microbiology.

References

Ahmed A, Engelberts MFM, Boer KR, Ahmed N, Hartskeerl RA, 2009. Development and Validation of a Real-Time PCR for Detection of Pathogenic Leptospira Species in Clinical Materials. PLoS One 4:4–11.

EFSA, 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA Journal 16:5500.

El-zamkan MA, Hameed KGA, 2016. Prevalence of Campylobacter jejuni and Campylobacter coli in raw milk and some dairy products. Vet World 9:1147–51.

Erdősi O, Szakmár K, Szili Z, Szita G, Bernáth S, Sövényi J, Laczay P, 2018. Rapid in-house detection method of campylobacter spp. from food by redox potential monitoring combined with Real-Time PCR. Acta Vet Hung 66:1–11.

European Commission, 2017. Commission Regulation (EU) 2017/1495 of 23 August 2017 amending Regulation (EC) No 2073/2005 as regards Campylobacter in broiler carcases. In: Official Journal of the European Union L 218/1.

Fabiani L, Delibato E, Volpe G, Piermarini S, De Medici D, Palleschi G, 2019. Development of a sandwich ELIME assay exploiting different antibody combinations as sensing strategy for an early detection of Campylobacter. Sensor Actuat B-Chem 290:318-325.

Gobert G, Cotillard A, Fourmestraux C, Pruvost L, Miguet J, Boyer M, 2018. Droplet digital PCR improves absolute quantification of viable lactic acid bacteria in faecal samples. J Microbiol Methods 148:64–73.

Hansson I, Sandberg M, Habib I, Lowman R, Engvall EO, 2018. Knowledge gaps in control of Campylobacter for prevention of campylobacteriosis. Transbound Emerg Dis 65, 1:30–48.

Hindson BJ, Ness KD, Masquelier DA Belgrader P, Heredia NJ, Makarewicz AJ, Bright IJ, Lucero MY, Hiddessen AL, Legler TC, Kitano TK, Hodel MR, Petersen JF, Wyatt PW, Steenblock ER, Shah PH, Bousse LJ, Troup CB, Mellen JC, Wittmann DK, Erndt NG, Cauley TH, Koehler RT, So AP, Dube S, Rose KA, Montesclaros L, Wang S, Stumbo DP, Hodges SP, Romine S, Milanovich FP, White HE, Regan JF, Karlin-Neumann GA, Hindson CM, Saxonov S, Colston BW, 2011. High-Throughput Droplet Digital PCR System for Absolute Quantitation of DNA Copy Number. Anal Chem 83:8604-10

ISO, 2017.Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. — Part 1: Detection method ISO Norm 10272-1:2017. International Standardization Organization ed., Geneva, Switzerland.

ISO, 2017. Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. — Part 2: Colony-count technique. ISO Norm 10272-2:2017. International Standardization Organization ed., Geneva, Switzerland.

Karki AB., Marasini D, Oakey CK, Mar K., Fakhr MK, 2018. Campylobacter coli From Retail Liver and Meat Products Is More Aerotolerant Than Campylobacter jejuni. Front Microbiol. 9:2951.

Persson S, Eriksson R, Lowther J, Ellström P, Simonsson M, 2018. Comparison between RT droplet digital PCR and RT real-time PCR for quantification of noroviruses in oysters. Int J Food Microbiol 284:73–83.

Peruzy MF, Murru N, Yu Z, Cnockaert M, Joossens M, Proroga YTR, Houf K 2019. Determination of the microbiological contamination in minced pork by culture dependent and 16S amplicon sequencing analysis. Int J Food Microbiol 290:27–35.

Peruzy MF, Murru N, Yu Z, Kerkhof P, Neola B, Joossens M, Proroga YTR, Houf K 2019. Assessment of microbial communities on freshly killed wild boar meat by MALDI-TOF MS and 16S rRNA amplicon sequencing. Int J Food Microbiol 301:51–60.

Peruzy MF, Murru N, Perugini AG, Capuano F, Delibato E, Mercogliano R, Korkeala H, Proroga YTR 2017. Evaluation of virulence genes in Yersinia enterocolitica strains using SYBR Green real-time PCR. Food Microbiol 65:231–5.

Porcellato D, Narvhus J, Skeie SB, 2016. Detection and quantification of Bacillus cereus group in milk by droplet digital PCR. J Microbiol Methods 127:1–6.

Ricke SC, Feye KM, Chaney WE., Shi Z, Pavlidis H, Yang Y, 2019. Developments in Rapid Detection Methods for the Detection of Foodborne Campylobacter in the United States. Front Microbiol 9:3280:

Tang H, Cai Q, Li H, Hu P, 2016. Comparison of droplet digital PCR to real-time PCR for quantification of hepatitis B virus DNA. Biosci Biotechnol Biochem 80,11:2159-64.

Wang M, Yang J, Gai Z, Huo S, Zhu J, Li J, Wang R, Xing S, Shi G, Shi F, Zhang L, 2018. Comparison between digital PCR and real-time PCR in detection of Salmonella typhimurium in milk. International Journal of Food Microbiology. Int J Food Microbiol 266:251–6.

Wang Y, Salazar JK, 2016. Culture-Independent Rapid Detection Methods for Bacterial Pathogens and Toxins in Food Matrices. Compr Rev Food Sci F 15.

Yu Z, Peruzy MF, Dumolin C, Joossens M, Houf ,K 2019. Assessment of food microbiological indicators applied on poultry carcasses by culture combined MALDI-TOF MS identification and 16S rRNA amplicon sequencing. Food Microbiol 82:53-61.

Zhao C, Ge B, Villena JD E, Sudler R, Yeh E, Zhao S, White DG, Wagner D, Meng J., 2001. Prevalence of Campylobacter spp., Escherichia coli, and Salmonella Serovars in Retail Chicken, Turkey, Pork, and Beef from the Greater Washington, D.C., Area. Appl Environ Microbiol 67:5431–6.