Preventive Veterinary Medicine 176 (2020) 104933

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Comprehensive evaluation and implementation of improvement actions in bovine abattoirs to reduce pathogens exposure

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ARTICLE INFO

Keywords:
Risk estimation
Salmonella spp.
Escherichia coli
Abattoirs
Meat

ABSTRACT

The slaughter process plays an important role in animal welfare, meat quality, safety and public health through the meat production chain. In this study, we performed a three-stage evaluation: I) comprehensive evaluation, II) implementation of improvement actions and III) verification of the success of the actions implemented in three abattoirs from Argentina during 2016-2018. Risk was estimated using two checklists, quantified on a 1–100 scale and classified as high (1–40), moderate (41–70) and low (71–100). In stages I and III, *Salmonella* spp., *E. coli* O157:H7 and non-O157 STEC were detected and isolated in samples from carcasses (n = 252), the environment (n = 252); head meat (n = 21) and viscera washing and chilling water (n = 105). Carcass samples were analyzed for mesophilic aerobic organisms, coliforms and *E. coli* enumeration. Of 201 water samples taken, 42.0–75.6 % were non-potable quality. After the implementation of improvement actions in stage II (building, processes, systems for water purification and training), the estimation of risk of contamination was reduced from high to moderate in all three abattoirs, the count of indicator microorganisms decreased in two abattoirs, and the presence of pathogens significantly decreased. *Salmonella* spp. was not isolated from any of the samples collected in two abattoirs. Isolation of *E. coli* O157:H7 decreased in carcass and was not isolated from viscera washing and chilling water. Isolation of non-O157 STEC decreased in carcass but not in environmental samples. Finally, 75.0–95.0 % of water samples were of potable quality. Although this was only the first step in the process of change and improvement of abattoirs, the assessment of the situation and the proposal of solutions to correct deviations in a joint effort with the health authorities helped to implement a work model for enhancing food safety before meat reaches consumers.

1. Introduction

The slaughter process plays an important role in animal welfare, meat quality, safety and public health through the meat production chain (Aghwan, 2019). During slaughtering, carcasses can be contaminated with foodborne pathogens directly by feces, in the process of evisceration, from contaminated hides, when they are removed during the dressing process, and as a result of cross contamination with other carcasses and line or cool chamber surfaces (Bosilevac et al., 2015; Brusa et al., 2019). Contamination can also occur through direct

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https://doi.org/10.1016/j.prevetmed.2020.104933
Received 17 October 2019; Received in revised form 22 January 2020; Accepted 17 February 2020

contact with the abattoir environment (Cap et al., 2018). In addition, water can be the main source of contamination when hygiene and sanitation conditions and good practices are not respected (Haijoubi et al., 2017).

In Argentina, there are different categories of abattoirs, depending on their slaughter capacity, the marketing area for meat and viscera, and the sanitary authority responsible for their control. The four main abattoir categories identified by the National Service of Agrifood Health and Quality of Argentina (SENASA, for its Spanish acronym) (SENASA 4238) include:

- exporter abattoirs, which distribute their product outside the country, have a Hazard Analysis and Critical Control Points (HACCP) system, comply with the sanitary requirements of SENASA and the countries of destination, and provide continuous food safety training;
- federal transit abattoirs, which distribute their product within the country, have a HACCP system and comply with the sanitary requirements of SENASA;
- provincial transit abattoirs, whose products are consumed within the area corresponding to each province, not always have an HACCP system, comply with the sanitary requirements of each provincial health authority, but do not implement microbiological verification of either product or the environment;
- rural market, in which case the animals slaughtered must be issued and consumed exclusively within the locality for which they were authorized, do not have an HACCP system, comply with the sanitary requirements of each health authority, but do not implement microbiological verification of either product or the environment.

Hygiene and sanitation standards differ among the abattoirs categories described above (Santángelo and Robert, 2013). Thus, people consuming beef from provincial and rural abattoirs are more exposed to diseases such as Salmonellosis or hemolytic uremic syndrome (HUS). In 2017, provincial abattoirs from Argentina working in the precarious conditions described previously were responsible for 16.0 % of the total slaughter in the country (Consortium ABC, 2019).

The aims of this work were to perform a comprehensive evaluation, which including risk estimation and bacteriological analysis of meat, viscera washing and chilling water, environment and water samples, implement improvement actions and verify the impact of those actions on the beef production chain of three provincial bovine abattoirs, aimed at reducing contamination with foodborne pathogens.

2. Materials and methods

The study was carried out in three abattoirs located in the province of Buenos Aires, Argentina (area, 307 571 km^2; 15 355 000 inhabitants). The project included three stages: I) evaluation, II) implementation of improvement actions, and III) impact verification. For this study, the provincial health authority selected three licensed abattoirs (identified as A, B and C) located at a distance of less than 100 km from the sample processing laboratory, with an average slaughter of 150–200 animals per day each. Abattoir participation was voluntary, supported and endorsed by the health authorities.

Stage I began in February 2016. Each abattoir was visited once a week for 10 consecutive weeks to perform comprehensive assessments and risk estimation based on checklists. Carcass and environmental samples (hands, knives, boots of the workers, platform, cool chambers and bathrooms) were taken for bacteriological analysis. The microbiological quality of water was also evaluated. In the last three visits, samples of head meat and viscera washing and chilling water (heart, sweetbread, liver, kidney and chitterlings) were also collected.

The results of this stage were delivered to the people in charge of each abattoir. Using the deviations found as starting point, a training plan was applied and improvement actions were implemented in an agreed period of time (6–8 months, Stage II). Finally, the three abattoirs were reevaluated using the same tools to verify the impact of improvement actions (Stage III, 2018).

2.1. Risk estimation

Risk was estimated using a preoperational and an operational checklist, developed by consensus of a group of two researchers from the National Council of Scientific and Technical Research (CONICET, Argentina) and three professionals from the Ministry of Agro-Industry of the province of Buenos Aires. The checklists were completed alternately during 10 weeks before and during the slaughter process, respectively. They were divided into six blocks that represented all areas of the abattoir. Each block was assigned a score related to the risk of contamination of the final product, based on current legislation. The four possible qualifications of abattoirs were acceptable (perfect condition), marginal (not ideal conditions), unacceptable (not corresponding conditions), and does not apply (conditions could not be evaluated but did not influence process outcomes). They were assigned a numerical value according to their relevance and by consensus of the working group.

The final block score (BS) was obtained with a formula that included the sum of the total acceptable and marginal grades obtained (TAM) multiplied by the importance (I) assigned to each block, divided by the sum of all acceptable scores (AA) minus total grades referred to as “does not apply” (TDNA).

$$BS = \frac{TAM \times I}{AA - TDNA}$$

The sum of all BS gave a final score of 100. Accordingly, risk was estimated on 1–100 scale as high (1–40), moderate (41–70) or low (71–100).

The preoperational checklist included the following blocks and scores: 1) pens (15.0); 2) slaughter area (35.0); 3) cool chambers (10.0); 4) quartering (10.0); 5) offal area (20.0); 6) outdoors (10.0). The operational checklist included the following blocks and scores: 1) pens (15.0); 2) slaughter area (25.0); 3) head and viscera area (10.0); 4) control points (15.0); 5) cool chambers (20.0); 6) offal area (15.0). Both checklists are presented as Supplementary Tables 1 and 2.

2.2. Sample collection

Carcass and environmental samples (Stage I, N = 180; Stage III, N = 72) were obtained using a sterile sponge (Whirl-Pak speci-sponge, Nasco, USA) soaked in 10 ml buffered peptone water (BPW) (Biokar, Zac de Ther, France). Carcass samples (n = 6 each) were taken during preoperational (previous workday, up to 6 days of storage in cool chambers) and operational (on the same workday, in airing chambers) visits. Two samples from each carcass were obtained. One was used for the count of indicator microorganisms by swabbing four carcass areas of 100 cm^2 each (chest, neck, buttock and posterior lateral hock). First, the chest and neck area was swabbed with one side of the sponge (ten strokes in two directions, from left to right and from top to bottom). The same sponge was then flipped to the other side to swab the buttock and posterior lateral hock as aforementioned. The other sample was used to detect pathogenic microorganisms by swabbing the carcass entire surface (external and internal side) with another sterile sponge (Whirl-Pak)

Table 1
Number of samples collection in Stages I and III.

	Stage I		Stage III	
	Pre operational	Operational	Pre operational	Operational
Carcass	90	90	36	36
Environment	90	90	36	36
Head meat	3	6	6	6
Viscera washing and chilling water	15	30	30	30
Water	73	72	28	28

Table 2
Comparison of the pre-operational and operational checklists in Stages I and III of the study. Only variables with highly significant differences are detailed [McNemar test (*p*)].

Pre-operational					Operational				
Block	Item	Abattoir (*p*)			Block	Item	Abattoir (*p*)		
		A	B	C			A	B	C
Pens	Building conditions	0.008	0.317	0.008	**Pens**	Staff clothing	0.032	0.055	0.024
	Shower and bath for sprinkling	0.050	1.000	0.014		Chlorine in water	0.046	0.005	0.074
	Animal welfare (management)	0.014	0.022	(*)		Building	0.025	0.264	0.005
	Total block I	0.046	0.074	0.051		**Total block I**	0.026	0.007	0.530
Slaughter area	Aprons	0.008	0.011	0.046	**Slaughter area**	Correct use of knife to slit the throat	0.006	0.008	0.005
	Sanitary filter	0.008	0.008	0.049		Cleanliness	0.006	0.024	0.005
	Platforms	0.049	0.008	0.008		Hand soap	0.007	0.028	0.006
	Total block II	0.024	0.019	0.024		**Total block II**	0.012	0.012	0.012
Cool chambers	Presence of condensation	0.008	0.013	0.237	**Head and viscera**	Washing and sterilizing of utensils for head	0.091	0.020	0.007
	Ligths	0.112	0.011	0.439		Head washing (N = 10)	0.005	0.005	1.000
	Structures and rails	0.008	0.350	0.008		Washing and sterilizing of utensils for viscum	0.025	0.009	0.005
	Total block III	0.032	0.015	0.207		**Total block III**	0.010	0.013	0.010
Quartering	Platforms	0.008	(*)	(*)	**Control points**	Sterilizer temperature (82 – 85 °C)	1.000	0.008	0.008
	Ligths	0.008	(*)	(*)		Correct washing of carcasses (N = 10)	0.005	0.036	1.000
	Layout of waste	0.014	(*)	(*)		Water chlorination (ppm)	(*)	0.008	0.120
	Total block IV	0.010				**Total block IV**	0.010	0.010	0.897
Offal area	Stoves	0.439	0.040	0.008	**Cool chambers**	Sanitary filter	1.000	0.005	0.007
	Containers	0.049	0.739	0.010		Condensation	0.028	0.074	0.180
	Aprons	(*)	0.011	0.014		Staff clothing	0.005	0.005	0.264
	Total block V	0.023	0.018	0.127		**Total block V**	0.019	0.010	0.521
Outdoors	Locker room	0.197	0.040	0.008	**Offal area**	Staff clothing	0.079	0.005	0.371
	Bathrooms and toilets	0.049	0.040	0.008		Building conditions	(*)	0.371	0.091
	Store of chemicals	0.008	0.008	0.008		Sanitary filter	1.000	0.005	1.000
	Total block VI	0.010	0.017	0.121		**Total block VI**	0.694	0.090	0.661
	Total	0.025	0.020	0.053		**Total**	0.014	0.013	0.013

(*) Variable not evaluated because it does not correspond in the establishment.

following the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) methodology (U.S. Department of Agriculture, Food Safety and Inspection Service, 2005). The external side was first swabbed with ten strokes of the sponge in two directions (from left to right and from top to bottom); the same sponge was flipped and the internal side was covered by another ten strokes in both directions, as mentioned previously.

Environmental samples were collected using six different sterile sponges (Whirl-Pak) as follows: 1) samples (n = 5) of all workers´ hand surfaces (front, back, interdigital spaces and nails); 2) samples (n = 5) from the entire surface of the knife blade and the intersection between the blade and the blade handle; 3) samples (n = 5) from workers´ boots, which were swabbed entirely from shaft to sole; 4) samples from platforms by swabbing carefully the areas where they rub against carcasses during slaughtering; 5) samples from cool chambers by passing the sponge through the walls, doors, latches and columns that could come in contact with carcasses; 6) samples from bathroom walls, toilets and faucets from washbasins where workers clean themselves. After swabbing, all sponges were placed into sterile stomacher bags, stored at 4 °C and immediately sent to the laboratory for analysis.

Viscera washing and cooling water was sampled by collecting 500 ml of water from each viscera in sterile bottles (Stage I, N = 45; Stage III, N = 60). In the case of head meat samples, a kilogram was taken each visit in sterile bags (Whirl-Pak).

To evaluate the microbiological quality and dose of residual chlorine in water, samples (Stage I, N = 145; Stage III, N = 56) were collected in sterile bottles at different points of the abattoir (water wells, exit of water collection tanks, pens, inside the abattoir and bathrooms). Sodium thiosulfate (0.3 ml, Mallinckrodt Baker, New Jersey, USA) was added to samples taken after the addition of chlorine. All faucets were sterilized before sampling using alcohol and fire. Samples were stored at 4 °C and immediately sent to the laboratory for analysis (Table 1).

2.3. Bacteriological analysis

Carcass samples were analyzed for mesophilic aerobic organisms, coliforms and *Escherichia coli* with 3M™ Petrifilms™ aerobic count plates (3M™, Minnesota, USA) and 3M™ Petrifilms™ *E. coli*/coliform count plates (3M™). Samples were placed in a stomacher bag and 15 ml BPW (Biokar) was added. After mixing for 30 s, 1 ml of sample was placed into each Petrifilm plate, incubated and counted according to the manufacturer's specifications. Results were expressed as log CFU/cm^2.

Salmonella spp., *E. coli* O157:H7 and non-O157 STEC were detected and isolated from carcass, environment, viscera washing and chilling water and head meat samples. The latter samples were washed with 500 ml BPW (Biokar). Sponges were aseptically cut in half and the 500 ml BPW were aseptically divided in two portions of 250 ml each to analyze the different bacteria.

All samples collected during Stage I were screened for *E. coli* O157:H7, non-O157 STEC and *Salmonella* spp. using GeneDisc® RT-PCR (Pall Corporation, New York, USA). After STEC and *Salmonella* spp. detection, the STEC Top 7 method was used to identify STEC serogroups according to the manufacturer's specifications. In Stage III, the corresponding isolation techniques were applied directly in all samples.

Detection and isolation of *Salmonella* spp., was carried out according to ISO 6579-1:2017 (ISO, 2017).

Detection and isolation of *E. coli* O157:H7 was carried out according to ISO 16,654:2001 (ISO, 2001), with some modifications. The rfb_{O157}, stx_1 and stx_2 genes were screened by multiplex-PCR (Leotta et al., 2005). Genes $fliC_{h7}$, *ehx*A and *eae* were characterized according to Leotta et al. (2008).

Detection and isolation of non-O157 STEC were carried out according to ISO/TS 13,136:2012 ISO, 2012), with some modifications. Screening for the *stx* gene was performed with multiplex-PCR (Leotta et al., 2005).

Table 3
Microorganism counts in carcass samples in stages I and III.

Abattoir	Mesophilic			Coliforms			*E. coli*		
	I	III	*p*	I	III	*p*	I	III	*p*
A	**3.6**	**3.81**	**0.048**	1.67	1.83	0.348	0.79	0.49	0.277
B	**3.48**	**2.94**	**0.001**	0.97	0.69	0.283	0.27	0.33	0.673
C	**3.83**	**2.44**	**0.001**	**1.47**	**0.57**	**0.001**	**0.57**	**0.39**	**0.012**

Data are presented as log CFU/cm^2.

2.4. Water

Water samples were evaluated according to the microbiological criteria of the Argentine Food Code (AFC) for potable water (coliform bacteria, ≤ 3 MPN/100 ml; *Escherichia coli,* not detected in 100 ml; *Pseudomonas aeruginosa,* not detected in 100 ml; mesophilic bacteria count, < 500 CFU/ml), following standard methods (Rice et al., 2017). Before collection, free chlorine levels were measured using a commercial kit (Merck KGaA).

2.5. Improvement actions

Based on the deviations identified with the checklists, improvements were recommended, regarding the regulatory framework that was not being met. The plan for the promotion of improvement actions in abattoirs included training meetings for workers and the personnel responsible for each plant. The report containing the microbiological results and the problems identified during risk estimation with checklists was used to make recommendations on facilities, good hygiene practices (GHP), good manufacturing practices (GMP) and standard operating procedures for sanitation (SSOP). Possible solutions to make the water in the plant potable were proposed. The guidelines for the implementation of improvement actions were submitted to the consideration of abattoir administrators/owners and the health authorities. All operators attended a mandatory official training course given by the provincial health authority to become certified food handlers.

2.6. Verification of the impact of improvement actions

From April to August 2018, the abattoirs analyzed during Stage I were retested to verify the impact of improvement actions. Sample type, sampling frequency and procedure, risk estimation and bacteriological analyses were carried out as described above for Stage I, except that only four visits were made to each abattoir, during which both the preoperational and operational checklists were implemented. The same samples (viscera washing and cooling water and head meat) were taken and water sampling and chlorine dosing were carried out at the same points as in Stage I.

2.7. Statistical analyses

McNemar test was used to evaluate the impact of improvement actions comparing changes in checklist-based risk estimation in Stages I and III. The microbiological quality of carcasses (counts of mesophilic aerobic organisms, coliforms and *E. coli* before and after implementing the improvement actions) was evaluated using Student's *t*-test for independent variables. Variance homogeneity was checked with Levene test. The impact of improvement actions on *Salmonella* spp., *E. coli* O157:H7 and non-O157 STEC isolation from all samples was evaluated using Chi^2 test. All statistical analyses were performed using IBM® SPSS® version 24. Significance was set at $p < 0.05$.

3. Results

3.1. Risk estimation

In Stage I, checklist-based risk estimation in preoperational and operational visits was 18–27 and 13–20 in abattoir A, 28–32 and 22–25 in abattoir B and 33–39 and 32–40 in abattoir C, respectively. All values corresponded to high risk.

The comparison of the results obtained in Stages I and III of the trial and McNemar test results are shown in Table 2. Only variables with highly significant differences are detailed. In abattoir A, pre-operational risk was between 40 (high) and 41 (moderate), whereas operational risk was between 39 (high) and 48 (moderate) ($p = 0.025$ and $p = 0.014$, respectively). In abattoir B, preoperational and operational risk was moderate, with values ranging between 48 and 60 ($p = 0.020$) and 66 and 69 ($p = 0.013$), respectively. In abattoir C, pre-operational risk was between 38 and 44 (moderate), showing a trend towards significance ($p = 0.053$), whereas operational risk results were between 48 and 56 (moderate) in all visits ($p = 0.013$).

Pre-operational analysis of the quartering block was carried out in abattoir A, as it was the only one performing this activity. Differences were significant in all blocks of abattoir A. In abattoir B, differences were significant in all blocks except for the pen, where a trend towards significance was observed ($p = 0.074$). In abattoir C, differences were significant only in the slaughter area ($p = 0.024$).

The operational analysis of abattoirs A and B showed significant differences in all blocks, excepting the offal area ($p = 0.05$). In abattoir C, differences were significant in blocks of the slaughter and head and viscera areas ($p < 0.05$).

3.2. Bacteriological analysis

Results of indicator microorganism counts in Stages I and III are presented in Table 3. After the implementation of improvement actions, differences in mesophilic counts were in abattoirs A and B ($p = 0.05$), whereas they were significant in the three determinations analyzed in abattoir C.

Results of the isolation of pathogenic microorganisms in Stages I and III were not enough to find statistical significance; therefore, the three abattoirs were analyzed jointly (Table 4). An important reduction in the presence of pathogens was observed. The characterization of serovars and sources of *Salmonella* spp. strains is depicted in Table 5.

Of the *E. coli* O157:H7/NM strains positive for *fliC*$_{h7}$, *ehxA* and *eae*, ten were positive for *stx*$_2$, eight for *stx*$_1$ and *stx*$_2$, and only one for *stx*$_1$. Interestingly, two *E. coli* O157:H21 strains were isolated and resulted positive for *stx*$_2$, *ehx*A and *saa.* The characterization of non-O157 STEC is presented in Table 6.

3.3. Water

Water samples were considered non-potable whenever any of the parameters tested were outside the limits established by the AFC. In Stage I, 75.6 % of samples were non-potable in abattoir A, finding free chlorine levels < 1.5 ppm in all visits. In abattoir B, 42.0 % of samples were non-potable and one of the three extraction wells was contaminated. In abattoir C, 62.0 % of samples were non-potable; values were outside the AFC limits in all sampling sites at least once. Contamination was detected in the extraction wells of all three abattoirs; the greatest deviation was in coliform counts (≤ 3 MPN/100 ml).

In Stage III, 75.0 % of water samples from abattoir A were potable, whereas five samples from the extraction wells (before adding chlorine) were non-potable (coliform counts > 3 MPN/100 ml), isolating *E. coli* from one of them. In abattoir B, 95.0 % of samples were potable and only one sample from the extraction wells had coliform counts (43 MPN/100 ml). In abattoir C, non-potable samples were detected in the first two visits, where the chlorine dosage was 0 ppm (*E. coli,*

Table 4
Salmonella spp., *E. coli* O157:H7 and non-O157 STEC isolated from carcasses, environmental samples, head meat and viscera in stages I and III.

Microorganism	Stage	Carcass				Environment				Head meat				Viscera			
		A	B	C	Total	A	B	C	Total	A	B	C	Total	A	B	C	Total
***Salmonella* spp.**	I	5/60 (8.3)	2/60 (3.3)	3/60 (5.0)	10/180 (5.5)	3/60 (5.0)	7/60 (11.6)	1/60 (1.6)	11/180 (6.1)	1/3 (33.3)	0/3	0/3	1/9 (11.1)	1/15 (6.6)	2/15 (13.3)	1/15 (6.6)	4/45 (8.8)
	III	0/24	0/24	0/24	0/72	3/24 (12.5)	0/24	0/24	3/72 (4.1)	0/4	0/4	0/4	0/12	3/20 (15.0)	0/20	0/20	3/60 (5.0)
***E. coli* O157:H7**	I	0/60	4/60 (6.6)	2/60 (3.3)	6/180 (3.3)	0/60	1/60 (1.6)	1/60 (1.6)	2/180 (1.1)	0/3	0/3	1/3 (33.3)	1/9 (11.1)	0/15	0/15	4/15 (26.6)	4/45 (8.8)
	III	0/24	2/24 (8.3)	0/24	2/72 (2.7)	1/24 (4.1)	1/24 (4.1)	0/24	2/72 (2.7)	1/4 (25.0)	0/4	0/4	1/12 (8.3)	0/20	0/20	0/20	0/60
non-O157 STEC	I	25/60 (41.6)	4/60 (6.6)	5/60 (8.3)	34/180 (18.8)	7/60 (11.6)	0/60	4/60 (6.6)	11/180 (6.1)	0/3	0/3	0/3	0/9	0/15	1/15 (6.6)	2/15 (13.3)	3/45 (6.6)
	III	4/24 (16.6)	3/24 (12.5)	3/24 (12.5)	10/72 (13.8)	3/24 (12.5)	1/24 (4.1)	1/24 (4.1)	5/72 (6.9)	0/4	0/4	1/4 (25.0)	1/12 (8.3)	2/20 (10.0)	1/20 (5.0)	1/20 (5.0)	4/60 (6.6)

Data are presented as N/N total (%).

Pseudomonas aeruginosa and coliform counts > 3 MPN/100 ml were obtained). Nevertheless, 75.0 % of samples were potable. After the implementation of improvement actions, significant differences could be observed in the three establishments ($p < 0.05$).

3.4. Improvement actions

In Stage II, the personnel from each abattoir was trained with reference to the following recommendations:

1) Building conditions: improvement of ceilings, walls, floors, windows, doors, lights and ventilation, among others. Installation and use of sanitary filters and sterilizers. Delimitation between clean and dirty areas. General repairs in the viscera area.

2) Equipment and utensils: reinforcement of the SSOP concept and need for a preoperational and operational sanitation plan. Importance of using hot water for cleaning. Incorporation of the use of pneumatic stunning.

3) Workers: correct personal hygiene. Proper use of work clothing. Training on prevention of foodborne diseases.

4) Processes: comprehensive GMP program. Reinforcement of concepts on cross contamination and integrated pest and waste management. Improvement in evisceration and dressing procedures.

5) Water: installation of chlorine dosing equipment. Daily chlorine dose and record. Periodic microbiological and physicochemical controls. Effluent monitoring and record.

Despite the proposed actions, some improvements were partially achieved.

4. Discussion

During Stage I of this study, structural and process deviations were identified in the three abattoirs analyzed. After that stage, improvement actions were proposed, implemented and successfully verified by comparing the results of both preoperational and operational checklists and microbiological analyses.

In Argentina, like in other countries (Festus Jaja et al., 2018; Essendoubi et al., 2019), there is a wide variety of meat processing plants, in which the application of quality systems depends their size and on market demands. This may occasionally result in multiple hygiene and sanitation standards for abattoirs (Santángelo and Robert, 2013). In previous studies conducted in butcher shops from Argentina, abattoirs were identified as a possible common source of contamination (Leotta et al., 2016; Brusa et al., 2017; Londero et al., 2019). In those works, checklists were useful to identify relevant deviations (Leotta et al., 2016). Here, checklists for risk estimation and microbiological analyses were adapted to the abattoir environment.

Process, structural and staff deviations have already been detected in provincial abattoirs of Argentina (Cendón and Unger, 2009) and worldwide (Adeolu et al., 2019; Bersisa et al., 2019). In the current study, we not only identified deviations through the implementation of improvement actions in a joint effort with the health authorities, we could reduce the risk of contamination from high to moderate in the three abattoirs studied. However, considering this was a pilot study, it was not possible to complete all the proposed improvements; therefore, there is still much work to be done in building and process improvements.

In addition to the results obtained with both checklists, bacterial counts in meat were also an acceptable indicator of hygiene quality, considering that abattoir characteristics influence the bacterial load of beef carcasses (Barco et al., 2015). Indicator microorganism counts were reduced in two abattoirs during Stage III, probably due to the implementation of improvement actions. However, increased counts were observed in abattoir A, which could be associated with the longer storage time in chambers, since preoperational sampling in stage III coincided with the maximum time for sampling (6 days). The continuous manipulation during those days may have influenced the

Table 5
Sources of *Salmonella* spp. serovars isolated during stages I and III.

Abattoir	Stage	Carcass	Environment	Head meat	Viscera
A	**I**	*S.* Anatum (n = 3)	*S.* Anatum (n = 2) (Boot)	*S.* Typhimurium	*S.* Anatum (Chitterlings)
		S. Give	*S.* Give (Hand)		
		S. Typhimurium			
	III		*S.* Montevideo (n = 2) (Platform)		*S.* Montevideo (Chitterlings)
			S. Anatum (Knives)		*S.* Newport (Kidney)
					S. Anatum (Sweetbread)
B	**I**	*S.* Cerro	*S.* Montevideo (n = 3) (Hands)		*S.* Montevideo (Sweetbread)
		S. Montevideo	*S.* Anatum (n = 2) (Hands)		*S.* Montevideo (Liver)
			S. Montevideo (Knives)		
			S. Montevideo (Boots)		
C	**I**	*S.* Anatum (n = 3)	*S.* Anatum (Boots)		*S.* Montevideo (Kidney)

development of microorganisms (Nychas et al., 2008; Doulgeraki et al., 2012). In this sense, Signorini et al. (2018) described at least two less orders of magnitude in the counts of indicators microorganism in Argentine exporting abattoirs, showing the importance of implementing a quality management system.

In this study, important reductions in the presence of pathogens could be observed in Stage III after the implementation of GMP and SSOP. *Salmonella* spp. was neither isolated from carcass and head meat samples in any of the abattoirs, nor from any of the samples collected in abattoirs B and C. Isolation of *E. coli* O157:H7 and non-O157 STEC decreased in carcass but not in environmental samples. Non-O157 STEC was isolated from viscera washing and chilling water, without detecting *E. coli* O157:H7.

The prevalence of pathogenic microorganisms on carcasses found by our group was similar to that reported world wide for *Salmonella* spp. (0.4–14.3 %) (Bersisa et al., 2019; Bosilevac et al., 2019) and O157:H7 (0.4–20.3 %) (Narvaez-Bravo et al., 2013; Loiko et al., 2016). However, non-O157 STEC prevalence was higher than reported elsewhere

Table 6
Sources of non-O157 STEC serotypes and genotypes isolated during stages I and III.

Abattoir	Stage	Carcass		Environment		Head meat		Viscera	
		Serotype	Genotype	Serotype	Genotype	Serotype	Genotype	Serotype	Genotype
A	I	O120:H19	*stx*$_1$/*stx*$_2$/*ehxA*/*saa*	O116:H49	*stx*$_2$/ *ehxA*/*saa*				
		O113:H21 (n = 3)	*stx*$_2$/*ehxA*/*saa*	O178:H19	*stx*$_2$				
		OND:HND	*stx*$_2$/*ehxA*/*saa*	O8:H19	*stx*$_2$/ *ehxA*				
		O91:H21 (n = 5)	*stx*$_2$/*ehxA*/*saa*	O174:H21	*stx*$_2$				
		O15:H27	*stx*$_2$	O145:NM	*stx*$_2$/*eae*/*ehxA*				
		O178:H19	*stx*$_2$/*ehxA*/*saa*	O91:H21	*stx*$_2$/ *ehxA*/*saa*				
		O130:H11(n = 2)	*stx*$_1$/*stx*$_2$/*ehxA*/*saa*						
		O174:H46	*stx*$_2$/ *ehxA*/*saa*						
		O174:H21(n = 3)	*stx*$_2$						
		O124:H19 (n = 3)	*stx*$_1$/*stx*$_2$/*ehxA*/*saa*						
		O178:H19	*stx*$_1$/*stx*$_2$/*ehxA*/*saa*						
		OND:NM	*stx*$_2$						
		O116:H49	*stx*$_2$/ *ehxA*/*saa*						
		O112:H2	*stx*$_2$/ *ehxA*/*saa*						
		O120:H7	*stx*$_2$						
	III	O163:H19	*stx*$_2$/ *ehxA*/*saa*	O163:H19	*stx*$_2$/ *ehxA*/*saa*			O163:H19	*stx*$_2$/ *ehxA*/*saa*
		OND:H7	*stx*$_2$	O168:H8	*stx*$_2$			O179:H8	*stx*$_2$/ *ehxA*/*saa*
		O113:H21	*stx*$_2$/ *ehxA*/*saa*	OND:H8	*stx*$_2$/ *ehxA*/*saa*				
		O171:NM	*stx*$_2$						
B	I	O113:H21	*stx*$_2$/ *ehxA*/*saa*					O178:H19	*stx*$_2$
		O116:H49	*stx*$_2$/ *ehxA*/*saa*						
		O120:H7	*stx*$_2$						
		O174:H21	*stx*$_2$						
	III	OND:H46	*stx*$_2$	OND:H7	*stx*$_2$			O174:H21	*stx*$_2$
		O171:NM	*stx*$_2$						
		O130:H11	*stx*$_2$/ *ehxA*/*saa*						
C	I	O130:H11	*stx*$_1$/*stx*$_2$/*ehxA*/*saa*	O145:NM	*stx*$_2$/*eae*/*ehxA*			O113:H21(n = 2)	*stx*$_2$/ *ehxA*/*saa*
		O116:H49	*stx*$_2$/ *ehxA*/*saa*	O8:H7 (n = 2)	*stx*$_2$/ *ehxA*				
		O174:H28	*stx*$_2$/ *ehxA*/*saa*	O130:H11	*stx*$_1$/*stx*$_2$/*ehxA*/*saa*				
		O8:H19	*stx*$_2$/ *ehxA*						
		O120:H7	*stx*$_2$						
	III	OND:H7	*stx*$_2$	O8:H19	*stx*$_2$/ *ehxA*	OND:NM	*stx*$_2$	O130:H11	*stx*$_1$/*stx*$_2$/*ehxA*/*saa*
		O171:NM (n = 2)	*stx*$_2$						

(0.8–8.9 %) (Barkocy-Gallagher et al., 2003; Varela-Hernandez et al., 2007). In the current work, *Salmonella* spp. and non-O157 STEC isolation reduced to 0.0 % and 13.8 %, respectively, after implementing improvement actions.

The isolation of pathogenic microorganisms in the abattoir environment and the associated risk of meat contamination from the environment have been reported by several authors (Aftab et al., 2012; Kore et al., 2017). In this study, the same *Salmonella* serovars and non-O157 STEC serotypes were detected in samples of carcasses, the environment and viscera washing and chilling water from the same abattoir. Future studies using subtyping techniques could validate the clonality of strains and confirm the evidence of cross contamination. However, the importance of pre-operational and operational SSOP implementation for risk reduction was confirmed when comparing stages I and III of this study.

The current research provides the first bacteriological information of viscera washing and chilling water and head meat in Argentinian provincial bovine abattoirs, suggesting they could be a possible source of contamination in the offal area, during transport and in butcher shops. The viscera are by-products sold at retail along with the rest of meat products, without any previous processing. Head meat is sometimes destined for minced meat, a matrix from which various authors have described, the isolation of *E. coli* O157:H7, non-O157 STEC, *Salmonella* spp., *Listeria monocytogenes* and *Staphylococcus aureus* in Argentina (Tanaro et al., 2010; Llorente et al., 2014; Leotta et al., 2016; Salinas Ibáñez et al., 2018; Barril et al., 2019).

In Argentina, *S.* Typhimurium is the most prevalent serovar in human beings, followed by *S.* Enteritidis and *S.* Newport, whereas cases of human disease by *S.* Montevideo are scarce (Caffer et al., 2010). In this work, the highest percentage of *Salmonella* isolates corresponded to serovars Anatum and Montevideo. However, *S.* Typhimurium was isolated from carcass and head meat samples and *S.* Newport was from viscera washing and chilling water. Although these serovars are associated with the ability to form biofilms on different surfaces (Xia et al., 2009), the use of sodium hypochlorite in appropriate times and concentrations and mechanical and abrasive cleaning allowed the elimination of biofilms (Wong et al., 2010; Rodrigues et al., 2011). Here, after the implementation of GMP and SSOP, the number of isolates was reduced from 26 in Stage I to only six in Stage III.

In Argentina, the most prevalent serogroups associated with severe disease correspond to *E. coli* O157:H7, and non-O157 STEC: O145 [H27; H−; NT]; O121 [H19]; O26 [H2;11; NT]; O174 [H8; 21; 28; H−] (Galli et al., 2016), all of them *eae*-positive. In Stage III of our study, the frequency of *E. coli* O157:H7 was 2.7 %, similar to that described 10 years ago in export abattoirs (2.6 %) (Masana et al., 2010). In the case of O157:H21, despite it has been described in Spain (Sanchez et al., 2010) and Japan (Nara, 2014), this is the first report of such strain in beef from Argentina. Non-O157 STEC isolation was still higher in provincial (13.8 %) than export (5.8–9.0 %) abattoirs (Masana et al., 2011; Brusa et al., 2017), even after implementing improvement actions. In the present study, non-O157 STEC isolates from carcass, head meat and viscera water samples did not belong to serogroups commonly associated with cases of human disease, and they were all *eae*-negative. However, two O145:NM (*eae* positive) strains were isolated from environmental samples, suggesting the possible cross contamination of carcasses from the environment.

The use of potable water in the food industry is required by the health authority of Argentina (SENASA, 1968); nevertheless, Cendón and Unger (2009) informed that this condition was not met by provincial abattoirs. We identified and rectified this problem after the installation of automated chlorinators, resulting in 75.0–95.0 % potable samples in Stage III. This improvement decreased risk estimation, considering that the use of non-potable water in the abattoir might contribute to carcass contamination (Bello et al., 2011). Other works in different countries (Sanna et al., 2016; Nienie et al., 2017; Kayembe et al., 2018) have also identified the presence of fecal coliforms as the main cause of well contamination. In our study, this situation was reverted with the correct chlorination of water before entrance to the slaughter plant and by registering the adequate daily dose of chlorine.

5. Conclusions

In Argentina, 16.0 % of the total slaughter is carried out in provincial abattoirs, which not always have an HACCP system or perform the microbiological verification of either products or the production environment. The present descriptive study based on the estimation of the risk of contamination in production environment, product, by-products and water in provincial abattoirs. The sanitary authority was absent prior to carrying out this collaborative work. A slight improvement in products and the production environment was seen after applying corrective actions, GMP and GHP in the production process. Considering that this was a pilot study, not all the proposed improvements were completed, and much work remains to be done in building conditions and processes to reduce the risk of contamination of meat with pathogens that could affect the health of consumers. The current research provides the first bacteriological information of viscera washing and chilling water and head meat in Argentinian provincial bovine abattoirs *i.e.*, *Salmonella* spp. and non-O157 STEC was isolated even after the implementation of improvement actions. This highlights the need to increase epidemiological surveillance on the human population consuming products from these abattoirs. One of the most negative aspects identified was the use of non-potable water in provincial abattoirs, which allowed to reverse this problem through the implementation of elementary strategies such as chlorination. We consider that all Argentine abattoirs should have the same sanitary requirements, *i.e.*, the same as in export abattoirs.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments

This study was carried out within the framework of a collaboration agreement between Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ministerio de Agroindustria de la Provincia de Buenos Aires, and División Higiene y Seguridad Alimentaria y Ambiental, Stamboulian Servicios de Salud. We thank A Di Maggio for correcting and editing the manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2020.104933.

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