MICROBIOLOGICAL QUALITY OF CATTLE CARCASS DURING SLAUGHTER AND OCCURRENCE OF *E. coli* O157: H7 IN BEEF

QUALIDADE MICROBIOLÓGICA DA CARCAÇA BOVINA DURANTE O PROCESSO DE ABATE E A OCORRÊNCIA DE E. coli O157:H7 NA CARNE

C. B. PRATA[1*]; M. V. F. LEMOS[1]; L. F. PRATA[1]; K. CASELANI[1]

SUMMARY

Escherichia coli O157:H7, an important bacillus strain associated with serious gastroenteritis in humans, is more frequently derived from the consumption of raw or poorly cooked beef. Cattle are important reservoirs suggesting the possibility that feedlot diet management influences the emergence of Shiga-toxigenic strains. This study evaluates the microbiological quality of carcasses and the occurrence of *E. coli* O157:H7 using the results from general indicator methods (total viable count, coliform rate and *E. coli* counts) and by an automated PCR method for the detection of *E. coli* O157:H7. Samples were collected from (industrially processed) meat trimmings and from carcasses of cattle finished on pasture or in feedlots so that sufficient data for the Hazard Analysis and Critical Control Points (HACCP) could be obtained. Samples of rectal swab for experimental detection of *E. coli* O157:H7 were also collected. One hundred rectal swabs, 100 samples retrieved from warm carcasses and 323 samples of meat trimmings were analyzed. With the exception of one sample of meat trim (0.31%), all the other samples from excreta and carcasses were negative for the O157:H7 *E. coli* strain. There were no significant differences between the methods used for cattle finishing. Indicator methods results were considered acceptable in 91%, 85% and 93% of tested samples of carcasses respectively for TVC, coliform and *E. coli* counts. These results agree with statistical data showing the low occurrence of O157:H7 strain.

KEY-WORDS: Bovine. *E. coli* O157:H7. Feedlot. Pasture. PCR.

RESUMO

A *Escherichia coli* O157: H7 é uma importante cepa associada a surtos graves de enfermidade em seres humanos, a maioria deles derivada do consumo de carne crua ou mal cozida. É provável que o gado atue como um importante reservatório, sugerindo-se a possibilidade de que a gestão da dieta no confinamento possa influenciar o aparecimento de cepas Shigatoxigênicas. Este estudo teve como objetivo verificar a qualidade microbiológica das carcaças e a ocorrência de *E. coli* O157: H7, por meio dos resultados obtidos por métodos indicadores (contagem total de microrganismos viáveis, contagem de Coliformes e de *E. coli*) e por um método automatizado de PCR para detecção de *E. coli* O157: H7. Foram colhidas amostras de retalhos de carne (carne industrial) e de carcaças de bovinos terminados em pastagem ou em confinamento, permitindo o fornecimento de subsídios necessários para a Análise de Perigos e Pontos Críticos de Controle (HACCP). Desses mesmos animais foram colhidas, também, amostras de swab retal para a detecção experimental de *E. coli* O157: H7 nas fezes. Um total de 100 swabs retais, 100 amostras de carcaças quentes, além de outras 323 amostras de aparas de carne (retalhos da desossa), foram analisados. Com exceção de uma amostra de retalhos de carne (0,31%), todas as demais, de fezes e de carcaças, foram negativas para a presença de *E. coli* O157: H7. Não houve diferenças significativas entre os tipos de terminação utilizada para o gado. Os resultados dos métodos indicadores foram considerados aceitáveis em 91%, 85% e 93% das amostras testadas, respectivamente, para a CTV, contagem de Coliformes e de *E. coli* de carcaças, dando suporte e em acordo com a baixa ocorrência da cepa O157: H7.

PALAVRAS-CHAVE: Bovino. Confinamento. *E. coli* O157:H7. Pasto. PCR.

[1] Universidade Estadual Paulista – UNESP. Faculdade de Ciências Agrárias e Veterinárias – FCAV

INTRODUCTION

The slaughter process stages, especially those related to skinning and gutting, are responsible for the contamination of carcasses by microorganisms with deteriorating potential or possibly by pathogens. However, it is impractical to monitor these pathogens due to their diversity; small number and uneven distribution, when present. Even results indicating the absence of a pathogen do not conclude the safety of food consumption.

During the slaughtering process, the hazards and risks associated with hygiene or safety must be referenced to lawful standards. These are known as indicator methods that include microorganisms Total Viable Count (TVC) and methods that determine the source of fecal contamination (Coliform bacteria and *Escherichia coli* counts). The presence of *E. coli* on carcasses may imply that other fecal microorganisms, including *E. coli* O157: H7 and *Salmonella* may be present (JARDIM et al, 2006).

Cattle are considered the main reservoir of *E. coli* O157: H7 (MERCADO, 2007). In several countries, this serotype is the most frequently identified (GOMEZ et al., 2005; VICENTE et al., 2004) and represents a threat to public health due to the severity of the disease caused by it (ROLDÁN et al., 2007).

The meat industry is responsible for producing safe foods that meet consumer expectations and importers requirements. To ensure the quality of the product, the industry is concerned with the occurring changes in the livestock production systems, mainly the rapid expansion of finishing cattle in feedlots as observed in recent years. Although the confinement period in Brazil is limited and ranges from 45 to 90 days just to give carcass finish, no information is available about these changes and their possible impact on meat quality. Therefore, there is a continuing need for results to support and sustain quality management and food safety.

This study aimed to monitor control practices applied during the slaughtering of cattle and their results through indicator methods. These monitoring methods are used to determine the occurrence frequency of *E. coli* O157: H7 on carcasses and meat, and to determine a possible relationship between these results and the animals raised and finished on pasture or in feedlots.

MATERIAL AND METHODS

Sampling: To determine the systematic occurrence of *E. coli* O157: H7, 323 samples of meat cuts (trimmings) were collected in the boning room. Of these, 256 samples were collected daily from November 2008 to October 2009, with 67 other samples taken during comparative experiments, as a pool from the industrial meat production. Normally these clippings are packed in 30-kg boxes while each piece must weigh more than 200g.

Before starting the routine work of the boning room, the meat contact surfaces (tables, knives, hooks and conveyor belts) were sampled for bacteria TVC as an indicator of hygiene, which requires corrective actions when results are unsatisfactory. An average of 38.6 monthly samples was analyzed for this purpose, totaling 463 contact surface samples.

For comparison of finishing systems, we selected 100 apparently healthy bulls, of which 50 were finished in the pasture and 50 in feedlots. From each property, five animals were randomly sampled, totaling 10 properties with extensive system (eight of them in São Paulo, and one in Minas Gerais and Goiás), and 10 farms with intensive systems (nine located in Minas Gerais and one in Goiás). The slaughter took place between November 2008 and February 2009, under similar conditions and processes in a slaughterhouse inspected by the Serviço de Inspeção Federal (SIF), and located in São Paulo, Brazil.

Samples of trimmings: during each production hour, 50-g samples were collected from five boxes randomly distributed, totaling 250 grams, which were stored in sterile plastic bags. A total of 67 samples with 250g of beef trimmings were collected from November 2008 to February 2009.

During the slaughter, one hundred (100) stool samples were collected to determine *E. coli* O157: H7 by automated PCR. From the hot carcass surfaces, another hundred (100) samples were collected to determine the occurrence of *E. coli* O157: H7 and to estimate the total microbial count. From the chilled carcass surfaces, one hundred (100) samples were collected to estimate coliform and *E. coli* count.

Stool: Stool samples were collected to determine *E. coli* O157: H7 by the automated PCR technique. One hundred rectal swabs were collected from the same animals previously selected, on the slaughter platform and before the occlusion of the rectum, and placed in sterile plastic bags containing 10 ml of TSB (Trypic Soy Broth).

Carcass surface samples: The samples were collected according to the criteria established by Regulation No. 2073/20005 of the European Commission, through non-destructive method using a cellulose sponge. Previously sterilized sponges were moistened and rubbed ten times in each direction, vertical and horizontal, covering an area of 10 x 10 cm (100 cm^2), bounded by a jig metal (stainless steel) in four regions of the chilled carcasses - neck, chest, flank and rump - and three regions for hot carcass - the chest, flank and rump (Food Standards Agency, UK, 2007). The sponges were placed in sterile plastic bags containing 10ml of TSB medium to determine *E. coli* O157: H7 presence and 10 ml of BPW to estimate TVC of coliforms and *E. coli*.

Polymerase chain reaction - PCR: 90 ml of TSB medium were added to the sterile plastic bags containing stool or carcasses samples. One milliliter of

novobiocin (20mg.U^{-1}) was added to the stool samples. Then the samples were mixed in a Stomacher for one minute at 200 rpm and incubated at 41.5°C for 24h. From each meat trimmings sample, 25g were removed and placed in sterile flasks containing 225 mL of TSB. The flasks were incubated at 41.5°C, for 18 to 24 h.

Respectively, the samples of carcasses, of meat trimmings and feces were subjected to a PCR reaction using the commercial kit system BAX ® from DuPont, Qualicon, following the manufacturer's instructions. This automated method for the detection of *E. coli* O157: H7 has received approval AOAC-RI - Performance Tested Method, Certificate 010 401, for the analysis of samples of ground beef and other food types. The experimental detection in stool samples followed the procedures set by DuPont Brazil while the positive control was performed using a strain of *E. coli* O157: H7, courtesy of Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil.

Microbiological Analysis: Based on ISO 4833:2003 and 21528-2:2004 protocols, the Petrifilm™ APC and Petrifilm™ *E. coli* Count Plates (ECC) were used to estimate the TVC and coliforms and *E. coli* counts, respectively. Following the AOAC methodology, 1mL of each solution contained in plastic bags was inoculated on the Petrifilm plate, according to the manufacturer's instructions. Then the plates were incubated at 35°C for 48h. The PCA Petrifilm plate has an indicator to facilitate colony counting. On the ECC plates, red colonies showing gas production were counted as coliforms, while blue colonies with gas production were counted as *E. coli.* The results were expressed as $\log_{10}$ colony forming units (CFU) per unit area (cm^2).

According to the Pathogen Reduction Program, regarding *E. coli,* the result is considered satisfactory when values are less than or equal to 100 CFU.cm^{-2}, which is equivalent to 2 $\log_{10}$ CFU.cm^{-2}. Total Viable Count of Coliform and *E. coli* were classified according to criteria of the meat industry Guidelines, 2007, from the Food Standards Agency, UK.

Statistical analysis - The results of the substrate surface indicators were statistically analyzed using SAS 9.1 (11) and Fisher exact test to assess the differences between groups finished on pasture and confinement, at significance level of $p < 0.05$ (25). According to the detection limits of microbiological analysis, when there were neither coliforms nor *E. coli,* a value of 0.8 $\log_{10}$ CFU/cm^2 was used for statistical analysis.

RESULTS AND DISCUSSION

Depending on the purpose, as a hygiene standard or food safety, the results for TVC bacteria can be interpreted differently. Thus, for samples of food-contact surface, the results are satisfactory when ≤ 1.0 $\log_{10}$ and unsatisfactory when exceeding this value, without an intermediate category known as acceptable. For the 463 contact surfaces samples analyzed, 219 (47.30%) were classified as satisfactory, while the remaining 244 (52.70%) were considered unsatisfactory. For many satisfactory samples, the result showed no bacterial colonies on plates. For the unsatisfactory samples, the results ranged from 1.1 to 2.48 $\log_{10,}$ with most of those very close to the limit value (1.0 $\log_{10}$) and some displaying much higher values.

For carcasses, the TVC results were satisfactory when $\log_{10}$ was lower than 2.8 ($<6.3x10^2$ CFU.cm^{-2}); acceptable, between 2.8 and 4.3 (between $6.3x10^2$ and $2.0x10^4$ CFU.cm^{-2}) and unsatisfactory when greater than 4.3 ($> 2.0x10^4$CFU.cm^{-2}) .CFU.cm^{-2}. The parameters used for classification of Coliforms and *E. coli* were satisfactory when $\log_{10}$ value was less than 0.8 (<6.3CFU.cm^{-2}), acceptable between 0.8 and 1.8 (6.3 to 6.3x10) and unsatisfactory when higher than 1.8 ($>$ 6.3 x10 CFU.cm^{-2}). This classification is shown in Table 1.

This last standard (for Coliforms and *E. coli*) is more rigorous than that established by the European Commission, in the Regulation No. 2073 (2005) stating as satisfactory an average lower than or equal to 1.5 $\log_{10}$CFU/cm^2; acceptable, between 1.5 and 2.5; and unsatisfactory, higher than 2.5 $\log_{10}$CFU/cm^2. In 1997, when the Ministry of Agriculture started the implementation of HACCP and Pathogen Reduction Program, the latter demanded by the United States as an additional requirement for export, the criteria for scoring indicators were even less stringent.

The TVC results for samples of pasture finished cattle were slightly higher than those from feedlot cattle, but these differences were not significant for the two groups. For the pasture finished cattle, the results ranged from 2.50 to $6.31x10^2$UFC.cm^{-2}, with a mean of $9.43x10 \pm 2.01x10^2$ while for feedlot, the results ranged from 1.50 to $6.31x10^2$ UFC.cm^{-2}, with an average of $5.26x10 \pm 1.48x10^2$.

Numerically, the opposite occurred for coliforms and *E. coli*. The confined animals showed higher values than the pasture cattle. The coliform count ranged from 0.08 to 1.81x10 UFC.cm^{-2} and from 0.08 to 7.50x10 UFC.cm^{-2} for pasture and confinement, respectively, averaging 1.34 UFC.cm^{-2} and 9.25 UFC.cm^{-2}. The *E. coli* counts for carcasses samples of animals finished on pasture or feedlot showed the same variation, from 0.08 to 1.25x10 UFC.cm^{-2}, but with averages of 0.78 and 1.97 UFC.cm^{-2}, respectively.

Unsatisfactory results were observed only for coliforms, which corresponded to 4 (8.0%) carcass samples of feedlot animals, but again, there was no statistically significant difference. According to Fischer exact test, the TVC ($p = 0.4870$), Coliforms ($p = 0.1334$) and *E. coli* ($p = 0.4360$) values were independent of termination type, pasture or feedlot. However, it should be emphasized that this comparison was performed for a small sampling. These results were consistent with those of Jardim et al. (2006), who observed similar levels of coliforms and *E. coli,* both in the skin and carcasses of either pasture or feedlot animals.

The average TVC values found for samples of grazing (1.41 log CFU/cm^2) and confined (1.12 log CFU/cm^2) cattle were slightly lower than the values

Table 1 - Classification of the results obtained from the evaluation of the hot and chilled carcasses for contamination indicators (total viable count of microorganisms, coliforms and *E. coli*), interpreted according to criteria recommended for the meat industry (Food Standards Agency, 2007).

Termination		**Confinement**		**Pasture**	
Number of Samples		50		50	
TVC	**Satisfactory**	47	94%	44	88%
	Acceptable	3	6%	6	12%
	Unsatisfactory	0	0%	0	0%
Total Coliforms	**Satisfactory**	40	80%	45	90%
	Acceptable	6	12%	5	10%
	Unsatisfactory	4	8%	0	0%
E. coli	**Satisfactory**	45	90%	48	96%
	Acceptable	5	10%	2	4%
	Unsatisfactory	0	0%	0	0%

reported by Zweifel et al. (2004), in Switzerland, whose average ranged from 2.1 to 3.1 log CFU/cm^2, and also lower than the value of 2.42 log CFU/cm^2 observed by Phillips et al. (2001). The decision of the European Union, through Regulation No. 471/2001, of indicating the non-destructive cellulose sponge method to sample carcass surface was considered adequate since it standardizes and simplifies this step for microbiological estimates, thus enabling comparisons.

E. coli O157: H7 results were all negative, except for one trimmings sample. Despite the small number of samples analyzed, these data are consistent with the results obtained by the standard methods for TVC, Coliforms and *E. coli,* which were acceptable in 91%, 85% and 93% of samples, respectively.

The frequency of 0.31% for *E. coli* O157: H7 in meat trimmings sample is closely related with the values found in studies with cattle carcasses during slaughter, 0.5% reported by Meichtri et al. (2004) in Argentina, 0.1% reported by Phillips et al. (201) in Australia, and 1.5% by Cerqueira et al. (199 9) in Rio de Janeiro. Roldan et al. (2007) isolated *E. coli* O157: H7 in 1.2% of 250 samples of sliced meat, nearly four times greater than the percentage found in this study.

Despite the experimental conditions of the evaluation and all stool samples being negative for *E. coli* O157: H7, other Brazilian authors have reported a low frequency of this serotype. In São Paulo, Irino et al. (2005) found 0.6% of *E. coli* O157: H7 in fecal samples of young dairy cattle. In the region of Ribeirão Preto, SP, Stella et al. (2008) isolated *E. coli* in 430 stool samples, of which two were confirmed as the O157: H7 strain (isolated from calves).

The changing frequency of *E. coli* O157: H7 may be due to different techniques (ARMSTRONG et al, 1996). The method used in this study, the BAX® system has a direct correlation, higher than 99%, with over 120 types of *E. coli* O157: H7 and demonstrates an excellent exclusion ability (>98%) against other *E. coli* strains and other enteric bacteria, conditions evaluated using positive and negative controls (DUPONT QUALICON, 2002).

Despite the low frequency of serotype O157: H7 in this study, studies with other serotypes that produce Shiga-toxins (STEC) showed that they are widespread in cattle. Sales et al. (2006) analyzed 100 fecal samples from cattle slaughtered in São Luís, Maranhão, and the isolation rate of STEC was 73%. Vincent et al. (2004) reported an even higher rate, with STEC strains identified in all herds examined in Jaboticabal, SP while the O157, O111 and O113 serotypes were observed in 40%, 50% and 90% of the samples, respectively.

CONCLUSION

The results according to the used indicator methods were satisfactory in 91%, 85% and 93% of the tested samples, respectively, for TVC, Coliform and *E. coli* counts. The reported data support and agree with the low prevalence of *E. coli* O157: H7, which was found in only one sample (0.31%) of meat trimmings. Despite the small sample size, the comparison of the results showed that the microbiological quality of carcasses of grazing cattle and feedlot were not significantly different. No strain of *E. coli* O157: H7 was detected in rectal swabs (fecal samples), which might indicate that sample size was not adequate and even the PCR automated methodology may not be suitable for this purpose.

REFERENCES

ARMSTRONG, G. L. et al. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the

developed world. **Epidemiologic Reviews**, v.18, n.1, p.29-51, 1996.

CERQUEIRA, A. M. et al. High occurence of shiga-producing *Eschericia coli* (STEC) in healthy cattle in Rio de Janeiro State, Brazil. **Veterinay Microbiology**, n.70, p.111-121, 1999.

DU PONT QUALICON. **Sistema BAX® - Análise de Reação em Cadeia de Polimerase (PCR) com detecção automatizada**. 2002.

FSA - FOOD STANDARDS AGENCY. **Red Meat Safety & Clean Livestock**. 2002. Disponível em: <http://www.food.gov.uk/multimedia/pdfs/redmeatsafety.pdf>. Acesso em 02 nov 2009.

GOMEZ, D. et al. Aislamiento de Escherichia coli productor de toxina Shiga durante um brote de gastroenteritis en un Jardín Maternal de la ciudad de Mar del Plata. **Revista Argentina de Microbiologia**, v.37, p.176-181, 2005.

IRINO, K. et al. Serotypes and virulence markers of Shiga toxin-producing *Escherichia coli* (STEC) isolated from dairy cattle in São Paulo State, Brazil. **Veterinary Microbiology**, v.105, p.29-36, 2005.

JARDIM, F. B. B. et al. Influência dos sistemas de pastagem e confinamento na contaminação microbiana de carcaças bovinas. **Ciência e Tecnologia de Alimentos**, v.26, n.2, p.277-282, 2006.

MEICHTRI, L. et al. Shiga toxin-producing *E. coli* in health young beef steers from Argentina: prevalence and virulence properties. **Journal of Food Microbiology**, v.96, p.189-198, 2004.

MERCADO, E. C. Síndrome Urémico Hemolítico: ¿por qué Argentina? **Revista Argentina de Microbiología**, v.39, p.191-192, 2007.

PHILLIPS, D.; SUMNER, J.; ALEXANDER, J. F.; DUTTON, K. M. Microbiological quality of Australian beef. **Journal of Food Protection**, v.64, n.5, p.692-696, 2001.

ROLDÁN, M. L. et al. Aislamiento, caracterización y subtipificación de cepas de *Escherichia coli* O157:H7 a partir de productos cárnicos y leche. **Revista Argentina de Microbiología**, v.39, p.113-119, 2007.

SALES, S. S. et al. Ocorrência de *E. coli* produtora de toxinas "Shiga" (STEC) na microbiota intestinal de bovinos destinados ao abate no município de São Luís–MA, Brasil. **Rev. Portuguesa de Ciências Veterinárias**, v.101, p.245-251, 2006.

STELLA, A. E. et al. Ocorrência e sensibilidade microbiana de linhagens de *E. coli* enteropatogênicas isoladas de propriedades leiteiras na região de Ribeirão Preto-SP, Brasil. **Veterinária e Zootecnia**, v.15, n.1, p.66-74, 2008.

VICENTE, H. I. G. et al. Shigatoxigenic *Escherichia coli* serogroups O157, O11 and O113 in feces, water and milk samples from dairy farms. **Brazilian Journal of Microbiology**, v.36, p.217-222, 2005.

ZWEIFEL, C. et al. Microbiological contamination of cattle and pig carcasses at five abattoirs determined by swab sampling in accordance with EU Decision 2001/471/EC. **Meat Science**, v.69, n.3, p.559-566, 2004.