Trabajos de investigación

Assessment of hygienic-sanitary status and implementation of an integral risk management program in butcher shops from Tafí Viejo city, Tucumán, Argentina

Evaluación del estado higiénico-sanitario e implementación de un programa de gestión integral de riesgos en carnicerías de la ciudad de Tafí Viejo, Tucumán, Argentina

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Abstract: Meat and meat products promote the growth of pathogens such as Shiga toxin-producing *Escherichia coli* and *Salmonella* spp. In this work, an evaluation was carried out through risk quantification and microbiological analysis on ground beef and environmental surfaces in butcher shops, of Tafí Viejo city,Tucumán province. The study was carried out in three periods: period 1 (description of the health-status), period 2 (implementation of improvement actions) and period 3 (verification of success). Ground beef samples and environmental surfaces were analyzed in the previously described periods. The improvement actions in each butcher shop were implemented on the deviations detected in the first period. An increase of 35.7% in low-risk butchers was observed. Microbial indicators in ground beef were significantly lower for total aerobic mesophilic microorganisms and *Stapylococcus aureus,* compared to the first period. In ground beef samples and environmental surfaces, the detection/isolation of STEC non-O157, presented a 100% decrease in the verification period. The detection of *Salmonella* spp. showed a reduction of 14.3% only on ground beef samples. This study confirms that the implementation of a risk management program in butcher shops would reduce contamination of ground beef and the environment with pathogens.

Keywords: Food borne illnesses, *Salmonella* spp., STEC, hygienic health risk, butcher shops.

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Resumen: La carne y los productos cárnicos promueven el crecimiento de patógenos como *Escherichia coli* productora de toxina Shiga y *Salmonella* spp. Se realizó una evaluación mediante cuantificación de riesgo y análisis microbiológicos en carne picada y superficies ambientales en carnicerías de Tafí Viejo, provincia de Tucumán. El estudio se realizó en tres periodos: periodo 1 (descripción del estado sanitario), periodo 2 (implementación de acciones de mejora) y periodo 3 (verificación del éxito). Se analizaron muestras de carne picada fresca y superficies ambientales en los períodos 1-3 previamente descritos. Las acciones de mejora en cada carnicería se implementaron siguiendo los desvíos detectados en el primer período. Se observó un aumento del 35.7% en carnicerías de bajo-riesgo. El recuento de los microorganismos indicadores en carne picada resultó significativamente menor para microorganismos mesófilos aerobios totales y *Stapylococcusaureus*, en comparación con el período 1. En las muestras analizadas, la detección/aislamiento de STEC no-O157, presentó una disminución del 100% en el período de verificación. La detección de *Salmonella* spp. mostró una reducción del 14.3% en muestras de carne picada. Este estudio confirma que la implementación de un programa de gestión de riesgos en carnicerías reduciría la contaminación de la carne y el medio ambiente con patógenos.

Palabras clave: Enfermedades transmitidas por alimentos, *Salmonella*spp., STEC, riesgo higiénico sanitario, carnicerías.

Introduction

Foodborne diseases represent a significant threat to global health, especially in developing countries (Scott, 2003) because of poor infrastructure and low level of awareness. The World Health Organization estimates that 550 million diarrhea episodes and 125.000 deaths in children under the age of 5 occur every year in the world, mainly by contaminated foodstuffs (WHO, 2020). Meat and meat products are conducive to the growth of a wide range of microorganisms including pathogens (Barril *et al.,* 2019; Leotta *et al.,* 2016; Ruiz *et al.,* 2022) routinely associated with food poisoning outbreaks, unless hygiene principles are applied (CDC, 2013; EFSA & ECDC, 2016). *Salmonella* spp. and Shiga toxin-producing *Eschericia coli* (STEC) have been responsible for several foodborne outbreaks related with these products' consumption (Torso *et al*., 2015; Wagner *et al.,* 2013).

In Argentina, nontyphoidal salmonellosis is not listed as a mandatory reporting event, unlike salmonellosis which is included in the acute diarrhea events and reported as one of the principal foodborne diseases (Boletín Epidemiológico Nacional, 2023). *Salmonella* spp. was the second causative agent of diarrhea, and the highest proportion corresponds to those not serotyping, followed by *S. enteritidis* and*S. typhimurium* and in a much lower percentage *S. newport*. In Tucumán province *Salmonella* spp. is also the second causative agent of diarrhea (Torrez Lamberti *et al.,* 2020). The incidence of hemolytic uremic syndrome (HUS) in Argentina (http://www.lusuh.org.ar/material.html) is high and shows an endemic pattern, with high morbidity and mortality rates were *E. coli* O157:H7 and STEC were identified as the primary etiological agent (Carbonari *et al.,* 2022; Torti *et al.,* 2021). However, in Tucumán province the impact of HUS is very low; only one case per year was reported during the last three years (Boletín Epidemiológico Nacional, 2024). Meat products may be vehicles of *Salmonella* and STEC, but only STEC was reported on beef from Tucumán retails (Jure *et al.,* 2015). In a recent study, Brusa *et al.* (2020), reported that only 10.0% of the Argentinian HUS cases could be related to beef.

Food contamination with microorganisms may occur at any stage in the process from food production to consumption (Brusa *et al.,* 2017) and may be the result of cross contamination in the retail environment contributing to an increased risk of foodborne illness (Sirsat *et al.,* 2014). Previous studies involved in a program called "Healthy Butcher Shops" were conducted in Argentina (Barril *et al*., 2019; Leotta *et al.,* 2016; Ruiz *et al.,* 2022). These studies confirm the

feasibility of implementing a comprehensive risk management program.

The aims of the present study were therefore a) to estimate the hygienic-sanitary risk and determine the microbiological quality of raw ground beef and meat contact surfaces in butcher shops from Tafí Viejo city; b) to implement improvement actions for both butcher shops and consumers; and c) to verify the impact of such improvement actions.

Materials and methods

Background

In April 2017, a pilot program called "Healthy Butcher Shops" was conducted in the city of Tafí Viejo (26°44′00″S 65°16′00″O) (https://geohack.toolforge.org/geohack.php?language=es&pagename=Taf%C3%AD_Viejo&params=-26.733333333333_N_-65.266666666667_E_type:city), Tucumán, Argentina. Tafí Viejo has 1210 km² and a population of 121.600. A total of 40 butcher shops were identified at the beginning of the program and, from this total, 28 butcher shops completed the program and were included in this study. Beef was provided to butcher shops by 8 abattoirs in the region, as follows: abattoir "A", 14 (50%) butcher shops; abattoir "B", 5 (18 %); abattoir "C", 3 (11%); abattoirs "D", "E" and "F", 2 (7%) butcher shops each one and abattoirs "G" and "H" did not distribute in the city. None of these abattoirs had a Hazard Analysis and Critical Control Points (HACCP) program.

Sampling was randomly performed and covered all the geographic areas of the city. Comprehensive evaluation and risk quantification using a checklist were made at each butcher shop. In addition, five samples from each butcher shop were collected for bacteriological analysis, including ground beef, meat tables, knives, meat mincing machines and manipulator hands. Ground beef samples presented the organoleptic and commercial characteristics established in the Argentine Food Code (AFC) (AFC, 2022). They were kindly provided by each butcher shop to carry out the "Healthy Butcher Shops" Program, in full agreement with the Tafí Viejo sanitary authorities. Results of this evaluation period were delivered to the person in charge of each butcher shop.

Thereafter, a training plan was designed using those results as starting point to implement the improvement actions. Consumers received information about foodborne disease prevention. We also delivered workshops for teachers of all kindergartens in the city. Finally, during the verification period (2019), all butcher shops were re-evaluated using the same tool for risk quantification and the same

bacteriological analysis to verify the impact of the improvement actions implemented.

The entire study period lasted from 2017 to 2019. Field work was authorized by the health and supervision authorities of Tafí Viejo. To this end, a cooperation agreement between the Tafí Viejo city authorities and the National University of Tucumán Faculty of Chemical Biochemistry and Pharmacy was signed.

Hygienic-sanitary risk quantification and sample collection

The checklist used for risk quantification included five groups of variables as described previously by Leotta *et al.* (2016).

From April to December 2017 (evaluation period), 28 butcheries were visited. To obtain a microbiological evaluation during the visits, 28 ground beef and 112 environmental samples were taken, and risk quantification was performed. All samples were collected 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. Environmental samples were obtained from meat contact surfaces using a sterile sponge (Whirl-Pak speci-sponge, Nasco, USA) soaked in 10 ml of buffered peptone water (BPW) (Biokar, Zac de Ther, France), according to the following protocol. In meat tables, three areas of 20 x 20 cm each (a total of 1,200 cm2) were sampled. The sponge was wiped 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 samples were ice-refrigerated and sent to the laboratory to be analyzed immediately.

Bacteriological analysis

Ground beef samples were analyzed for mesophilic aerobic organisms, *Escherichia coli* and coagulase-positive *Staphylococcus aureus* enumeration (Maturin & Peeler, 2001; ISO 16649-2, 2001; ISO 6888–1, 2021). All media were from Biokar Diagnostics (Beauvais, France). The Argentinean Food Code (AFC, 2022) microbiological criteria for fresh ground beef were used, i.e.: mesophilic aerobic organisms (n:5 c:3 m:106 M:107), *E. coli* (n:5 c:2 m:100 M:500), and coagulase-positive *S. aureus* (n:5 c:2 m:100 M:1000). In addition, the search and isolation of *Salmonella* spp., *E. coli* O157:H7 and STEC were carried out in ground beef and

environmental samples. In environmental samples all the sponges were aseptically divided into three portions and each sponge portion was used to analyze the different pathogens.

Salmonella spp.

Twenty-five g of ground beef and one portion of the sponge from each environmental sample were cultured in 225 ml and 100 ml of buffered peptone water (Merck)(https://www.sigmaaldrich.com/AR/es/product/mm/107228), respectively, for 24±2 h at 35°C (ISO 6579-1, 2017). After the pre-enrichment step, 0.1 ml of the broth was put onto 0,1 ml of Rappaport-Vassiliadis broth (Biokar Diagnostics), and 1 ml onto 10 ml of tetrathionate broth (Acumedia Manufacturers, USA) for 24±2 h at 42±0.2°C and 37±0.2°C, respectively. Ten microliters were plated into bismuth sulfite agar (Becton Dickinson, Le Pont de Claix, France), xylose-lysine-desoxycholate plus tergitol agar (Oxoid, Basingstoke, UK), and Hektoen enteric agar (Laboratorios Britania, Buenos Aires, Argentina) and incubated for 24±2 h at 35°C. After incubation, presumptive colonies were selected and screened for *invA* gene by PCR (Malorny et al., 2003). The characterization was made by biochemical tests following ISO 6579-1 (2017) and Salmonella serotyping was performed according to the White-Kauffmann-Le Minor scheme by slide (O antigen) and tube (H antigen) agglutination, using specific antisera (Instituto Nacional de Producción de Biológicos (INPB) - ANLIS "Dr. Carlos G. Malbrán", Argentina).

Escherichia coli O157:H7.

Sixty-five g of ground beef samples and one portion of sponge from each environmental sample were incubated onto 585 ml and 100 ml of modified trypticase soy broth (Acumedia), respectively, for 20 h at 41.5°C (ISO 16654, 2001). After enrichment, a specific O157 concentration was made using immunomagnetic separation (Dynal Biotech, Oslo, Norway), streaked into chromogenic agar for *E. coli* O157 (CHROMagar™ O157) and cefixime-tellurite MacConkey sorbitol agar (Oxoid, Hampshire, UK), and incubated for 20 h at 37°C. After incubation, presumptive colonies were selected and screened for rfb_{O157}, stx_1 and stx_2 genes by multiplex-PCR (Leotta *et al.*, 2005). The characterization was made by biochemical tests (ISO 16654, 2001) and the genotypic profile testing: $fliC_{h7}$, stx_1, stx_2, ehx_A, and eae genes (Gannon *et al.*, 1997; Karch *et al.*, 1993; Leotta *et al.*, 2005; Schmidt *et al.*, 1995).

Shiga toxin-producing Escherichia coli (STEC).

Twenty-five g of ground beef samples and one portion of sponge from each environmental sample were incubated in 225 ml and 100 ml, respectively, of modified *Escherichia coli* broth (Acumedia) for 20 h at 37°C. MK-PCR screening (Leotta, 2006) was used after the enrichment step. One milliliter from all MK-PCR-positive samples was plated onto Mac-Conkey agar (Becton Dickinson Co., Sparks, MD, USA) and Levine-eosyne methylene blue agar (Biokar). All plates were incubated for 18 h at 37°C. Fifty colonies with *E. coli* morphology were selected from each plate and point-inoculated on nutrient agar (Britania Laboratory). After incubation, five pools of 10 colonies were screened for stx_1 and stx_2 genes by multiplex-PCR (Leotta *et al.*, 2005). Colonies from positive pools were analyzed individually by multiplex-PCR to detect the *stx*-positive colony. The characterization of the isolated strains was made by biochemical tests. STEC serotyping of O and H antigens and *eae*, *ehxA* and *saa* genes detection were performed as previously described (Karch *et al.*, 1993; Paton *et al.*, 2001; Schmidt *et al.*, 1995).

Improvement actions and consumer information

A structured planning was prepared to assess hygienic and sanitation practices being experienced in this study. The training plan for workers in the butcher shops was conducted in a period between the evaluation and verification periods and was focused to implement the improvement actions. The workers received: 1- Results of the first evaluation period, 2- A guideline including national, provincial, and local regulations about meat sale and 3- Recommendations about Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP), raw food handling and meat preservation.

In consumers the strategy was based on activities that included 1- Training to kindergarden teachers of the Tafí Viejo schools, highlighting their role as primary educators of children, in personal hygiene and food handling, 2- Teaching children, through workshops, plays, videos and educational games about the importance of good hygiene habits to prevent foodborne diseases, 3- Transfer of what children had been learned to other members of the family group, and 4- Design and production of teaching materials, kindly provided by a non-governmental organization for HUS mitigation (http://www.lusuh.org.ar/material.html) from which we are delegate in the northwest region of the country (NOA), as an strategy to

ensure that children adopt the five keys of food safety and transmit them at home.

Verification of the impact of improvement actions

From April to December 2019, the same 28 butcher shops analyzed during the 2017 evaluation period were retested to verify the program impact. Quantity and type of samples, sampling frequency and procedure, risk quantification and bacteriological analysis were performed as described previously in the evaluation period.

Statistical analyses

The McNemar and Student's tests were used to evaluate the impact of the improvement actions after comparing the results of both periods. The facilities, GMP, SSOP, raw food handling/meat preservation and risk quantification were evaluated by the McNemar test. The microbiological quality of meat sold in butcher shops, determined by the counts of mesophilic aerobic organisms, *S. aureus* and *E. coli* were evaluated using Student's paired t-test with a two-tailed distribution. The presence or absence of *Salmonella* spp., *E. coli* O157:H7 and STEC in ground beef and environmental surfaces (counter tops, knives, meat grinders, handlers' hands) were evaluated by the McNemar test.

All statistical evaluation and improvement actions in butcher shops analyses were performed using Info Stat software with a significance of 0.05.

Association between indicators/pathogenic microorganisms and the estimated risk level

To demonstrate whether there was a direct relationship between risk quantification vs. counts of indicator microorganisms vs. detection of pathogens, butcher shops were grouped by type of risk, (regardless of the period), with the results of counts outside the limits established by the AFC and the detection of at least some of the pathogens sought in this work.

Results

Twenty-six shops (93%) were meat stores and 2 (7%) were butcher shops at supermarkets. Six abattoirs provided meat to the 28 butcher shops.

Comprehensive evaluation of butcher shops in the 2017 period

Risk quantification

During this evaluation period, risk quantification in all butcher shops (n=28) resulted in 6 (21.4%) high-risk, 19 (67.8%) moderate-risk and 3 (10.7%) low-risk shops. Results for each group of five variables were as follows: situation and conditions of construction, 4.42/10.0; equipment and tools, 4.73/15.0; manipulator hands, 18.3/25.0; raw materials and products for sale, 14.34/20.0; and production flow, 13.5/30.0. Individual variables and average risk are shown in Table 1.

Table 1

Risk quantification and microbiological analysis on ground beef and environmental surfaces in butcher shops of Tafí Viejo cityTucumán

Groups of variables and average risk	Individual variable	2017	2019	p-value
1- Building situation and conditions	Access to drinking water	96.4%	100.0%	0.0164
	SSOP in the water supply tank	0.0%	0.0%	
	Hot water	14.3%	10.7%	
	Suitable floors	14.3%	35.7%	
	Suitable roofs	21.4%	39.3%	
	Suitable walls	25.0%	46.4%	
	Suitable windows	50.0%	75.0%	
	Protected windows	7.1%	10.7%	
	Adequate lighting	32.1%	35.7%	
	Adequate ventilation	78.6%	92.9%	
	Adequate staff changing room	14.3%	7.1%	
	Adequate staff sanitation area	92.9%	100.0%	
	Waste management	25.0%	60.7%	
	SSOP in work environment	7.1%	35.7%	
	AVERAGE RISK (10.0)a	4.42	5.37	
2- Equipment and tools	Quantity of tools	71.4%	89.3%	0.1728
	Proper conservation of tools	21.4%	71.4%	
	Good conditions of tools	35.7%	78.6%	
	Sufficient refrigeration equipment	53.6%	64.3%	
	SSOP application on equipment and tools	7.1%	32.1%	
	AVERAGE RISK (15.0)a	4.73	5.82	
3- Handlers	Proper hygiene habits	60.7%	96.4%	0.0341
	Health verification	96.4%	89.3%	
	AVERAGE RISK (25.0)a	18.30	20.92	
4- Raw materials and products for sale	Raw material receipt control	92.9%	100.0%	0.8613
	Control of organoleptic properties in products for sale	71.4%	85.7%	
	Proper conservation of raw materials and products for sale	39.3%	46.4%	
	AVERAGE RISK (20.0)a	14.34	14.87	
5- Production flow	Linear flow of meat in one direction	50.0%	50.0%	0.014
	Control of cross-contamination	32.1%	46.4%	
	Protection of meat products	10.7%	10.7%	

Conservation at adequate temperatures	53.6%	78.6%
Food storage by product type	25.0%	53.6%
Pest management	85.7%	92.9%
Qualified personnel for handling meat	7.1%	14.3%
AVERAGE RISK (30.0)a	13.54	17.36

Average risk of each group of variables during the evaluation (2017) and verification (2019) periods. Reference.
[a]Maximum value assigned to each group of variables.p-value = McNemar test.

Bacteriological analysis

Results of microorganism enumeration in the 28 ground beef samples analyzed showed absence of *S. aureus*>10^3 UFC/g in all samples, and mesophilic counts >10^7 UFC/g in 12 (42.8%) samples, distributed as follows: 3 from high-risk butchers, 8 from moderate-risk and 1 from low-risk butchers. *E. coli* >500 UFC/g was detected in 4 (14.3%) samples, 2 from high-risk butchers, and 2 from moderate risk.

Salmonella spp. was detected in 5 (17.9%) ground beef samples and isolated in 2 (7.1%) of them. STEC was detected in 8 (28.6%) samples and isolated in 2 (7.1%) samples. Results are shown in Table 2A.

Table 2A

Salmonella spp., and STEC, detected and isolated from ground beef (A) and environmental samples (B) of butcher shops, in the evaluation (2017) and verification (2019) periods.

Microorganism	[1]Period	Ground beef n=28			
		Detection %(n)	[2]*p*-value	Isolation %(n)	[2]*p*-value
Salmonella spp.	1	17.9 (5)	0.220	7.1 (2)	1.000
	3	3.6 (1)		3.6 (1)	
STEC	1	28.6 (8)	0.013	7.1 (2)	0.479
	3	ND		ND	

Reference. ND: Not detected. [1]Period: 1= Evaluation (2017), before implementing improvement actions; 3=Verification (2019), after implementing improvement actions. [2]*p-value* = McNemar test.

Salmonella spp. was detected in 4/112 (3.6%) environmental samples and isolated from 2 samples. STEC was detected in 32/112 (28.6%) environmental samples and isolated from 4 (3.6%) (knife and meat mincing machine). Results are shown in Table 2B.

E. coli O157:H7 was not isolated from ground beef and environmental samples.

Table 2B

Salmonella spp., and STEC, detected and isolated from ground beef (A) and environmental samples (B) of butcher shops, in the evaluation (2017) and verification (2019) periods.

Microorganism	Period1				
		Knives n=28			
		Detection % (n)	p-value2	Isolation % (n)	p-value2
Salmonella spp.	1	3.6 (1)	0.479	3.6 (1)	0.479
	3	3.6 (1)		ND	
non-O157 STEC	1	35.7 (10)	0.004	14.2 (4)	0.313
	3	ND		ND	
		Manipulator hands n=28			
		Detection% (n)	p-value2	Isolation% (n)	p-value2
Salmonella spp.	1	3.6 (1)	1.000	ND	-
	3	ND		ND	
non-O157 STEC	1	25 (7)	0.023	ND	-
	3	ND		ND	
		Meat tables n=28			
		Detection% (n)	p-value2	Isolation% (n)	p-value2
Salmonella spp.	1	7.1 (2)	0.479	3.6 (1)	1.000
	3	ND		ND	
non-O157 STEC	1	25 (7)	0.023	ND	-
	3	ND		ND	
		Mincing machines n=28			
		Detection% (n)	p-value2	Isolation% (n)	p-value2
Salmonella spp.	1	ND	0.479	ND	-
	3	7.1 (2)		ND	
non-O157 STEC	1	28.6 (8)	0.013	ND	-
	3	ND		ND	

Reference. ND: Not detected. [1]Period: 1= Evaluation (2017), before implementing improvement actions; 3=Verification (2019), after implementing improvement actions. [2]*p-value* = McNemar test.

Improvement actions and consumer information

We promoted the implementation of a GMP program in all butcher shops because of 8 collective training meetings and 28 individual training sessions in each butcher shop. The owners and handlers of meat products belonging to the butchers implemented improvement measures considering the results of risk and microbiological analysis.

Butchers can optimize operational control during the production and sale chain of fresh ground beef. To this end, the concepts of food handling, cold chain, types of contamination, disinfection, and cleaning, among others, were reinforced. Corrective measures were applied on the variables associated with the production line, the

SSOP and the raw material reception. Waste treatment was also reinforced. The guidelines for the implementation of improvement actions addressing butchers are available at http://www.ipcva.com.ar/files/manualcarniceros.pdf

Building conditions were adjusted, bathrooms and changing rooms for work clothing were sectorized for workers. Other building improvements included lighting and ventilation, and conditioning of the ceiling, walls, floors, windows, and doors.

The procedures relating to good hygiene practices (GHP) of staff included personal hygiene and correct hand washing. Liquid soap, paper towels and wastebaskets were distributed at different points of the establishment. Regarding dress standards, appropriate clothing was provided to each operator. Although emphasis was placed on its correct use and washing, the results were not always satisfactory. Other topics included pest control and the sanitary filters installation in strategic sites.

Within the framework of this project 53 teachers received informative brochures to prevent foodborne diseases, later transferred also to 1800 children, distributed to 40 school canteens, and exposed in food fairs. These trainings are part of a multisectoral effort including the Tafí Viejo Municipality and the Bromatology department.

Impact verification of the implemented improvement actions

Risk quantification and evaluation

Risk was reassessed in 2019 in all butcher shops, obtaining the following results: 3 (10.7%) high-risk, 12 (42.8%) moderate-risk and 13 (46.4%) low-risk shops. All groups of variables investigated improved in 2019 compared with the 2017 period (Table 1). Specifically, 1) the situation and condition of buildings improved significantly, (p=0.016) except for the implementation of SSOP in the water supply tank , hot water and adequate staff changing room ; 2) all the individual variables evaluated in equipment and tools did not show significant differences but improved (p=0.173); 3) the aspects concerned with handlers improved as compared with that observed in the 2017 period (p=0.034) except health verification; 4) in raw materials and products for sale, all the variables improved considerably in 2019 compared with 2017 period although they did not present significant differences (p=0.861); 5) most individual variables related with the production flow improved (p=0.014). However, further efforts are needed to introduce guidelines and procedures following GMP and SSOP programs, to ensure non-recurrence of deficiencies.

Bacteriological analysis

Results of microorganism enumeration in the 28 ground beef samples analyzed in 2019, showed *S. aureus*> 10^3 CFU/g in 1 (3.6%) sample from a moderate-risk butcher, mesophilic counts >10^7 CFU/g in 3 (10.7%) samples, 1 from a moderate-risk butcher and 2 from low-risk butchers and *E. coli*>500 CFU/g in 3 (10.7%) samples, 2 from moderate-risk and 1 from low-risk butchers.

The average count of indicator microorganisms in ground beef between both periods was statistically significant in total mesophylls (p=0.004) and in S. aureus (p=0.018); in *E. coli* no significant differences were observed (p=0.343). In ground beef samples a reduction in the number of positive samples for pathogenic microorganisms was observed between both periods (Table 2A). *Salmonella* spp. was isolated from 1 (3.6%) ground beef sample, showing no statistically significant differences when compared with that observed before the implementation of improvement actions (p = 0.221). *E. coli* O157:H7 was not isolated from ground beef and environmental samples. The decrease in the detection rate of STEC showed significant differences from one period to another (p=0.013).

A significant reduction of STEC isolates was observed after implementation of improvement actions in all environmental samples, but mainly in knife samples (p=0-.004). Additionally, STEC were not isolated from environmental samples (meat tables, knives, mincing machines, manipulator hands) in the 2019 sampling period. Co-contamination with STEC and *Salmonella* spp. was detected and isolated from one environmental sample (knives) from a butcher shop.

The proportion of samples from ground beef, meat tables, knives, mincing machines and manipulator hands tested positive for *Salmonella* spp. and STEC is showed in Table 2B.

The association between indicators/pathogenic microorganisms and the estimated risk level (butcher shops) qualified as high risk a count of high microbial indicators associated with the detection of pathogenic microorganisms and in low-risk butcher shops, a low count of microbial indicators without detection of pathogenic microorganisms.

Discussion

In the present study, a first descriptive hygienic-sanitary risk assessment in butcher shops, of Tafí Viejo city, Tucumán province, using a checklist and standard microbiological analysis was completed. The results obtained were used to apply improvement

actions, and subsequently verified using the same descriptive hygienic-sanitary risk and microbiological analysis. In Argentina, other authors (Barril *et al.,* 2019; Leotta *et al.,* 2016; Ruíz *et al.,* 2022) previously used this methodology to applied improvements in manipulation of meat products and facilities.

In this study, the butcher shops applied improvement with success. In Tafí Viejo, after applying improvements, 46.4% of the butcher shops presented low risk, 42.8% moderate risk and 10.7% high-risk. In 73 butcher shops analysed in Neuquén province, 53.4% showed low risk, 45.2% moderate risk and 1.4% high-risk. However, in Tandil (Buenos Aires province) 100% of the butcher shops analysed showed low risk. It is possible to identify great differences between butcher shops from different cities. The established criteria and the rigor of the auditors when applying the check list could affect the risk result.

With regards to raw meat products, safety and quality can be estimated using indicator microorganisms, and obtaining the total aerobic mesophilic, *S. aureus*, and *E. coli* count (de Souza Couto Oliveira & Freitas Brilhante de Sao José, 2019; Siriken, 2004). A higher total aerobic mesophilic count is usually an indication of poorer quality and reduced shelf life and could be associated with handling practices in abattoirs (Pérez Terrazzino *et al.,* 2023), transport (Milojevic *et al.,* 2018), discharge in butcher shops or in environment and manipulation in the butcher shops (Leotta *et al.,* 2016).

In this study, prior to application of improvement actions, 42.9% and 14.3% of the analysed samples showed total aerobic mesophilic and *E. coli* above AFC reference values. After improvement actions were applied, 10.7%, 3.6% and 10.7% of the analysed samples of raw ground beef showed above reference values to total aerobic mesophilic, *S. aureus* and *E. coli*, respectively. These results could be explained by the inefficiency in the applied of SSOP, GHP and GMP procedures in the butcher shops; subsequently these were adjusted, showing lower total aerobic mesophiles values. However, the *E. coli* counts remained the same, and the number of samples that exceeded the count limits established in the AFC for *S. aureus* increased. Probably, it is necessary to reinforce improvement actions in butcher shops, especially due to the deviations found with *S. aureus* that could be originated during handling (Ho *et al.,* 2015), although we must also consider the quality of the product during the production process in the abattoirs. During 2016-2018, two Tucumán abattoirs that supply the Tafí Viejo butcher shops were analysed (Pérez Terrazzino *et al.,* 2023) and qualified both as high risk. Indicators count on carcasses (approximately 8.000 cm^2) for total aerobic

mesophilic (3 log CFU/cm^2) and *E. coli* (0.45 log CFU/cm^2), were reported. These findings allow us to hypothesize abattoirs as a possible source of contamination. Moreover, it is interesting to compare the indicators results obtained in other reports on ground beef from butcher shops in Argentina. In this study, 53.6% (n=28) of the fresh ground beef samples presented indicator counts above the reference limit.

Several studies carried out around the world on fresh meat reported results that differed from those of Tafí Viejo (Abreu *et al.*, 2011; Bersisa *et al.*, 2019; Maciel Sousa *et al.*, 2012; Tonjo *et al.*, 2022). 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, *et al.,* 2008). In this study, we were able to demonstrate that in butcher shops where the risk was high, the pattern repeated was related to unacceptable indicator microorganisms counts and pathogenic microorganisms detection. Kang *et al.* (2018) reported that the counts of indicator microorganisms are mostly affected by temperature regulation during meat processing. The processing conditions, flow and distribution structure of meat are also identified as key factors to reduce microbiological loads. Therefore, meat markets should focus on maintaining raw materials and meat products at low temperatures, preventing cross-contamination, and avoiding the proliferation of microorganisms during distribution (Kang *et al.*, 2018).

The relationship between indicator microorganisms and presence of foodborne pathogens in raw meats remains unclear (Wu *et al.,* 2011). Several studies have shown the presence of *Salmonella* spp., *E. coli* O157:H7, and STEC in ground beef from butcher shops (Bai *et al.,* 2015; Bosilevac *et al.,* 2007, Bosilevac & Koohmaraie, 2011; EFSA & ECDC, 2022; Fayemi *et al.,* 2021; Momtaz *et al.,* 2013; Toro *et al.,* 2018). In our study, 50% of the butcher shops presented, at least, one positive sample for the pathogenic microorganisms studied. Difference about rate of STEC reports in beef may be due to the methodologies used (PCR, isolation, immunopotentiation, hybridization), serotype search (O157:H7, O111, O145, O26, O121, O103) or all STEC, type of sample (ground beef or raw meat), year season, and controls performed on retails, among others. In this study, before reinforcing GPH, GHM and SSOP procedures, the proportion of ground beef samples that tested positive for STEC was 7.1%, and the proportion of positive environmental samples was 3.6%. After improvement actions in Tafí butchers, all samples were negative.

Prevalence studies of pathogens in commercially acquired meat products provide estimates that reflect the consumer exposure level

(Barril *et al.,* 2019). The reported rates of contamination with *Salmonella* spp. in beef at retail are also different depending on the country (Cabrera Díaz *et al.,* 2020; EFSA (European Food Safety Authority) & ECDC (European Centre for Disease Prevention and Control), 2022; Siriken *et al.,* 2020; Vipham *et al.,* 2012; Yan *et al.,* 2010). In this study, before reinforcing GPH, GHM and SSOP, the proportion of ground beef samples screened positive for *Salmonella* spp. resulted 17.9%, and the proportion of environmental samples positive to *Salmonella* spp. by isolation was 1.8%. After improvement actions in Tafí butchers, 3.6% and 3.6% of ground beef and environmental samples were found positive, respectively. Unlike previous reports, in which different *Salmonella* serovars were found, in this study the unique *Salmonella* serovar identified was Typhimurium. These were isolated from ground meat and environmental samples, before and after implementing improvement actions. *S.* Typhimurium is associated with human diseases worldwide (Duong *et al.,* 2020; Mather *et al.,* 2018; Scallan *et al.,* 2013; Sun *et al.,* 2020; Vithayasai *et al.,* 2011; WHO, 2015). In 2017-2019, of 2.8% acute diarrhea cases reported in Tucumán province (N=1167), were confirmed as salmonellosis (Base de datos cómputos, Hospital del Niño Jesús, Tucumán, Argentina). The etiological agent was identified as *S.* Typhimurium. In this context, the transmission of *S.* Typhimurium in Tucumán province through bovine ground beef should be considered. A possible source of contamination could be the abattoirs that supplied these butcher shops.

The HUS is endemic in Argentina (Rivas *et al.,* 2006) and recently the annual incidence was 0.6 cases per 100,000 inhabitants considering the total population (Boletín Epidemiológico Nacional 718, 2024). Brusa *et al.* (2020) published a Quantitative Microbial Risk Assessment due to beef consumption in Argentina. According to these authors, it would be expected that 32 annual cases (10%) of HUS in Argentina were originated after beef consumption, being ground beef the meat product with the highest risk. During the present study, only a few cases of HUS were reported in Tafí Viejo/ Tucumán, according with low reports of STEC and *E. coli* O157:H7 in butcher shops.

In Tucumán, reinforcing epidemiological studies in clinical cases to prevent salmonellosis are need. In addition, the effort on butcher shops must be continuous to consolidate the GHP, GMP and SSOP procedures, to reduce the indicators microorganisms charge and pathogenic bacteria.

All risk variables investigated after implementing improvement actions had a positive evolution. In building situation and conditions, handlers and production flow, significative differences were observed.

However, in the equipment and tools and raw materials and products for sale significative differences were not observed.

The prevention of zoonotic diseases associated with meat consumption can be improved through collaboration and cooperation under the "One Health" concept, since meat contamination can occur at various points involved in production and processing (in the farm) to consumption (on the table) (Ali & Alsayeqh, 2022). Understanding health in global terms, considering the microorganisms circulating among the population, but also between animals and the environment, is the key to success in the control of present and future infectious conditions. After the improvements actions applied in Tafí Viejo butcher shops, it was only possible to reduce the total aerobic mesophilic count, *Salmonella* was isolated in ground beef from butcher shops classified as high-risk, and STEC was not isolated. The most problematic points detected can be improved with the implementation of an integrated GMP program. In addition, the application of SSOP should be reinforced. Emphasis should be placed mainly on specific training in the preparation of meat according to species, not mixing cooked products with raw materials or using the same refrigeration chamber for the storage of merchandise other than meat. Therefore, improvement of GMP is critical to reduce cross-contamination between the environment and beef products, and ultimately, to avoid foodborne illness. 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 Tucumán.

Conclusions

This is the first study carried out in Tucumán province, with a focus on the effective implementation of strategies based on detecting fails in the use of GMP, GHP and SSOP. The feasibility of implementing this comprehensive risk management program with the sampling to validate their application and the statistical verification of the improvements, were useful tools to identify and correct the sanitary and hygienic state of the butcher shops in Tafí Viejo city. However, there is a need to expand collaborative efforts to improve food safety at home and retail level to prevent foodborne illness.

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Author agreement and contributions

Moreno Mochi, María Paula: Conceptualization, data curation, formal analysis, investigation, methodology, roles/writing-original draft, writing-review & editing. Vargas, Juan Martín: Conceptualization & methodology. Costa, Magdalena: writing-review & editing. Solorzano, María Victoria; Vega, Silvia; Condori, Marina Susana: Resources. Leotta, Gerardo Aníbal: Conceptualization & Supervision. Jure, María Angela: Conceptualization, supervision, roles/writing -original draft, writing-review & editing.

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Assessment of hygienic-sanitary status and implementation of an integral risk management program in butcher shops from Tafí Viejo city, Tucumán, Argentina

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