Direct Detection and Quantification of Bacterial Pathogens from Broiler Cecal Samples in the Slaughter Line by Real-Time PCR

ABSTRACT

Chicken meat is an important source of foodborne pathogens, including *Salmonella*, *Campylobacter*, and *Clostridium perfringens*. These bacteria can occur in the intestinal microbiota of broilers and contaminate chicken carcasses in industrial meat processing. This study aimed to develop and evaluate a procedure based on real-time PCRs for the direct detection and quantification of these three bacteria in broilers’ ceca collected in poultry slaughter houses and demonstrate the occurrence of these important foodborne pathogens in Brazilian poultry production flocks. Cecal contents were collected from 45 different broiler flocks in three different slaughterhouses in the state of Paraná, Brazil, totaling 45 samples (in pools of 10 different ceca/chickens per broiler flock). Then, these samples were tested for the detection and quantification of *Salmonella*, *Campylobacter*, and *Clostridium perfringens* by real-time PCRs. The results demonstrated the occurrence of three (6.7%) positive pools for *Salmonella*, 20 (44.4%) for *Campylobacter*, and 32 (71.1%) for *C. perfringens*. Mean bacterial concentrations in the positive samples were 4.3log₁₀ cells/g for *Salmonella*, 6.4 log₁₀ cells/g for *Campylobacter*, and 5.5 log₁₀ cells/g for *C. perfringens*. In conclusion, *Salmonella*, *Campylobacter*, and *C. perfringens* could be detected and quantified directly from the broilers cecal contents collected in the slaughter line. This procedure will be certainly useful to more quickly detect these foodborne pathogens and prevent their occurrence in chicken meat and other poultry food products.

INTRODUCTION

Foodborne diseases can result in more than two million deaths per year worldwide. Chicken meat is a possible carrier of foodborne pathogens that cause human infectious diseases (Saif, 2008; FAO-WHO, 2009; WHO, 2020). The most common bacterial pathogens are *Salmonella* and thermotolerant *Campylobacter* (*C. jejuni*, *C. coli*, and *C. lari*), accounting together for 94.1% of total foodborne outbreaks (EFSA, 2019). *Clostridium perfringens* is also a concerning bacterial pathogen due to the production of toxins that can cause food poisoning, mainly in children, elderly, and immunosuppressed people (Van Immerseel et al., 2004; Lindström et al., 2011; CDC, 2017).

Reduction of the risk of foodborne diseases infection can be pursued with measures of biosecurity and pathogen control throughout the food chain (Gölz et al., 2014; Rivera et al., 2018). The total absence or the occurrence of low levels of pathogenic bacteria in foods is the main goal to minimize human risk. This is also imperative in broilers flocks processed to produce chicken meat in slaughterhouses (Brazil, 2011; EFSA, 2011; Hermans et al., 2011). Besides broiler infection...
in any step of the poultry production chain, cross-contamination by pathogenic bacteria can also occur in food processing at the slaughterhouse. Evisceration in the slaughter line is considered the point of highest risk for carcass contamination by enteric pathogens. Therefore, animal and environmental samplings in all slaughterhouse steps (mainly in the evisceration) have to be collected to further perform laboratory analyses aimed at the detection of specific pathogens (Allaart et al., 2013; Rajan et al., 2017). Additionally, several different biological samples can be used for pathogen detection: fecal drop, feces, drag swabs, etc. It is noteworthy that a previous study demonstrated that cecal drops reflect chickens’ cecal microbiome (including pathogenic bacteria) better than any other animal sample and should be preferentially used to estimate the contamination risk in slaughterhouses and foods (Pauwels et al., 2015).

Foodborne bacteria are usually detected in laboratories by bacterial isolation in different culture media, followed by additional biochemical and serological characterization of bacterial colonies (Lee et al., 2015). Although these traditional microbiological methods are considered the gold standard, they are laborious and require several steps and reagents. These time-consuming analytical processes can take days to obtain a final result, which reduces the effectiveness in controlling the contamination of poultry flocks. Additionally, some fastidious bacteria such as Campylobacter are difficult to isolate in culture media, requiring other analytical procedures (Schnider et al., 2005; Rasschaert, 2007). These methods are also usually not quantitative, making the effective estimation of the contamination risk of positive foods for any bacteria impossible (Navidshad et al., 2012; Ricke et al., 2019).

Thus, DNA-based methods like real-time polymerase chain reaction (qPCR) and loop-mediated isothermal amplification (LAMP) have been increasingly developed and used to detect and quantify foodborne pathogens in the poultry production chain (Souza et al., 2019; Waldman et al., 2020). Other important advantage of DNA-based methods is the possibility of performing qualitative and quantitative analysis of food pathogens in biological samples simply with prior enrichment or even directly from biological samples (Albini et al., 2008; Rodgers et al., 2012; Park et al., 2014; Ricke et al., 2019).

The present study aimed to develop and evaluate a procedure based on real-time PCRs for the direct detection and quantification of the foodborne pathogens Salmonella, Campylobacter, and C. perfringens in broilers’ ceca collected in poultry slaughterhouses.

**MATERIAL AND METHODS**

**Samples**

A total of 450 griller broilers with 30 days of production (weight of birds ~ 1.4 kg) from 45 flocks in 39 different farms located in the state of Paraná, Brazil, were selected by convenience (10 broilers per flock) in three different slaughterhouses between 2017 and 2019. The cecum of each broiler was collected aseptically in the slaughter line after mechanical evisceration, placed in 45 sterile plastic bags (pools of 10 ceca per flock), and maintained at 4°C for a maximum of 24 h until laboratorial processing (Stern et al., 2005; Rasschaert et al., 2007).

**Samples pre-processing**

The 45 pools of ceca collected in slaughterhouses were prepared in the laboratory before analytical processing. First, cecal contents were released from the pools manually and homogenized. Then approximately 50 mg of each sample was collected with a sterile spatula and mixed with 1,250 μL of lysis solution (NewGene Prep, Simbios Biotecnologia, Rio Grande do Sul, Brazil). It was then incubated at 60°C for 10 min and centrifuged for 1 min at 9,410 x g. Tubes containing pre-processed samples were separated for DNA extraction, which was performed immediately after.

**DNA extraction**

DNA extraction of cecal content samples was performed using a commercial kit for total nucleic acid purification (NewGene Prep and Preamp, Simbios Biotecnologia). This procedure was performed according to the manufacturer’s instructions with some modifications. Initially, 100 μL of supernatant from the pre-processed sample prepared in the previous step was transferred to another tube (1.5 mL) containing 400 μL of cell lysis solution. After homogenization, 20 μL of silica suspension was added into the tube, which was incubated at room temperature for 10 min and shaken by inversion every 2 min. Then each sample was centrifuged for 1 min at 9,410 x g and the pellet was washed once with 150 μL of wash solution A, once with wash solution B, and once with wash solution C from the extraction kit. All tubes were placed in a thermoblock at 60°C to remove water. Then, 50 μL of elution solution was added and each sample tube
was centrifuged for 3 min at 9,410 x g. The liquid with soluble DNA was removed to another tube and stored at -20°C until DNA amplification.

**Real-time quantitative PCRs (qPCRs)**

Real-Time PCRs were carried out using commercial kits SALAmp, CPBAmp and CPERAmp (NewGene, Simbios Biotecnologia). All these kits are TaqMan® based-PCRs (including only one primers pair and one probe) with the following specific molecular targets: a) *invA* gene for *Salmonella* spp. (Hoorfar et al., 2000); b) 16S rRNA target for thermotolerant *Campylobacter* (C. *jejuni*, C. *coli* and C. *lari*) (Josefsen et al., 2004); and c) *plc* gene for *C. perfringens* (Abildgaard et al., 2010). Three gBlocks™ Gene Fragments (Integrated DNA Technologies, Iowa, USA) were synthesized with DNA sequences specific for all three targets and used as positive controls in the qPCRs. All assays were carried out on the 7300 Real-Time PCR equipment (Applied Biosystems, Massachusetts, USA) with the following cycling conditions: one initial denaturation cycle of 3 min at 95°C, followed by 40 cycles of 15 s at 95°C and 60 s at 60°C. Also, the three gBlocks™ were serially ten times diluted (initial concentrations of standard samples: 4,000,000 copies) and five dilutions with known loads (40 to 400,000 copies) of each gBlocks™ were used as standard samples for preparing standard curves for each bacterium in all runs of qPCR. CTs (cycle threshold) observed in all positive samples were compared with CTs of the standard curve to determine the bacterial load and to estimate the number of bacteria cells. All results were converted to log$_{10}$ cells/gram of cecal content.

**Data analysis**

Analytical sensitivity of the assays (LOD, limit of detection) was calculated using the PROBIT procedure based on the methodology described by Waldman et al., 2020. In summary, replicates of positive control samples of each pathogen with loads of specific bacteria DNAs (*Salmonella* 5,000,000 copies; *Campylobacter* 4,000,000 copies; and *C. perfringens* 3,000,000 copies) were serially ten times diluted (diluted 10$^0$ to 10$^4$ fold). The procedure was repeated on three different days by the same operator and equipment to evaluate reproducibility, totaling 9 replicates of each dilution. The number of cells of each dilution, the total number of repetitions, and number of positive repetitions were used to calculate the 50% and 95% cut-off values, which represented the LOD of the assays.

Assessments of possible statistical differences between qualitative variables were verified by Pearson's chi-square test or Fisher's exact test as appropriate. Distributions of quantitative variables were verified by the Kolmogorov-Smirnov test with Lilliefors correction and comparisons between groups were performed by Student's t-test and ANOVA. All analyses were bilateral with a pre-established significance level of 5% alpha error (p<0.05). Data were compiled and analyzed using SPSS® software (23.0 version, Chicago, IL Statistical Package for the Social Sciences).

**RESULTS**

**Development of the pre-processing procedure and limit of detection of qPCRs assays**

First, pre-processing conditions were evaluated by testing different volumes of samples and cell lysis solutions to avoid the occurrence of amplification inhibitors. Three DNA extractions protocols were developed: A, B, and C (Table 1). All the 45 samples collected in this study were submitted to these DNA extraction protocols and amplified with the specific qPCR for *Salmonella*. The final quantities of cecal content per qPCR reaction (mg/qPCR) for each protocol were 0.077, 0.0154, and 0.00308 mg/qPCR, respectively. The results demonstrated the occurrence of 1, 3, and 2 PCR positive samples for *Salmonella* in protocols A, B, and C, respectively. So, we assumed protocol B (0.0154 mg/qPCR) to be the most sensitive (Table 1).

**Table 1 – Adjustment of the DNA extraction protocol to avoid amplification inhibitors.**

| DNA extraction protocol | Volume of pre-processed sample extracted (µL) | Volume of cell lysis solution added in the DNA extraction step (µL) | Final quantity of cecal content per qPCR reaction (mg/qPCR) | Number of *Salmonella* positive samples/total samples
|-------------------------|---------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|-----------------------------------------------|
| A¹                      | 500                                         | 0                                                            | 0.077                                                         | 1/45                                        |
| B                       | 100                                         | 400                                                          | 0.0154                                                        | 3/45                                        |
| C                       | 20                                          | 480                                                          | 0.00308                                                       | 2/45                                        |

qPCR= quantitative real-time PCR.

¹ Original protocol, as described by the manufacturer’s instructions purification (NewGene Prep and Preamp, Simbios Biotecnologia).

²Repeated three times.
PROBIT analysis demonstrated a limit of $2.8 \log_{10}$ cells/g of cecal content of *Salmonella*, $2.7 \log_{10}$ cells/g of cecal content of thermotolerant *Campylobacter* and $2.6 \log_{10}$ cells/g of cecal content of *C. perfringens* to obtain 50% positive results, and $3.8 \log_{10}$ cells/g of cecal content of *Salmonella*, $3.7 \log_{10}$ cells/g of cecal content of thermotolerant *Campylobacter* and $3.6 \log_{10}$ cells/g of cecal content of *C. perfringens* to obtain 95% positive results for the qPCR, with a 95% confidence level (Table 2).

**Table 2** – PROBIT analysis – Analytical sensitivity of the qPCR assays in cecal content samples.

| Assay            | Confidence level (%) | Sensitivity (log_{10} cells/g) |
|------------------|----------------------|--------------------------------|
| qPCR *Salmonella*| 50%                  | 2.8                            |
|                  | 95%                  | 3.8                            |
| qPCR *Campylobacter* | 50%                  | 2.7                            |
|                  | 95%                  | 3.7                            |
| qPCR *Clostridium perfringens* | 50%                  | 2.6                            |
|                  | 95%                  | 3.6                            |

qPCR= quantitative real-time PCR.

**Prevalence of foodborne pathogens in the slaughterhouses**

The complete procedure described above, its mean qPCR for the three foodborne pathogens, was used in the analysis of the broiler flocks’ cecal content pools collected in the slaughter line. *Salmonella* was detected in 3 (6.7%), thermotolerant *Campylobacter* in 20 (44.4%), and *C. perfringens* in 32 (71.1%) poultry flocks in the three Brazilian slaughterhouses. The detection frequencies were statistically different for the three bacterial pathogens ($p<0.01$).

All flocks were evaluated for single or multiple infections. Five flocks (11.1%) showed negative results for the three bacteria analyzed, 8 flocks (17.8%) were positive only for thermotolerant *Campylobacter*, 18 flocks (40.0%) were positive only for *C. perfringens*, 2 flocks (4.4%) tested positive for *Salmonella* and *C. perfringens*, 11 flocks (24.4 %) tested positive for thermotolerant *Campylobacter* and *C. perfringens*, and only one flock (2.2 %) tested positive for the three bacteria analyzed.

Bacterial loads were determined in all positive samples. Overall results ranged from 2.8 to 6.4 $\log_{10}$ cells/g of cecal content (mean 4.3± 1.9) to *Salmonella*, 3.8 to 10.0 $\log_{10}$ cells/g (mean 6.4± 1.7) to thermotolerant *Campylobacter* and 3.7 to 7.8 $\log_{10}$ cells/g (mean 5.5± 1.0) to *C. perfringens* (Figure 1).

**DISCUSSION**

To increase food security it is necessary to reduce the occurrence of foodborne pathogens. For poultry products, it is also important to control bacterial contamination levels in all production chain processes, from farm to slaughterhouses, distribution, and storage (Brazil, 2011, EFSA, 2011; Back, 2014). Complete elimination of pathogens in chicken meat and other poultry products is an extremely difficult task, as they can become persistent in food processing environments (Rivera et al., 2018), but some measures can be taken.
to reduce the risk of food contamination, such as the rapid identification of infected broiler flocks to avoid sending contaminated chicken products to the market (Josefsen et al., 2004; Park et al., 2014).

Risk of chicken carcasses contamination by pathogenic bacteria could be assessed by evaluating the intestinal microbiota content, including the detection and quantitation of *Salmonella*, *Campylobacter*, and *C. perfringens*. Furthermore, pathogen tracking is more efficient when broilers are analyzed immediately after evisceration in the slaughter line, thus monitoring intestinal poultry microbiota and providing safer food products to human consumption (Selviworstow et al., 2015; Yeh et al., 2019). Traditionally, feces and fecal material have been the samples collected in poultry farms as well as in slaughterhouses, respectively. However, cecal drop and/or cecal content are better samples to evaluate the broilers’ intestinal microbiota because birds’ ceca retain the microbial content longer than the small and large intestines, including high concentrations of bacteria, fungi, and other microorganisms (Clench, 1999; Pauwels et al., 2015; Stanley et al., 2015; Ijaz et al., 2018). Moreover, feces and fecal material are not good estimators of the bacterial community of the cecal content (Pauwels et al., 2015; Selviworstow et al., 2015; Stanley et al., 2015). For these reasons, cecum was already known as a spot for bacterial isolation in poultry (Pauwels et al., 2015).

The present study evaluated the application of a procedure to detect and quantify three important foodborne pathogens (*Salmonella*, *Campylobacter*, and *C. perfringens*) in broiler’s cecal samples by real-time PCR. First, PROBIT assays were carried out to determine the LOD of the qPCRs in cecal samples and the results demonstrated that between 100 and 1000 bacterial cells/g could be detected as previously reported (Waldman et al., 2020). In addition, all these three bacterial pathogens were detected in more than one sample, demonstrating the good performance of the complete procedure. Previous studies have already demonstrated that PCR methods can be used to detect foodborne pathogens in chicken and other poultry samples (Alonso et al., 2011; Park et al., 2014). However, they have been carried out only after bacterial pre-enrichment and/or isolation (Borges et al., 2019; Souza et al., 2019; Borges et al., 2020). The present study demonstrated that *Salmonella*, *Campylobacter*, and *C. perfringens* can be detected directly from broilers’ cecal samples obtained in slaughter houses. With the removal of the pre-enrichment step in BPW, the whole procedure could be reduced in more than 12 hours (Park et al., 2014; Ricke et al., 2019). Additionally, quantitative data could be used to estimate the risk of contamination of chicken meat.

Additionally, it was possible to estimate the prevalence of these bacterial pathogens in the evaluated chicken flocks. *Salmonella* was detected in 6.7% of the ceca from the sampled flocks. Some previous studies in Brazil described a wide range of *Salmonella* frequencies in chicken samples collected at different stages of slaughter: 48.9% in samples obtained during the slaughtering process in the Rio Grande do Sul state (Borges et al., 2019); 9.33% of slaughterhouse samples (whole carcasses, cuts, viscera, and chiller water) in Mato Grosso do Sul state (Boni et al., 2011); 0.2% of cloacal swabs collected from broilers chicken flocks in the metropolitan region of Fortaleza (Bezerra et al., 2016); 3.7% of chicken carcasses from a slaughterhouse in the state of Mato Grosso (Cunha-Neto et al., 2018); and 1.66% of cloacal swabs and 26.66% of chicken carcasses in the state of Rio de Janeiro (Baptista et al., 2018). Moreover, 37.5% of raw chicken legs sold at retail in the state of São Paulo were positive for *Salmonella* (Ristori et al., 2017).

Bacterial loads were estimated in all positive samples and the results ranged from 2.8 log_{10} to 6.4 log_{10} with a mean of 4.3 log_{10} bacteria/g of cecal content. This result could be compared to another Brazilian study that presented *Salmonella* loads ranging from 1.16 to 3.64 log_{10} CFU/mL in chicken carcasses (Borges et al., 2019).

*Campylobacter* was observed in 44.4% of the broiler flocks analyzed here. Previous studies have also demonstrated the high frequency of *Campylobacter* in cecal samples from broiler flocks, ranging from 50% to 73% of positive flocks (Rasschaert et al., 2007; Reich et al., 2008; Boer et al., 2015; Selviworstow et al., 2015). Unfortunately, there are no studies in Brazil regarding cecal content samples, making more detailed comparisons impossible. However, a previous study with cecal drops demonstrated a high frequency (81.8%) of *Campylobacter* (Kuana et al., 2008). Some Brazilian studies tested other types of samples (water, cloacal swabs, carcasses, organs, among others) and reported a wide range of bacterial frequencies: 69.4% (Borges et al., 2020); 71.3% (Francini et al., 2007); 4.9% (Cortez et al., 2006); 16.8% (Hungaro et al., 2015); and 8.73% (Castro Chaves et al., 2010). It is noteworthy that all these studies were performed in different farms and also different Brazilian geographic regions. At the retail, two studies conducted at
Southern Brazil found *Campylobacter* in 17% of poultry meat (Silva et al., 2016) and 91.7% of poultry meat products (Würfel et al., 2018), while a study conducted at São Paulo state detected this bacterium in 7.7% of raw chicken (Lopes et al., 2018).

Previous studies have also shown that *Campylobacter* spreading is frequently fast in poultry flocks, with most birds from the same flock hosting this bacterium (Berndtson et al., 1996; Rudi et al., 2004). Also, a direct correlation between *Campylobacter* concentrations in broiler intestinal content and carcass is known and when a previously negative poultry flock is slaughtered after a positive flock, the more recent processed chickens become positive by cross-contamination (Rosenquist et al., 2006; Reich et al., 2008). These findings reinforce the importance of monitoring and decreasing *Campylobacter* intestinal concentrations in broilers at the flock level to reduce the transmission risk of this microorganism to humans through food consumption (Rudi et al., 2004; Rosenquist et al., 2006; Franchin et al., 2007; Reich et al., 2008).

In this study, bacterial loads of *Campylobacter* ranged from 3.8 log_{10} to 10 log_{10}, with a mean of 6.4 log_{10} bacteria/g of cecal content. According to a previous study (Seliwiorstow et al., 2015), highly contaminated broiler flocks (> 7.5 log_{10} CFU/g) should be excluded to reduce the potential risk of campylobacteriosis transmission to humans. The present study found five broiler flocks (11.1%) with loads > 7.5 log_{10} bacteria/g of cecal content, i.e., presenting a high transmission risk of campylobacteriosis through poultry product consumption. Another study quantified *Campylobacter* in other different types of samples collected during slaughter in Brazil, with bacterial loads ranging from 0 to 2.1 log CFU/mL (Borges et al., 2020). In worldwide studies that analyzed cecal samples, bacterial concentrations ranged from 6.1 log_{10} to 11.1 log_{10} with means ranging from 6.9 log_{10} to 8.5 log_{10} CFU/g (Rudi et al., 2004; Reich et al., 2008; Seliwiorstow et al., 2015; Vinueza-Burgos et al., 2018).

*C. perfringens* was found in 71.1% of broiler flocks analyzed in this study. A study conducted in Swedish slaughterhouses found *C. perfringens* in 18% of broiler carcasses after chiller (Lindblad et al., 2016). To our knowledge, this is the first Brazilian study to evaluate the occurrence of *C. perfringens* in poultry slaughterhouses. At the retail level, when analyzing chicken liver sold in USA markets, Cooper et al. (2013) detected *C. perfringens* in 69.6% of samples. *C. perfringens* loads ranged from 3.7 to 7.8 log_{10}, with a mean of 5.5 log_{10} log_{10} bacteria/g of cecal content. *C. perfringens* is a major cause of human food poisoning. Bacteria survive in the animal body when food is contaminated with very high concentrations (> 6 log_{10} cells/g of food) because viable bacterial cells can form spores in vivo (Shrestha et al., 2018). The present study found 10 broiler flocks (22.2%) with loads ≥ 6.1 log_{10} bacteria/g of cecal content, demonstrating concern in regards to contamination of chicken meat.

Brazil is an important poultry-producing country and exports chicken meat to different countries of the world (ABPA, 2020). The results reported in this study are concerning, as they show that three important bacterial pathogens are present in chickens from broiler flocks in commercial slaughterhouses and could contaminate poultry food products. It is noteworthy that dissemination of these pathogens in Brazilian poultry is widely variable depending on the region, poultry establishment, poultry flock, and type of sample analyzed (Cortez et al., 2006; Bezerra et al., 2016; Würfel et al., 2018; Borges et al., 2019).

Specific hygiene and biosecurity measures to control these foodborne pathogens throughout the poultry production chain are extremely necessary to reduce economic losses and minimize the risk of exposure of consumers to these bacteria.

In conclusion, a procedure for detection and quantitation of the main poultry bacterial pathogens in cecal content samples was developed and used in the routine analysis of three slaughterhouses to assess the risk for bacterial contamination in chicken meat. The complete procedure, from collection of samples to the analysis of the results in the laboratory, takes less than one day (approximately 5 h if the industry has a molecular biology facility). So it is possible to predict the risk of food infection, take sanitary measures before the food reaches the end of the production chain, and provide safe food for consumers. This procedure can also be useful to detect the occurrence and to determine the prevalence of these pathogens in both broiler flocks and slaughter houses from different poultry-producing regions in Brazil.

**ACKNOWLEDGMENTS**

This study was supported by Simbios Biotecnologia. MNS and JMW were supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) [Finance Code 001]; and VRL was supported by the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) [Process number 311010/2017-2].
REFERENCES

Abildgaard L, Hofberg O, Schramm A, Balle KM, Engberg RM. The effect of feeding a commercial essential oil product on Clostridium perfringens numbers in the intestine of broiler chickens measured by real-time PCR targeting the α-toxin-encoding gene (plc). Animal Feed Science and Technology 2010;157(3-4):181-9.

ABPA - Associação Brasileira de Proteína Animal. Relatório Anual 2020 ABPA. São Paulo; 2020 [cited 2020 May 12]. Available from: https://abpa-br.org/wp-content/uploads/2020/05/abpa_relatorio_anual_2020_portugues_web.pdf.

Albini S, Brodard I, Jaussi A, Wollschläger N, Frey J, Miserez R, et al. Real-time multiplex PCR assays for reliable detection of Clostridium perfringens toxin genes in animal isolates. Veterinary Microbiology 2008;127(1):179-85.

Allaart JG, van Asten AJ, Gröne A. Predisposing factors and prevention of Clostridium perfringens-associated enteritis. Comparative Immunology, Microbiology and Infectious Diseases 2013;36(5):449-64.

Alonso MZ, Padola NL, Parma AE, Lucchesi PMA. Enteropathogenic Escherichia coli contamination at different stages of the chicken slaughtering process. Poultry Science 2011;90(11):2638-41.

Back A. Monitoria Sanitária para frangos de corte. In: Macari M, Mendes Alonso MZ, Padola NL, Parma AE, editors. Produção de frangos de corte. 2nd ed. Campinas: FACTA; 2014. p. 371-6.

Baptista DQ, Santos AF, Aquino MHC, Mendes AA, Menten JF, Alencar NI, editors. Produção de frangos de corte. 2nd ed. Campinas: FACTA; 2014. p. 371-6.

Baptista DQ, Santos AF, Aquino MHC, Abreu DL, Rodrigues DP, Nascimento ER, et al. Prevalence and antimicrobial susceptibility of Salmonella spp. serotypes in broiler chickens and carcasses in the state of Rio de Janeiro, Brazil. Pesquisa Veterinária Brasileira 2018;38(7):1278-85.

Berndtson E, Daniellsson-Tham ML, Engvall A. Campylobacter incidence on a chicken farm and the spread of Campylobacter during the slaughter process. International Journal of Food Microbiology 1996;32(1-2):35-47.

Bezerra WGA, Da Silva ING, Vasconcelos RH, Machado DN, Souza Lopes E de, Lima SVG, et al. Isolation and antimicrobial resistance of Escherichia coli and Salmonella enterica subsp. enterica (O: 6, 8) in broiler chickens. Acta Scientiae Veterinariae 2016;44:1364.

Boer P, Rahouai H, Leer RJ, Montijn RC, Van der Vossen JMBM. Real-time PCR detection of Campylobacter spp.: a comparison to classic culturing and enrichment. Food Microbiology 2015;31:96-100.

Boni HFK, Carrijo AS, Fascina VB. Ocorrência de Salmonella spp. em aviaários. Revista Brasileira de Saúde e Produção Animal 2011;12(1):84-95.

Borges KA, Cisco IC, Furian TQ, Tedesco DC, Rodrigues LB, Nascimento VP do, et al. Detection and quantification of Campylobacter spp. in Brazilian poultry processing plants. The Journal of Infection Developing Countries 2020;14(1):109-13.

Borges KA, Martelo, EB, Dos Santos LA, Furian TQ, Cisco IC, Manto L, et al. Detection and quantification of Salmonella spp. in poultry slaughterhouses of southern Brazil. The Journal of Infection Developing Countries 2019;13:455-60.

Brazil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Manual técnico de diagnóstico laboratorial de Campylobacter: gênero Campylobacter: diagnóstico laboratorial clássico e molecular. Brasília; 2011.

Castro Chaves SO, De Souza CO, De Aritmaité Freitas J, Dos Santos DD, De Araújo CV, Da Silva RR. Ocorrência de Campylobacter em granjas e abatedouro avícolas na mesorregião metropolitana de Belém, PA, Brasil. Ciência Animal Brasileira 2010;11(3):554-60.

CDC - Centers for Disease Control and Prevention. Food safety. Clostridium perfringens. 2017 [cited 2018 Jan 01]. Available from: https://www.cdc.gov/foodsafety/diseases/clostridium-perfringens.html.

Clench MH. The avian cecum: update and motility review. Journal of Experimental Zoology 1999;283:441–7.

Cooper KX, Bueschel DM, Songer JG. Presence of Clostridium perfringens in retail chicken livers. Anaerobe 2013;21:67-8.

Cortez AL, Carvalho AC, Scarcelli E, Miyashiro S, Vidal-Martins A, Bürger KP. Survey of chicken abattoir for the presence of Campylobacter jejuni and Campylobacter coli. Revista do Instituto de Medicina Tropical de São Paulo 2006;48(6):307-10.

Cunha-Neto AD, Carvalho LA, Carvalho RCT, Prazeres Rodrigues D dos, Mano SB, Figueiredo EEDS, et al. Salmonella isolated from chicken carcasses from a slaughterhouse in the state of Mato Grosso, Brazil: antibiotic resistance profile, serotyping, and characterization by repetitive sequence-based PCR system. Poultry Science 2018;97(4):1373-81.

EFSA - European Food Safety Authority and ECDC - European Centre for Disease Prevention and Control. The European Union one health 2018 zoonoses report. EFSA Journal 2019;17(12):5926.

EFSA - European Food Safety Authority. EFSA panel on biological hazards (BIOHAZ). Scientific opinion on Campylobacter in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA Journal 2011;9(4):2105-245.

FAO-WHO - Food and Agriculture Organization, World Health Organization. Salmonella and Campylobacter in chicken meat. Microbial Risk Assessment Series 2009;19:1-69.

Franchin PR, Ogliari PJ, Batista CRV. Frequency of thermophilic Campylobacter in broiler chickens during industrial processing in a Southern Brazil slaughterhouse. British Poultry Science 2007;48(2):127-32.

Götz G, Rosner B, Hofreuter D, Josenhans C, Kreienbrock L, Löwenstein A, et al. Relevance of Campylobacter to public health - The need for a One Health approach. International Journal of Medical Microbiology 2014;304(7):817–23.

Hermans D, Van Deun K, Messens W, Martel A, Van Immerseel F, Haesebroeck F, et al. Campylobacter control in poultry by current intervention measures ineffective: urgent need for intensified fundamental research. Veterinary Microbiology 2011;152(3-4):219–28.

Hoorfar J, Ahrens P, Radstrom P. Automated 5’ nuclease PCR assay for identification of Salmonella enterica. Journal of Clinical Microbiology 2000;38:3429-35.

Hungaro HM, Mendonça RCS, Rosa VO, Badaró ACL, Moreira MAS, Chaves JBP. Low contamination of Campylobacter spp. on chicken carcasses in Minas Gerais state, Brazil: molecular characterization and antimicrobial resistance. Food Control 2015;51:15-22.

Ijaz UZ, Sivaloganathan L, Mckenna A, Richmond A, Kelly C, Linton M, et al. Comprehensive longitudinal microbiome analysis of the chicken cecum reveals a shift from competitive to environmental drivers and a window of opportunity for Campylobacter. Frontiers in Microbiology 2018;15(9):2452.

Josefson MH, Jacobsen NR, Hoorfar J. Enrichment followed by quantitative PCR both for rapid detection and as a tool for quantitative risk assessment of food-borne thermotolerant campylobacters. Applied and Environmental Microbiology 2004;70(6):3588-92.

Knappik AM, Santos LR, Rodrigues LB, Borsari A, Moraes HLS, Salle CTP, Nascimento VP. Occurrence and characterization of Campylobacter in the Brazilian production and processing of broilers. Avian Diseases 2008;52(4):680-4.
Lee KM, Runyon M, Herrmann TJ, Phillips R, Hsieh J. Review of Salmonella detection and identification methods: aspects of rapid emergency response and food safety. Food Control 2015;47:264-76.

Lindblad M, Lindmark H, Lambertz ST, Lindqvist R. Microbiological baseline study of broiler chickens at Swedish slaughterhouses. Journal of Food Protection 2006;69(12):2875-82.

Lindström M, Heikinheimo A, Lahti P, Korkeala H. Novel insights into the epidemiology of Clostridium perfringens type A food poisoning. Food Microbiology 2011;28(2):192-8.

Lopes GV, Landgraf M, Destro MT. Occurrence of Campylobacter in raw chicken and beef from retail outlets in São Paulo, Brazil. Journal of Food Safety 2018;38(3):e12442.

Maurischat S, Baumann B, Martin A, Malorny B. Rapid detection and specific differentiation of Salmonella enterica subsp enterica Enteritidis, Typhimurium and its monophasic variant 4,5,12:i:- by real-time multiplex PCR. International Journal of Food Microbiology 2015;193:8-14.

Nagpal R, Ogata K, Tsuji H, Matsuda K, Takahashi T, Nomoto K, et al. Sensitive quantification of Clostridium perfringens in human feces by quantitative real-time PCR targeting alpha-toxin and enterotoxin genes. BMC Microbiology 2015;15(1):219-31.

Navidshad B, Liang JB, Jahromi MF. Correlation coefficients between different methods of expressing bacterial quantification using real time PCR. International Journal of Molecular Sciences 2012;13(2):2119-32.

Park SH, Aydin M, Khattwara A, Dolan MC, Gilmore DF, Bouldin JL, et al. Current and emerging technologies for rapid detection and characterization of Salmonella in poultry and poultry products. Food Microbiology 2014;36:250-62.

Pauwels J, Taminiau B, Janssens GPJ, Beenhouwer M de, Delhalle L, Daube G, et al. Cecal drop reflects the chickens’ cecal microbiome, fecal drop does not. Journal of Microbiological Methods 2015;117:164-70.

Rajan K, Shi Z, Ricke SC. Current aspects of Salmonella contamination in the US poultry production chain and the potential application of risk strategies in understanding emerging hazards. Critical Reviews in Microbiology 2017;43(3):370–92.

Rasschaert G, Houf K, Van Hende J, Zutter L de. Investigation of the Campylobacter jejuni concurrent colonization with other bacterial pathogens in poultry and cecal samples by integrated cell concentration and DNA purification. Applied and Environmental Microbiology 2004;70(2):790-7.

Rudí K, Heidal HK, Katla T, Johansen BK, Nørdal J, Jakobsen KS. Direct real-time PCR quantification of Campylobacter jejuni in chicken fecal and cecal samples and its application for food safety. Food Control 2015;47(3):264-76.

Saif YM. Diseases of poultry. 12th ed. Iowa: Blackwell Publishing; 2008.

Scheidegger R, Rosenthal A, Garillot S, Swoyer JR, Kudelka S, Nissenkorn J, et al. Evaluation of the PCR test for Campylobacter jejuni and Campylobacter coli in poultry products by international collaborative study and comparison with the PFGE method. Veterinary Research 2008;39(5):548-56.

Shrestha A, Utzal FA, McClaine BA. Enterotoxigenic Clostridium perfringens enteric diseases. Microbiology Spectrum 2018;6(5):1-17.

Silvius J, Tejada TS, Blum-Menezes D, Dias PA, Timm CD. Campylobacter species isolated from poultry and humans, and their analysis using PFGE in southern Brazil. International Journal of Food Microbiology 2016;217:189-94.

Souza MN, Lehmann FKM, Carli S de, Kipper D, Fonseca ASK, Ikuta N, et al. Molecular detection of Salmonella serovars Enteritidis, Heidelberg and Typhimurium directly from pre-enriched poultry samples. British Poultry Science 2019;60(4):388-94.

Stanley D, Geier MS, Chen H, Hughes RJ, Moore RJ. Comparison of fecal and cecal microbiotas reveals qualitative similarities but quantitative differences. BMC Microbiology 2015;15(1):51.

Sterk NJ, Reersen L, Lowman R, Biasilione JR, Frickhöft G, Gunnarsson E, et al. Campylobacter jejuni in different cattle and poultry origins. International Journal of Food Microbiology 2016;219:37-45.

Van Meersema F, Buck JD, Pasmans F, Huysgebaert G, Haesebroeck F, Ducatelle R. Clostridium perfringens in poultry: an emerging threat for animal and public health. Avian Pathology 2004;33(6):537-49.

Vinueza-Burgos C, Cevallos M, Cisneros M, Domene I van, Zutter L de. Quantification of the Campylobacter contamination on broiler carcasses during the slaughter of Campylobacter positive flocks in semi-industrialized slaughterhouses. International Journal of Food Microbiology 2018;269:75-9.

WHO - World Health Organization. Food safety. 2020 [cited 2020 Oct 10].