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Microbiological risk characterization in butcher shops from the province of Neuquen, Patagonia Argentina

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ABSTRACT

Meat products may be vehicles of bacterial pathogens to humans. In this study, we determined both hygienic-sanitary risk and microbiological quality of raw ground beef and meat contact surfaces in butcher shops from Neuquén Province, Argentina. The hygienic-sanitary risk of the butcher shops was characterized based on the quantitative results of a checklist. A total of 44 raw ground meat and 49 meat contact surfaces were sampled. Most butcher shops presented low/moderate hygienic-sanitary risk, and one had high-risk. Counts of indicator microorganisms in ground meat samples were as follows: mesophilic aerobic microorganisms, 6.6 log CFU/g; *S. aureus,* 1.1 log CFU/g; *E. coli,* 1.5 log CFU/g. Pathogen microorganisms were found in 15.9% of ground beef samples (*Salmonella* spp., 6.8%; *E. coli* O157:H7, 2.3%; non-O157 STEC, 6.8%) and 28.6% of environmental samples (*Salmonella* spp., 6.1%; non-O157 STEC, 2%; *L. monocytogenes*, 22.4%). Concomitantly, *Salmonella* spp. was detected in raw ground beef and meat contact surface samples from two butcher shops. Ribotyping of these strains revealed cross-contamination. Risk quantification was useful to identify failures in different areas of the butcher shops and recognize potential improvements to reduce the risk of pathogenic bacteria contamination of meat and ready-to-eat products.

1. Introduction

Foodborne diseases as a result of poor infrastructure and low level of awareness are one of the most important issues all over the world, especially in developing countries (Scott, 2003). The World Health Organization (WHO) estimates that 1800 million diarrhea episodes and 3 million of deaths in children under the age of 5 occur every year in the world, mainly by contaminated foodstuffs.

Among foods intended for human consumption, those of animal origin are more likely to be hazardous in terms of pathogen content, unless hygiene principles are applied (CDC, 2013; EFSA & ECDC, 2016). Meat and meat products are routinely associated with food poisoning outbreaks. During production, processing and storage, these products are subject to contamination by pathogenic bacteria, including some of serious risk to health. *Salmonella* spp. and Shiga toxin-producing *E. coli* (STEC) have been responsible for several foodborne outbreaks related with the consumption of ground meat or products prepared from ground meat (Torso et al., 2015; Wagner, Silveira, & Tondo, 2013).

The clinical manifestations of STEC infections can vary from asymptomatic infections or mild to moderate diarrhea to severe disease, such as hemorrhagic colitis and hemolytic uremic syndrome (HUS) (Kaper, Nataro, & Mobley, 2004). While sporadic or massive HUS outbreaks have been reported in several developed countries (Böhnlein, Kabisch., Meske, Franz, & Pichner, 2016), in Argentina HUS shows an endemic pattern, representing a serious public health problem with high morbidity and mortality rates (Ministerio de Salud de la Nación, 2016). *Escherichia coli* O157:H7 is the dominant serotype associated with HUS worldwide, although non-O157 STEC serogroups can cause a similar disease (Gould et al., 2013).

Several studies have demonstrated bacterial attachment onto stainless steel and other meat contact surface materials. In particular, the attachment of *Listeria monocytogenes* to processing machines and the surrounding environment is of great concern (Veluz, Pitchiah, & Alvarado, 2012) since it is responsible for the highest hospitalization

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rates among known foodborne pathogens (Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017) and may lead to a serious and potentially life-threatening illness (Posfay-Barbe & Wald, 2004). Despite the complex structure of processing machines makes cleaning difficult, the general severity of human clinical disease caused by *L. monocytogenes*, coupled with its high case fatality rate, emphasizes the critical importance of effective control measures against this food pathogen (Jemmi & Stephan, 2006).

The aims of the present study were to estimate the hygienic-sanitary risk and determine the microbiological quality of raw ground beef and meat contact surfaces in butcher shops from three cities of the province of Neuquén in Patagonia, Argentina.

2. Materials and methods

2.1. Background

In Argentina, a pilot program called "Healthy Butcher Shops" was conducted in the city of Berisso during the period 2010–2013 (Leotta et al., 2016), in an effort to improve the hygienic-sanitary quality of butcher shops and the microbiological quality of commercialized products, thereby reducing the impact of foodborne diseases. After the success of the program in Berisso, it was transferred to the province of Neuquén, in which it was conducted during December 2015–January 2017 in three cities: Neuquén city (38°57′26″S 68°02′44″W), Junín de los Andes (39°55′00″S 71°04′00″W) and Piedra del Águila (40°03′00″S 70°04′40″W).

2.2. Hygenic-sanitary risk quantification

For the risk quantification analysis 49 butcher shops from Neuquén city were selected, choosing 13 butchers from the north of the city, 13 from the south, 12 from the east and 11 from the west. After a coordination visit to each butcher shop during December 2015–February 2016, risk quantification was performed during inspection visits in March–May 2016. The same applied to butcher shops from Junín de los Andes (n = 15; coordination visits, November 2016; inspection visits, December 2016) and Piedra del Águila (n = 9; coordination visits, January 2017; inspection visits, January–February 2017). All butchers were invited to participate voluntarily. The checklist for risk quantification included five groups of variables (total value, 100): 1) situation and condition of the building (10.0), 2) equipment and tools (15.0), 3) handlers (25.0), 4) raw materials and products for sale (20.0), and 5) production flow (30.0). Risk assessment on a 1–100 scale was quantified as high-risk (0-40), moderate-risk (41–70) or low-risk (71–100) (Leotta et al., 2016).

2.3. Sample collection

Samples were collected from 49 butcher shops, as follows: Neuquén city (n = 25, 25 ground beef and 25 pools of environmental samples), Junín de los Andes (n = 15, 12 ground beef and 15 pools of environmental samples) and Piedra del Águila (n = 9, 7 ground beef and 9 pools of environmental samples). All samples were taken during the day (operational process) before the sanitation step. One kilogram of ground beef was collected in a plastic bag provided by the butcher, under the same conditions as those used for selling the product. From each butcher shop, pools of environmental samples were obtained by taking samples from four meat contact surfaces: meat tables, knives, meat mincing machines and manipulator hands. Meat contact surface samples were obtained using a sterile sponge (Nasco, U.S.) soaked in 10 ml of buffered peptone water (BPW) (Scharlau Chemie, Spain), according to the following protocol: Three areas of meat tables (20 × 20 cm each) were sampled; wiping the sponge 10 times over each sampling area. The entire surface of the knife blade and the intersection between the blade and the blade handle were sponged. The meat mincing machine was disassembled and the sample was taken from the meat container, the worm meat grinder and the screw ring. In the case of manipulator hands, the sterile sponge sampled all hand surfaces, including front, back, interdigital spaces and nails. All ground beef and environmental samples were ice-refrigerated and sent to the laboratory in an insulated container to be analyzed immediately. All samples were analyzed within 8 h of sample collection.

2.4. Microbiological analysis

Ground beef samples were analyzed for mesophilic aerobic organisms, *E. coli* and coagulase-positive *S. aureus* enumeration (CFU/g) in accordance to ISO 4833–1 (Anonymous, 2013), 16649–2 (Anonymous, 2001a) and ISO 6888–1 (Anonymous, 1999) (Scharlau Chemie, Spain). They were also inspected for *Salmonella* spp., *E. coli* O157:H7 and non-O157 STEC detection. Pools of the four environmental samples taken at each butcher shop were analyzed for *Salmonella* spp., *E. coli* O157:H7, non-O157:H7 STEC and *L. monocytogenes*. Each pool of environmental sponges was aseptically divided into two portions, one for *Salmonella* spp., *E. coli* O157:H7 and non-O157 STEC detection and the other for *L. monocytogenes* detection.

2.4.1. Salmonella spp.

Twenty-five grams of ground beef and one portion of the sponge from each environmental sample were cultured in 225 ml and 200 ml of BPW, respectively, for 18 ± 2 h at 37 °C in accordance to ISO 6579–1 (Anonymous, 2017a). After the pre-enrichment step, 1 ml of the broth was heated at 95 °C for 10 min to extract DNA. The extracted DNA was amplified by real-time PCR (screening) with the commercial kit PATHfinder *Salmonella* Spp Assay (Generon, Italy). The samples identified as *Salmonella* spp. were isolated and characterized following the ISO 6579–1:2017 guidelines (Anonymous, 2017a). The isolated colonies were characterized by automated ribotyping with the restriction enzyme PvuII using the RiboPrinter® System (DuPont Qualicon, U.S.) and reagents from the DuPont Qualicon ribotyping kit, according to the manufacturer's instructions. Using the RiboPrinter software, PvuII patterns were compared against the DuPont *Salmonella* PvuII database. The top match was used to predict the serovar of a tested isolate.

2.4.2. Escherichia coli O157:H7

Sixty-five grams of ground beef samples were incubated onto 585 ml of modified Trypticase Soy Broth with 20 mg/L of novobiocin and casaminoacids (Acumedia, U.S.) for 18–24 h at 42 °C in accordance to ISO 16654 (Anonymous, 2001b). After the pre-enrichment step, ground meat and sponge samples were processed by immunomagnetic separation with *E. coli* O157:H7 immunomagnetic beads (Neogen, U.S.) according to the manufacturer's instructions, and plated onto CT-SMAC (Oxoid, England) and Fluoroclut (Merck, Germany). Suspect colonies were confirmed by indole production and latex agglutination with *E. coli* O157:H7 antiserum (Remel, U.S.).

2.4.3. STEC

Sixty-five grams of ground beef samples were incubated in 585 ml of BPW for 18–24 h at 37 °C in accordance to ISO 13136 (Anonymous, 2012). After the enrichment step, DNA was extracted with GENERlex Extraction Buffer 6% (Generon, Italy). Real-time PCR screening for stx_1, stx_2 and *eae* genes was carried out with the commercial kit PATHfinder *E. coli* VTEC stx1-stx2 & eae-IAC Duplex Assay (Generon, Italy). One milliliter from all positive samples was plated onto MacConkey agar (Merck, Germany) and Levine-Eosyne Methylene Blue agar (Merck, Germany) and incubated for 18 h at 37 °C. Fifty colonies with *E. coli* morphology were selected from each plate and point-inoculated on nutrient agar (Scharlau Chemie, Spain). After incubation, five pools of 10 colonies were screened for stx_1, stx_2 and *eae* genes by real-time PCR. Colonies from positive pools were analyzed individually by real-time PCR to detect the *stx*-positive colony.

2.4.4. Listeria monocytogenes

One portion of sponge from each environmental sample was cultured in 200 ml of half Fraser broth (Merck, Germany) for 24 h at 30 °C in accordance to ISO 11290–1 (Anonymous, 2017b). After the pre-enrichment step, 0.1 ml was put onto 10 ml Fraser broth (Merck, Germany) for 48 h at 37 °C. Ten microliters were plated into ALOA agar (Merck, Germany), another 10 µl were plated into Oxford Agar Base (Scharlau Chemie, Spain) and incubated during 24–48 h at 37 °C. The presumptive colonies were identified by real-time PCR with the commercial kit PATHfinder *Listeria monocytogenes*/IPC Detection Assay (Generon, Italy).

2.5. Statistical analysis

Differences in the counts of mesophilic aerobic organisms, *S. aureus* and *E. coli* were evaluated using Student's paired *t*-test with a two-tailed distribution. Data for enumerations were log-transformed before the analysis of variance. Differences in the detection rate of pathogen microorganisms were evaluated using Kruskal-Wallis test. All statistical analyses were performed using InfoStat software (Di Rienzo et al., 2014) with a significance of $P < 0.05$.

3. Results

3.1. Hygenic-sanitary risk quantification

Most butcher shops presented moderate or low-risk, and one resulted with high-risk (Table 1). Results for each group of the five variables included in the checklist used for the risk quantification and the average risk of the butcher shops from Neuquén Province are shown in Table 2.

In Neuquén city, building and equipment conditions were acceptable, but implementation of good manufacturing practices (GMP) should be reinforced. The most problematic point detected was the misuse and lack of maintenance of tools and machineries, namely, the presence of meat remains. Butchers from Junín de los Andes showed acceptable conditions of building and equipment, and better hygienic conditions of tools. Product traceability and merchandise flow should be reinforced, but in general shops followed a linear flow, preventing cross-contamination. Implementation of an integrated pest management program and GMP should be reinforced. In Piedra del Águila, butcher shops should strengthen the education of butchers and reinforce the implementation of GMP. Moreover, building improvements, such as hot water services and windows protection, should be applied.

3.2. Microbiological quality of ground beef and meat contact surfaces

Based on the results of the risk quantification analysis, 25 butcher shops from Neuquén city (those having high and moderate-risk and three with low-risk), 15 from Junín de los Andes and 9 from Piedra del Águila were selected for the microbiological study, collecting a total of 44 ground beef and 49 environmental samples.

The microbiological profile of the ground beef samples is presented in Table 3a. Counts of mesophilic aerobic microorganisms were significantly higher in butcher shops from Junín de los Andes than in those from Neuquén city ($P = 0.012$) and Piedra del Águila ($P = 0.005$), and also higher in Neuquén city than in Piedra del Águila ($P = 0.048$). On the other hand, counts of *S. aureus* and *E. coli* were similar in butcher shops from the three cities. Pathogen microorganisms were found in 7 (15.9%) ground meat samples. Samples from Piedra del Águila revealed higher detection rates of pathogenic microorganisms than those from Neuquén ($P > 0.05$) and Junín de los Andes ($P = 0.036$).

A total of 14 (28.6%) environmental samples revealed pathogenic bacteria contamination (Table 3b). Co-contamination with *L. monocytogenes* and *Salmonella* spp. was detected in one environmental sample from Piedra del Águila. *L. monocytogenes* was the most frequently detected pathogen (22.4%), followed by *Salmonella* spp. (6.1%), and one sample showed presumptive non-O157 STEC contamination (2%). Unfortunately, none of the presumptive non-O157 STEC samples from ground beef and environment could be isolated.

Overall, the level of pathogenic bacteria contamination of ground

Table 1
Average risk of the butcher shops analyzed.

Hygenic-sanitary risk	Neuquén city	Junín de los Andes	Piedra del Águila
High[a]	1	0	0
Moderate[b]	21	5	7
Low[c]	27	10	2

[a] 0–40 points.
[b] 41–70 points.
[c] 71–100 points.

Table 2
Average risk of each group of variables of the butcher shops analyzed.

Groups of variables	Neuquén city	Junín de los Andes	Piedra del Águila
Situation and conditions of building (%)			
Waste in the exterior area	53.1	80	55.5
Suitable floors	89.8	60	66.7
Suitable roofs	88.8	70	94.4
Suitable walls	92.8	70.8	87.5
Suitable windows	95.9	73.3	100
Protected windows	57.1	66.7	22.2
Adequate lighting	85.7	80	100
Adequate ventilation	95.9	53.3	88.9
Adequate staff sanitation area	55.1	33.3	55.5
Adequate staff changing room	34.0	10	11.1
Access to drinking water	100	93.3	88.9
Hot water	81.6	56.7	27.8
SSOP in the water supply tank	47.6	70	72.2
SSOP in the work environment	70.4	80	66.7
Average risk (10.0)[a]	**6.8**	**6**	**5.5**
Equipment and tools (%)			
Proper conservation of tools	93.9	86.7	100
Good conditions of equipments	15.2	78.3	72.2
Quantity of tools	46.9	83.3	88.9
Sufficient refrigeration equipment	93.9	80	100
SSOP application on equipment and tools	41.7	80	88.9
Average risk (15.0)[a]	**11.6**	**11.8**	**12.3**
Handlers (%)			
Appropriate clothes	44.9	60	11.1
Clean clothes	61.2	80	55.5
Proper hygiene habits	71.4	86.7	44.4
Health verification	85.7	73.3	100
Average risk (25.0)[a]	**18.3**	**19.8**	**15.6**
Raw materials and products for sale (%)			
Raw material receipt control	87.7	92.8	88.9
Control of organoleptic properties in products for sale	75.5	92.8	55.5
Proper conservation of raw materials and products for sale	91.8	82.1	94.4
Average risk (20.0)[a]	**17.1**	**17.5**	**16.1**
Production flow (%)			
Linear flow of meat in one direction	42.8	73.3	11.1
Control of cross-contamination	65.3	86.7	66.7
Protection of meat products	51	93.3	11.1
Conservation at adequate temperatures	93.9	100	100
Food storage by product type	67.3	83.3	27.7
Pest management	63.3	26.7	0
Qualified personnel for handling meat	77.5	80	44.4
Meat ground at the moment	40.8	40	75
Average risk (30.0)[a]	**19.9**	**24.1**	**15.5**

[a] Maximum value assigned to each group of variables.

Table 3
Microbiological profile of ground beef (a) and environmental samples (b) obtained in butcher shops from Neuquén Province, Argentina.

a)

Location	Microorganisms in ground beef						
	n	Counts: log CFU/g ± SD			Detection: % (n)		
		Mesophiles	S. aureus	E. coli	Salmonella spp.	E. coli O157:H7	Non-O157 STEC
Neuquén city	25	6.4 ± 1.0	1.1 ± 0.3	1.5 ± 0.8	4.0 (1)[a]	4.0 (1)	8.0 (2)
Junín de los Andes	12	7.5 ± 0.7	1.1 ± 0.3	1.6 ± 1.0	ND	ND	ND
Piedra del Águila	7	5.8 ± 1.2	1.0 ± 0.0	1.7 ± 0.9	28.6 (2)[a,b]	ND	14.3 (1)[b]
Neuquén Province	**44**	**6.6 ± 1.1**	**1.1 ± 1.0**	**1.5 ± 0.9**	**6.8 (3)**	**2.3 (1)**	**6.8 (3)**

b)

Location	Microorganisms in butcher environment				
	n	Detection: % (n)			
		Salmonella spp.	E. coli O157:H7	Non-O157 STEC	L. monocytogenes
Neuquén city	25	4.0 (1)[a]	ND[c]	ND	8.0 (2)
Junín de los Andes	15	ND	ND	ND	33.3 (5)
Piedra del Águila	9	22.2 (2)[a,b]	ND	11.1 (1)[b]	44.4 (4)
Neuquén Province	**49**	**6.1 (3)**	**ND**	**2.0 (1)**	**22.4 (11)**

[a] Pathogenic bacteria detected in ground beef and environmental samples from the same butcher shop.
[b] Pathogenic bacteria detected in ground beef or environmental samples from different butcher shops.
[c] ND: Not detected.

meat and/or meat contact surfaces was significantly higher in butcher shops from Piedra del Águila compared with those from Neuquén city ($P = 0.003$) and Junín de los Andes ($P = 0.014$).

3.3. Characterization of Salmonella strains

Salmonella spp. was concomitantly detected in raw ground beef and meat contact surfaces of one butcher shop from Neuquén city and another from Piedra del Águila. Serovar Enteritidis was isolated from both samples from Neuquén (0.99–1.00 similarity) and serovar Senftenberg from both samples from Piedra del Águila (0.91–1.00 similarity) (Fig. 1), indicating that cross-contamination occurred between ground meat and the environment. Ribotyping of the other *Salmonella* strains isolated in this study revealed serovar Typhimurium in a ground beef and serovar Rissen in a meat contact surface (Fig. 1).

3.4. Microbiological quality of butcher shops with different risk level

The microbiological profile of ground beef and meat contact surfaces in butcher shops with different hygienic-sanitary risk is shown in Table 4. As only one butcher shop revealed high-risk, it was not possible to statistically compare its microbiological quality with that of low or moderate-risk butchers. Mesophilic aerobic microorganism counts in ground meat from low-risk butcher shops were statistically higher

Table 4
Microorganism counts (mean $\log_{10}$ CFU/g ± SD) and pathogenic bacteria detection frequency (%, n) in raw ground beef and environmental samples at different risk-level butcher shops from Neuquén Province, Argentina.

Sample type	Microorganisms	Hygenic-sanitary risk		
		High	Moderate	Low
Ground beef	Mesophiles	4.4	6.3 ± 1.0	7.4 ± 0.6
	S. aureus	1.0	1.1 ± 0.2	1.1 ± 0.3
	E. coli	1.0	1.6 ± 0.9	1.5 ± 0.9
	Salmonella spp.	ND[a]	6.7 (2)	7.7 (1)
	E. coli O157:H7	ND	3.3 (1)	ND
	Non-O157 STEC	ND	10.0 (3)	ND
Environment	*Salmonella* spp.	ND	9.1 (3)	ND
	E. coli O157:H7	ND	ND	ND
	Non-O157 STEC	ND	3.0 (1)	ND
	L. monocytogenes	100.0 (1)	18.2 (6)	26.7 (4)

[a] ND: Not detected.

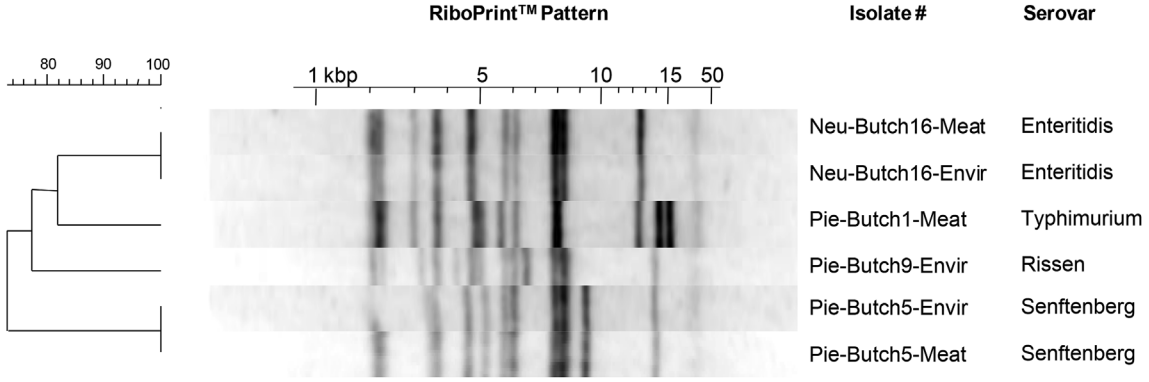

Fig. 1. Dendrogram of *Salmonella* enterica patterns obtained by ribotyping isolates recovered from ground meat and meat contact surfaces in butcher shops from the province of Neuquén, Argentina.

($P = 0.001$) than in moderate-risk butchers. Significant differences in *S. aureus* and *E. coli* counts and in pathogenic microorganisms detection rates were not observed neither in ground meat nor in environment from butcher shops with low or moderate-risk.

4. Discussion

A descriptive hygienic-sanitary risk assessment of butcher shops from Neuquén Province was carried out using a simple checklist. The assay revealed that most butcher shops (98.6%) presented low or moderate-risk. This methodology allowed identifying failures in different areas and potential improvements that might be applied in both practices and facilities. Hygienic practices of meat sellers at the three studied cities did not meet the levels for handling meat products recommended by the WHO and the Food and Agriculture Organization (FAO) Joint Committee (WHO & FAO, 2009, pp. 8–22). Deficiencies in GMP were the common denominator in all butcher shops and 32.7% of respondents had no training in food hygiene. Emphasis should be placed mainly on specific training in the preparation of meat according to species, not mixing ready-to-eat (RTE) products with raw materials or using the same camera for the storage of merchandise other than meat.

Non-pathogenic organisms counts are useful indicators to evaluate handling practices and process control during beef processing. These microorganisms can reach meat from different sources, namely, carcasses, manipulator hands, equipment and the environment. Aerobic mesophilic enumeration provides an estimate of the overall population of microorganisms present in meat, reflecting the existence of favorable conditions for the multiplication of microorganisms (Nyenje, Odjadjare, Tanih, Green, & Ndip, 2012). In this study, 54.5% of the samples analyzed showed bacteria counts above reference values (Argentinean Food Code, 2017), which indicate inefficiency in the cleaning techniques adopted in butcher shops. Butcher shops from Junín de los Andes showed the highest counts of mesophilic aerobic microorganisms, whereas butchers from Piedra del Águila resulted with the lowest levels of contamination. Differences in contamination levels were probably due to differences in study areas, geographic characteristics of animal feeding systems and, mainly, personal hygiene practices of food handlers (Kegode, Doetkott, Khaitsa, & Wesley, 2008).

Differences in indicator microorganism counts and pathogenic bacteria detection rates were not significant among low and moderate-risk butcher shops. Ground meat contamination from these butcher shops may have had the same origin because meat was purchased from the same abattoir.

Prevalence studies of pathogens in commercially acquired meat products provide estimates that reflect the consumer exposure level. In this study, the percentage of ground meat samples screened positive for *Salmonella* spp. (6.8%) was lower than the 71% found in Mexico (Martínez-Chávez et al., 2015), but higher than the 0% of Brazil (Ristori et al., 2017) and the 3.8% in the U.S. (Vipham et al., 2012). The differences in the prevalence of *Salmonella* could be due to differences in the sanitation of butcher shops and the hygienic standards of meat handlers, and also due to different methodologies applied for *Salmonella* detection. *Salmonella* spp. was found in 6.1% of meat contact surfaces in butcher shops from Neuquén Province. Cross-contamination was observed in a butcher from Neuquén city (serovar Enteritidis) and one from Piedra del Águila (serovar Senftenberg). Serovars Typhimurium and Rissen were also detected in ground meat and environmental samples. It should be noted that serovars Senftenberg and Typhimurium were also detected in butcher shops from Berisso (Leotta et al., 2016), suggesting that these serovars could be present in the Argentine beef production chain. All *Salmonella* serovars isolated in the present work are associated with human diseases worldwide (Galanis et al., 2006; Jackson, Griffin, Cole, Walsh, & Chai, 2013). Therefore, improvement of GMP is critical to reduce cross-contamination between the environment and meat and RTE products, and ultimately, to avoid foodborne illness.

In this study, the proportion of ground beef samples testing positive for *E. coli* O157:H7 (2.3%) was similar to the 0–0.5% and 0–2.8% reported by Rhoades, Duffy, & Koutsoumanis (2009) for ground beef from U.S. and Europe, and also similar to the 3.8% reported by Chinen et al. (2001) in Gualeguaychu city, Argentina, but lower than the 3.5–11.6% reported by Leotta et al. (2016) in Berisso. This pathogenic microorganism was not detected in the meat contact surfaces monitored in the present work. In Argentina, post-enteric HUS is endemic, representing the leading cause of acute kidney failure in children and the second leading cause of chronic renal failure, and *E. coli* O157:H7 is the dominant serotype. In the province of Neuquén, HUS incidence is above the national average, with a maximum of 28.6 cases per 100,000 children less than 5 years old (Pianciola et al., 2014). On the other hand, no HUS cases were reported in Berisso during the 2010–2013 study period (Leotta et al., 2016). The main HUS risk factors identified in earlier studies were dietary behaviors related to beef consumption, including eating undercooked beef (Rivas et al., 2008). However, in recent years, other risky exposures have also emerged, like the consumption of raw vegetables and sprouts, living, working or camping in rural areas, being in contact with farm animals, and person-to-person transmission (Rivas, Chinen, Miliwebsky, & Masana, 2014). In this sense, from the comparison of results of Neuquén and Berisso, it appears that ground meat would not be the main source of *E. coli* O157:H7 transmission.

Presumptive non-O157 STEC was found in 6.8% of the ground beef and 2% of the environmental samples analyzed. These values are higher than those reported by Liao et al. (2014), who found 0.8% presumptive STEC in ground beef from U.S. Numerous studies have reported varying prevalence of STEC in ground beef samples from retailers and processors, but most of them applied isolation followed by PCR for STEC detection. Rhoades et al. (2009) summarized the presence of non-O157 STEC in 5.7–16.8% of ground meat samples from U.S. and 1.1–15.5% from Europe. Unfortunately, none of the presumptive non-O157 STEC samples identified in this study could be confirmed by isolation. It is important to highlight that STEC isolation is problematic. Indeed, numerous reports have demonstrated poor correlation between the number of *stx*-positive samples and those confirmed by isolation (Bosilevac & Koohmaraie, 2011; Pradel et al., 2000). This could probably be due to the high sensitivity of the PCR technique, which can detect *stx* genes even in samples where nonpathogenic *E. coli* was by far dominant (Piérard, Stevens, Moriau, Lior, & Lauwers, 1997). Moreover, other variables such as volume of samples plated, amount of plates necessary to achieve STEC isolates and number of colonies selected per plate might affect STEC isolation from meat samples.

The presence of *L. monocytogenes* in meat processing environments is a microbiological hazard to the final products. This pathogen may remain in the environment for months or even years due to its ability to form biofilms on different materials and under various conditions, and to resist a range of environmental stresses, leading to the possible contamination of the final product and potential exposure to pathogenic species (Law, Ab Mutalib, Chan, & Lee, 2015). In this study, *L. monocytogenes* was found in 22.4% of the environmental samples of butcher shops from Neuquén, similar to the frequency rates reported by others in meat processing environments (Leotta et al., 2016; Silva et al., 2016). Significantly lower levels of *L. monocytogenes* were observed in the butchers from Neuquén city than in those from Junín de los Andes and Piedra del Águila. The main risk of the presence of this bacterium in butcher shop environments is the possibility of cross-contamination of RTE products (Luo et al., 2017).

5. Conclusions

Risk quantification was useful to identify relevant facts that should be corrected in order to improve the microbiological quality of ground meat. This study revealed that although pathogenic bacteria were

detected in ground beef and environmental samples, the situation of butcher shops from the province of Neuquén was better than in other districts of the country. However, more attention should be paid to GMP and handler training on the basis of the problems identified in each butcher shop in order to reduce the risk of pathogenic bacteria contamination of meat and RTE products. In this sense, it is necessary to reinforce and consolidate the step of implementation of improvement actions and the verification of the processes in all the butcher shops from the province of Neuquén.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2019.02.074.

References

Anonymous (1999). *Microbiology of food and animal feeding stuffs - horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) - Part 1: Technique using Baird-Parker agar medium.* ISO 6888-1:1999.

Anonymous (2001a). *Microbiology of food and animal feeding stuffs - horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli - Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.* ISO 16649-2:2001.

Anonymous (2001b). *Microbiology of food and animal feeding stuffs — horizontal method for the detection of Escherichia coli O157.* ISO 16654:2001.

Anonymous (2012). *Microbiology of food and animal feed – Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens – Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups.* ISO 13136:2012.

Anonymous (2013). *Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 1: Colony count at 30 degrees C by the pour plate technique.* ISO 4833-1:2013.

Anonymous (2017a). *Microbiology of the food chain - horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp.* ISO 6579-1:2017.

Anonymous (2017b). *Microbiology of the food chain – Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method.* ISO 11290-1:2017.

Argentinean Food Code (2017). http://www.anmat.gov.ar/alimentos/normativas_alimentos_caa.asp/, Accessed date: 7 September 2018.

Böhnlein, C., Kabisch, J., Meske, D., Franz, C. M., & Pichner, R. (2016). Fitness of EHEC/EAEC O104:H4 in comparison to EHEC O157: Survival studies in food and in vitro. *Applied and Environmental Microbiology, 82*, 6326–6334.

Bosilevac, J. M., & Koohmaraie, M. (2011). Prevalence and characterization of non-O157 shiga toxin-producing Escherichia coli isolates from commercial ground beef in the United States. *Applied and Environmental Microbiology, 77*(6), 2103–2112.

Buchanan, R. L., Gorris, L. G. M., Hayman, M. M., Jackson, T. C., & Whiting, R. C. (2017). A review of Listeria monocytogenes: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control, 75*, 1–13.

Centers for Disease Control and Prevention (CDC) (2013). Incidence and trends of infection with pathogens transmitted commonly through food - foodborne diseases active surveillance network, 10 U.S. sites, 1996-2012. *Morbidity and Mortality Weekly Report, 62*, 283–287.

Chinen, I., Tanaro, J. D., Miliwebsky, E., Lound, L. H., Chillemi, G., Ledri, S., et al. (2001). Isolation and characterization of Escherichia coli O157:H7 from retail meats in Argentina. *Journal of Food Protection, 64*(9), 1346–1351.

Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M., & Robledo, C. W. (2014). *InfoStat version 2014. Grupo InfoStat, FCA.* Argentina: Universidad Nacional de Córdoba. http://www.infostat.com.ar.

European Food Safety Authority, European Centre for Disease Prevention and Control (et al., 2016 European Food Safety Authority, European Centre for Disease Prevention and Control (EFSA & ECDC) (2016). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA Journal, 14*, 4634–4865.

Galanis, E., Lo Fo Wong, D. M., Patrick, M. E., Binsztein, N., Cieslik, A., Chalermchikit, T., Aidara-Kane, A., Ellis, A., Angulo, F. J., Wegener, H. C., & World Health Organization Global Salm-Surv (2006). Web-based surveillance and global Salmonella distribution, 2000-2002. *Emerging Infectious Diseases, 12*(3), 381–388.

Gould, L. H., Mody, R. K., Ong, K. L., Clogher, P., Cronquist, A. B., Garman, K. N., & White, P. L. (2013). Griffin for the emerging infections program Food-Net Working Group PM. Increased recognition of non-O157 Shiga toxin-producing Escherichia coli infections in the United States during 2000-2010: Epidemiologic features and comparison with E. coli O157 infections. *Foodborne Pathogens and Disease, 10*, 453–460.

Jackson, B. R., Griffin, P. M., Cole, D., Walsh, K. A., & Chai, S. J. (2013). Outbreak-associated Salmonella enterica serotypes and food Commodities, United States, 1998-2008. *Emerging Infectious Diseases, 19*(8), 1239–1244.

Jemmi, T., & Stephan, R. (2006). Listeria monocytogenes: Food-borne pathogen and hygiene indicator. *Revue Scientifique et Technique, 25*(2), 571–580.

Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic Escherichia coli. *Nature Reviews Microbiology, 2*(2), 123–140.

Kegode, R. B., Doetkott, D. K., Khaitsa, M. L., & Wesley, I. V. (2008). Occurrence of Campylobacter species, Salmonella species and generic Escherichia coli in meat products from retail outlets in the Fargo metropolitan area. *Journal of Food Safety, 28*, 111–125.

Law, J. W., Ab Mutalib, N. S., Chan, K. G., & Lee, L. H. (2015). An insight into the isolation, enumeration, and molecular detection of Listeria monocytogenes in food. *Frontiers in Microbiology, 6*, 1227.

Leotta, G. A., Brusa, V., Galli, L., Adriani, C., Linares, L., Etcheverría, A., Sanz, M., Sucari, A., Peral García, P., & Signorini, M. (2016). Comprehensive evaluation and implementation of improvement actions in butcher shops. *PLoS One, 11*(9), e0162635.

Liao, Y. T., Miller, M. F., Loneragan, G. H., Brooks, J. C., Echeverry, A., & Brashears, M. M. (2014). Non-O157 Shiga toxin-producing Escherichia coli in U. S. retail ground beef. *Journal of Food Protection, 77*(7), 1188–1192.

Luo, L., Zhang, Z., Wang, H., Wang, P., Lan, R., Deng, J., Miao, Y., Wang, Y., Wang, Y., Xu, J., Zhang, L., Sun, S., Liu, X., Zhou, Y., Chen, X., Li, Q., & Ye, C. (2017). A 12-month longitudinal study of Listeria monocytogenes contamination and persistence in pork retail markets in China. *Food Control, 76*, 66–73.

Martínez-Chávez, L., Cabrera-Diaz, E., Pérez-Montaño, J. A., Garay-Martínez, L. E., Varela-Hernández, J. J., Castillo, A., Lucia, L., Ávila-Novoa, M. G., Cardona-López, M. A., Gutiérrez-González, P., & Martínez-Gonzáles, N. E. (2015). Quantitative distribution of Salmonella spp. and Escherichia coli on beef carcasses and raw beef at retail establishments. *International Journal of Food Microbiology, 210*, 149–155.

Ministerio de Salud de la Nación (Argentina) (2016). *Boletín Integrado de Vigilancia N° 328. Informe Especial: Síndrome Urémico Hemolítico y enfermedad producida por Escherichia coli productor de verocitotoxina (VTEC)/E. coli productor de toxina de Shiga (STEC).* http://www.msal.gob.ar/images/stories/boletines/Boletin-Integrado-De-Vigilancia-N328-SE38.pdf/, Accessed date: 4 October 2018.

Nyenje, M. E., Odjadjare, C. E., Tanih, N. F., Green, E., & Ndip, R. N. (2012). Foodborne pathogens recovered from ready-to-eat foods from roadside cafeterias and retail outlets in Alice, eastern Cape province, South Africa: Public health implications. *International Journal of Environmental Research and Public Health, 9*(8), 2608–2619.

Pianciola, L., Chinen, I., Mazzeo, M., Miliwebsky, E., González, G., Müller, C., Carbonari, C., Navello, M., Zitta, E., & Rivas, M. (2014). Genotypic characterization of Escherichia coli O157:H7 strains that cause diarrhea and hemolytic uremic syndrome in Neuquén, Argentina. *International Journal of Medical Microbiology, 304*(3–4), 499–504.

Piérard, D., Stevens, D., Moriau, L., Lior, H., & Lauwers, S. (1997). Isolation and virulence factors of verocytotoxin-producing Escherichia coli in human stool samples. *Clinical Microbiology and Infections, 3*(5), 531–540.

Posfay-Barbe, K. M., & Wald, E. R. (2004). *Listeriosis. Pediatrics in Review, 25*(5), 151–159.

Pradel, N., Livrelli, V., De Champs, C., Palcoux, J. B., Reynaud, A., Scheutz, F., Sirot, J., Joly, B., & Forestier, C. (2000). Prevalence and characterization of Shiga toxin-producing Escherichia coli isolated from cattle, food, and children during a one-year prospective study in France. *Journal of Clinical Microbiology, 38*(3), 1023–1031.

Rhoades, J. R., Duffy, G., & Koutsoumanis, K. (2009). Prevalence and concentration of verocytotoxigenic Escherichia coli, Salmonella enterica and Listeria monocytogenes in the beef production chain: A review. *Food Microbiology, 26*(4), 357–376.

Ristori, C. A., Rowlands, R. E. G., Martins, C. G., Barbosa, M. L., Dos Santos, L. F., Jakabi, M., & de Melo Franco, B. D. G. (2017). Assessment of consumer exposure to Salmonella spp., Campylobacter spp., and shiga toxin-producing Escherichia coli in meat products at retail in the city of Sao Paulo, Brazil. *Foodborne Pathogens and Disease, 14*(8), 447–453.

Rivas, M., Chinen, I., Miliwebsky, E., & Masana, M. (2014). Risk factors for shiga toxin-producing Escherichia coli-associated human diseases. *Microbiology Spectrum, 2*(5).

Rivas, M., Sosa-Estani, S., Rangel, J., Caletti, M. G., Vallés, P., Roldán, C. D., Balbi, L., Marsano de Mollar, M. C., Amoedo, D., Miliwebsky, E., Chinen, I., Hoekstra, R. M., Mead, P., & Griffin, P. M. (2008). Risk factors for sporadic Shiga toxin-producing Escherichia coli infections in children, Argentina. *Emerging Infectious Diseases, 14*(5), 763–771.

Scott, E. (2003). Food safety and foodborne disease in 21st century homes. *Canadian Journal of Infectious Diseases, 14*(5), 277–280.

Silva, D. A., Dias, M. R., Cossi, M. V., Castilho, N. P., Camargo, A. C., & Nero, L. A. (2016). Hygiene and safety in the meat processing environment from butcher shops: Microbiological contamination and Listeria monocytogenes. *Journal of Food Protection, 79*(4), 628–634.

Torso, L. M., Voorhees, R. E., Forest, S. A., Gordon, A. Z., Silvestri, S. A., Kissler, B., Schlackman, J., Sandt, C. H., Toma, P., Bachert, J., Mertz, K. J., & Harrison, L. H. (2015). Escherichia coli O157:H7 outbreak associated with restaurant beef grinding. *Journal of Food Protection, 78*, 1272–1279.

Veluz, G. A., Pitchiah, S., & Alvarado, C. Z. (2012). Attachment of Salmonella serovars and Listeria monocytogenes to stainless steel and plastic conveyor belts. *Poultry Science, 91*(8), 2004–2010.

Vipham, J. L., Brashears, M. M., Loneragan, G. H., Echeverry, A., Brooks, J. C., Chaney, W. E., & Miller, M. F. (2012). Salmonella and Campylobacter baseline in retail ground beef and whole-muscle cuts purchased during 2010 in the United States. *Journal of Food Protection, 75*(12), 2110–2115.

Wagner, V. R., Silveira, J. B., & Tondo, E. C. (2013). Salmonelloses in the state of Rio Grande do Sul, southern Brazil, 2002 to 2004. *Brazilian Jorunal of Microbiology, 44*, 723–729.

World Health Organization, Food and Agriculture Organization (2009). *Food hygiene basic texts* (4th ed.). Rome, Italy.