Detection and isolation of Shiga toxin-producing *Escherichia coli* and microbial population counts on Argentinean Kosher beef for export to Israel

Detección y aislamiento de *Escherichia coli* productora de toxina Shiga y conteo de poblaciones microbianas en carne bovina Kosher argentina para exportar a Israel

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Abstract: The aim of this study was to provide data on the frequency of Top 7 Shiga toxin-producing *Escherichia coli* (STEC) and microbial population counts on processed beef from Argentinean Kosher cattle abattoirs authorized to export to Israel. A total of 480 samples (forequarters, primal cuts, trimmings) were taken and analyzed for Top 7 STEC detection and isolation and for mesophilic aerobic organism, coliform and *E. coli* enumeration. Differences in *stx* detection and microbial population counts on forequarter samples before and after salting were not statistically significant (P >0.05). All samples were negative for Top 7 STEC. Differences were significant for all microbial counts in primal cuts (P <0.001). Neck samples showed a higher level of contamination with the three groups of microorganisms than fore shank and brisket samples. The prevalence of *stx* was lower than that reported worldwide and in Argentinean export abattoirs. Salting did not significantly reduce the microbial load on forequarters.

Keywords: STEC, microbial counts, beef, Kosher, abattoir.

Resumen: El objetivo de este estudio fue determinar la frecuencia de *Escherichia coli* productora de toxina Shiga (STEC) Top 7 y realizar recuentos de poblaciones microbianas en carne Kosher de frigoríficos argentinos autorizados para exportar a Israel. Se tomaron 480 muestras (cuartos delanteros, cortes y recortes) y se realizó la detección y aislamiento de STEC Top 7 y el recuento de microorganismos aerobios mesófilos, coliformes y *Escherichia coli*. No se identificaron diferencias estadísticamente significativas (P > 0,05) en la detección de *stx* y en los recuentos de poblaciones microbianas en las muestras de cuartos delanteros antes y después del salado. Todas las muestras fueron negativas para STEC Top

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7. Se hallaron diferencias estadísticamente significativas ($P < 0,001$) en todos los recuentos de poblaciones microbianas de los cortes. Las muestras de cogote tuvieron recuentos de poblaciones microbianas mayores a los de las muestras de brazuelo y pecho. La prevalencia de *stx* obtenida en el presente estudio fue menor a las reportadas en el resto del mundo y en frigoríficos exportadores de Argentina. El salado no redujo significativamente la carga microbiana de los cuartos delanteros bovinos.

Palabras clave: STEC, conteo microbiano, carne bovina, Kosher, frigorífico.

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens that can cause bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS). Incidence of HUS is lower in Israel than in most countries, especially because STEC-HUS is very rare (Alfandary *et al.*, 2020).

STEC strains carried by cattle are unavoidably transferred to carcasses during slaughter (Duarte *et al.*, 2016). Since beef can be contaminated at different stages from the abattoir to consumption (EFSA & ECDC, 2018), the current food safety preventive systems comply with the hazard analysis and critical control point (HACCP) principles (Sofos, 2008).

Slaughter performed under Jewish law is referred to as Kosher slaughter. It is performed by a specially trained person known as a "Shochet" and, in contrast to non-religious slaughter, the animal is not stunned prior to slaughter. The presence of defects is verified according to religious definitions (Farouk *et al.*, 2014). If the carcass qualifies as Kosher, beef must go through the salting process to remove all the remaining blood (Zurek, *et al.*, 2022). Only forequarters are intended for consumption.

Even though STEC frequency in Kosher beef production was unknown, in July 2019 the State of Israel implemented the zero-tolerance criteria for Top 7 STEC serogroups (O26, O45, O103, O111, O121, O145 and O157) on beef products.

The aim of this study was to provide data on the frequency of Top 7 STEC in processed beef from Argentine abattoirs authorized to export to Israel. In addition, microbial populations (total aerobic counts, E. coli and coliforms) were counted.

2. Materials and methods

2.1. Study design

The study was conducted between November 2020 and January 2021 in Argentinean Kosher cattle abattoirs authorized to export to Israel (IVSHA; SENASA). Twelve abattoirs complying with the current legal regulations for the

religious practice of Kosher slaughter were invited and accepted to participate voluntarily in this study.

All abattoirs observed the following salting process (Akshará): 1) 30 min soaking (Ashraiá) of 1.5 l/kg of meat with cold (7 ºC) potable water using; 2) salting (Amlajá) applying coarse salt for 60 to 75 min; and 3) final washing (Akshrá) by immersing forequarters in three consecutive pools with cold (2 ºC) potable water. The entire process was monitored by each abattoir and verified by the National Service of Agrifood Health and Quality of Argentina (SENASA, for its Spanish acronym).

2.2. Sample collection

The following material was collected from each abattoir: 10 bovine forequarter samples before the salting process and 10 after 30-60 min salting; 18 Kosher primal cut samples before packaging (brisket n = 6; neck n = 6; fore shank n = 6); and 2 pools of trimmings (1kg each). From this material, a total of 480 samples were evaluated. They were distributed as follows: 60 right and 60 left bovine forequarter samples corresponding to the same animal before the salting process (n = 120); 60 right and 60 left bovine forequarter samples from the same animal after 30-60 min salting (n = 120); 216 Kosher primal cut samples before packaging (brisket n = 72; neck n = 72; fore shank n = 72); and 24 pools of trimmings (1-kg each) placed in sterile bags.

Two samples from each forequarter (before and after salting) and from each primal cut were used for microbial population counts (mesophilic aerobic organism, coliform and *E. coli*) and for Top 7 STEC search. For forequarter microbial population counts, a combination of two areas of 100 cm^2 each (brisket and neck) was sponged. First, the brisket area was swabbed with one side of the sponge (ten strokes in two directions, from left to right and from the top to bottom) (Whirl-Pak Speci-Sponge, Nasco, US). The same sponge was then flipped to the other side to swab the neck area as described above. For primal cut microbial population counts, one area of 100 cm^2 was sponged. For Top 7 STEC search, the total forequarter surface, including the external and internal surfaces, and the total primal cut surfaces were swabbed with another sponge. All sponges were sterile (Whirl-Pak) and had been previously soaked in 15 ml buffered peptone water (BPW, Biokar, Zac de Ther, France). After swabbing, sponges were placed into sterile stomacher bags. All sponges and trimming bags were stored at 4 °C and immediately sent to the "División Higiene y Seguridad Alimentaria y Ambiental, Stamboulian Servicios de Salud, Buenos Aires, Argentina".

Sample size was enough to detect differences in stx prevalence of at least 16.0% (95.0% confidence level; 80.0% statistical power) on forequarters before and after the salting process. In primal cuts, sample size was sufficient to detect differences of at least 20% in *stx* prevalence (95.0% confidence level).

2.3. Microbiological analyses

2.3.1. Top 7 STEC detection and isolation

Ninety ml of BPW broth were added to each sponge and 400 ml of BPW broth was added to trimmings, following the ISO (2012) standard. Sponges were incubated for 24 h at 37 °C. The Top 7 STEC were detected using the bioMerieux Gene Up system (GENE-UP® STEC – TOP 6, GENE-UP® *E. coli* O157:H7 2, GENE-UP® STEC – *stx & eae* 2). If samples were positive for one of the serogroup-associated genes in the scope of the method, isolation was performed with immuno-concentration (VIDAS® UP E. coli serogroups) and streaked onto suitable medium.

2.3.2. Microbial population counts

Ninety ml of BPW broth were added to each sponge and mixed in the stomacher bag for 2 min. An aliquot (1 ml) was used for enumerations. Mesophilic aerobic organism (MAO) enumerations were performed plating in duplicate and incubating 72 h at 30 °C (ISO, 2013). Coliform enumerations were performed plating in duplicate and incubating 24 h at 37 °C (ISO, 2006). *E. coli* enumerations were performed plating in duplicate, using 5-bromo-4-chloro-3-indolyl-b-D-glucoronide and incubating 24 h at 44 °C (ISO, 2001).

2.4. Statistical analysis

Bacterial counts were log-transformed before statistical analyses. The effect of salting on forequarter stx detection and microbial population counts was assessed using a Generalized Linear Mixed Model (GLMM). Salting stages (before and after salting) were used as fixed effect. Forequarters nested to abattoir were the random term. Binomial distribution using a logarithmic link function and normal distribution were used for stx detection and microbial population counts, respectively. Variation on primal cut microbial population counts was described using GLMM with normal distribution, using type of primal cut as fixed effect and abattoir as random factor. All statistical analyses were performed using the Statistical Package for the Social Sciences Software (SPSS, IBM-SPSS, Inc., Chicago, IL, US), with a significance of P <0.05.

3. Results

3.1. Top 7 STEC detection and isolation

All samples were negative for Top 7 STEC serogroups. However, *stx* gene was detected in 8.3% of forequarters before salting, 6.7% of forequarters after salting, 2.8% of necks, 8.3% of briskets, 11.1% of fore shanks and 20.8% of trimmings. Differences in stx detection before and after forequarter salting were not statistically significant (P = 0.690) (Table 1).

3.2. Microbial population counts

The frequencies of samples with microbial population count on forequarters before and after salting were as follows: MAO, 104/120 and 106/120; coliforms, 51/120 and 46/120; *E. coli*, 31/120 and 27/120, respectively. Differences before and after salting were not significant (MAO $P = 0.765$; coliforms $P = 0.068$; *E. coli* $P = 0.131$). In the case of primal cuts, differences were significant for all microbial counts ($P < 0.001$). Neck samples showed a higher level of contamination with the three groups of microorganisms than fore shank and brisket samples. Additionally, brisket samples showed higher coliform counts than fore shank samples (Table 1).

Table 1

Results of laboratory determinations and statistical analysis of Argentinean Kosher beef samples

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Analysis (N = 480)	Forequarters (N = 240)			Primal cuts (N = 216)				Trimmings (N = 24)
	Before salting (N = 120)	After salting (N = 120)	$P =$ [3]	Neck (N = 72)	Brisket (N = 72)	Fore shank (N = 72)	$P =$ [3]	
stx detection[1]	10 (8.3)	8 (6.7)	0.690	2 (2.8)	6 (8.3)	8 (11.1)	-	5 (20.8)
Top 7 STEC isolation[1]	0	0	-	0	0	0	-	0
Mesophilic aerobic organisms[2]	2.51 (0.29)	2.47 (0.28)	0.140	1.65 (0.25)[a]	1.10 (0.17)[b]	1.12 (0.16)[b]	0.000	-
Coliforms[2]	1.76 (0.23)	1.14 (0.16)	0.068	0.72 (0.12)[a]	-0.06 (0.03)[b]	-0.25 (0.03)[c]	0.000	-
E. coli[2]	1.59 (0.22)	0.61 (0.12)	0.131	-0.16 (0.05)[a]	-0.6 (-0.01)[b]	-1.02 (-0.03)[b]	0.000	-

[1]N positive (% positive); [2]Mean (standard error) (log CFU/cm^2); [a b c] Values with no common letter are significantly different ($P < 0.05$);

[3] Generalized Linear Mixed Model

[1]N positive (% positive); [2]Mean (standard error) (log CFU/cm^2); [a b c] Values with no common letter are significantly different (P < 0.05); [3] Generalized Linear Mixed Model

4. Discussion

Scientific publications on the microbiology of Kosher beef are scarce worldwide, and none of them refer to STEC (Hajmeer *et al.*, 2004; Zuckerman & Mannheim, 2001). For this reason, the results of the present study are discussed with reference to those of other authors who analyzed non-Kosher carcasses, primal cuts and trimmings.

In the present study, Top 7 STEC was not recovered from forequarters and primal cuts. The Top 7 STEC isolation rate on carcasses from the United States (US) (Barkocy-Gallagher *et al.*, 2003; Cobbold *et al.* 2008; Stromberg *et al.*, 2018) and Canada (Bohaychuk *et al.*, 2011) was 0.09-57.0%. In Argentina, Masana *et al.* (2010; 2011) reported 7.02% of Top 7 STEC isolated from carcasses, and Brusa *et al.* (2017) isolated Top 7 STEC from 0.02% of primal cuts in Argentinean export abattoirs. Differences between studies could be attributed to variables such as cattle STEC load, and intervention measures and HACCP application at abattoir.

The *stx* detection rate on forequarters reported in this study was lower than described in the European Union (EU) and the US , and similar to other

reports from Argentina. In the EU and the US, *stx* detection was in the range of 13.4-60.6% on carcasses from abattoirs (Barkocy-Gallagher *et al.*, 2003; Cobbold *et al.*, 2008; Stromberg *et al.*, 2018). In Argentina, previous studies have determined stx prevalence on carcasses from export abattoirs (Brusa et al., 2019, 2022; Signorini *et al.*, 2018). In the present study, *stx* detection on forequarters before and after salting was similar to that reported by Brusa *et al.* (2019) (6,7%). In all forequarters, *stx* detection was higher than reported by Brusa *et al.* (2022) (3%), but lower than reported by Signorini *et al.* (2018) on carcasses (37.5%).

On primal cuts, *stx* was identified in 11-36% of samples from the EU (Nobili *et al.*, 2017), the US (Cobbold *et al.*, 2008) and Uruguay (Baeza Quiroz *et al.*, 2013; Bosilevac *et al.*, 2007). The mean *stx* detection from primal cuts reported in the present study (7.4%) was lower than the international rate and comparable to the 8.1% reported in Argentinean export abattoirs (Brusa *et al.*, 2022).

The frequency of isolation of *stx* genes and *E. coli* O157:H7 in trimming samples from the US, Australia and New Zealand was 9.7-30 and 0.9-2.4%, respectively (Bosilevac *et al.*, 2007; Carney *et al.*, 2006). Detection frequency of *stx* gene in trimmings from the present study fell within the international range. In Argentina, Top 7 STEC were isolated in 0.03% of trimmings from export abattoirs (Brusa *et al.*, 2017). In contrast with international and previous Argentinean publications, in the present study all trimming samples were negative for Top 7 STEC. These results are consistent with those informed by the Israel Veterinary Inspection Service (IVIS), whose analysis of 349 Argentine beef samples between July 2019 and December 2020 found that they were all negative for Top 7 STEC (IVIS, personal communication, March, 2021).

The effect of Kosher slaughter on beef microbial population counts has been previously evaluated at laboratory scale (Hajmeer *et al.*, 2004; Zuckerman & Mannheim, 2001). Hajmeer *et al.*, (2004) analyzed 10 Kosher briskets and 15 non-Kosher briskets, concluding that the salt applied during Koshering had a potential for microbial reduction and might prove beneficial for controlling *E. coli* O157:H7. The process had little success in reducing MAO, coliform and *E. coli* counts and the authors did not search for *E. coli* O157:H7. In another laboratory assay, eight non-Kosher primal cuts were processed with salt to emulate the Kosher treatment process (Zuckerman & Mannheum, 2001). The lower MAO counts found in salted as compared with unsalted primal cuts were explained by the effect of salt on the surface bacteria and by the effect of the massive soaking and rinsing involved in Koshering. Hajmeer *et al.* (2004) mentioned that an abattoir-based study cannot provide a valid scientific evaluation for line effectiveness of Koshering and the performance of laboratory scale studies. We consider that, despite the usefulness of laboratory scale studies , those carried out at abattoirs demonstrate the real effect of the treatment on the beef produced. The current results of 240 forequarter samples from export abattoirs applying all Kosher control measures suggest that the salting process did not significantly reduce either MAO, coliform and *E. coli* counts or *stx* detection.

It is interesting to consider that between 2010 to 2017, Argentinean export abattoirs raised concern to reduce the presence of STEC throughout the process of slaughter (HACCP-STEC) and marketing of beef products (Brusa 2017; Masana 2010, 2011). Improvement actions implemented at abattoir level, allowed to reduce, and even achieve zero STEC detection at Argentine abattoirs

(Brusa 2017; 2019). In addition, in Argentina the effectiveness of applying interventions to reduce STEC was also evaluated (Brusa *et al.,* 2019; Signorini *et al.*, 2018), which would explain the absence of Top 7 STEC in this study. The current results provide evidence to predict low contamination with STEC and no contamination with the Top 7 STEC. However, it would be interesting to evaluate new interventions, such as high-pressure processing, ultrasound, radiation, ozone, organic acids, or other emerging processing techniques (Galli *et al.*, 2016) that tend to reduce indicator microorganism counts in Kosher meat products, to extend shelf life.

5. Conclusions

Few studies on Kosher beef microbiology are available, and to our knowledge none exist on STEC. In this study, microbiological counts on Kosher beef were similar to those reported on non-Kosher beef and within the reference limits of international regulations. Although the prevalence of *stx* was lower than reported globally and in Argentinean export abattoirs, it was not possible to conclude that the absence of Top 7 STEC was due to the religious ritual process or the use of salt. Likewise, salting was not identified as a practice with significant effect to reduce the microbial load on forequarters. Other studies are necessary to evaluate the effect of Koshering on spoilage and pathogenic bacteria.

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Declaration of competing interest

Findings presented in this article are the interpretation from authors and do not necessarily represent the views of the funding agency. The authors declare they have no competing interest.

Author contributions

The authors contributed to this article as follows: Brusa,Victoria: Conceptualization, data curation, formal analysis, investigation, methodology, roles/writing - original draft, writing - review & editing. Blainq, Luis; Brasesco, Hebe; Bruzzone, Mariana; Buezas, Joaquín; Contardi, Ignacio; García, Diego; Gómez, Elda; Mariame, Mariela; Medici, Laura; Moretti, Georgina; Ochoa, Gonzalo; Petroli, Sandra and Superno, Valeria: Conceptualization & Methodology, Sucari, Adriana: Methodology. Vinelli, Francisco, and Albanese, Román: Conceptualization. Dolev, Sergio: Conceptualization, supervision,

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