Food Control 40 (2014) 320–323

Contents lists available at ScienceDirect

Food Control

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

Low occurrence of *Salmonella* in the beef processing chain from Minas Gerais state, Brazil: From bovine hides to end cuts

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ARTICLE INFO

Article history:
Received 26 August 2013
Received in revised form
10 December 2013
Accepted 17 December 2013

Keywords:
Salmonella
Bovine
Slaughtering
PFGE

ABSTRACT

The present study aimed to track possible contamination sources of *Salmonella* spp. during bovine slaughtering. Three slaughterhouses located in Minas Gerais state, Brazil were selected and 836 samples were obtained by surface swabbing of 209 bovine carcasses at four steps of slaughtering: I) after bleeding (from the hide), II) after skinning, III) after evisceration, and IV) after end washing (performed with cold water). Samples were subjected to *Salmonella* spp. detection according to ISO 6975, and the suspected isolates were identified by PCR as *Salmonella* by targeting the *ompC* gene and performing serotyping. Twenty isolates were confirmed as *Salmonella* and subjected to *Xba*I macrorestriction and pulsed-field gel electrophoresis (PFGE). *Salmonella* spp. was detected in the hides of six animals, during slaughtering after skinning (one carcass), after evisceration (two carcasses), and after end washing (three carcasses). Isolates were serotyped as *S.* Dublin ($n = 7$), *S.* Derby ($n = 8$), *S.* Infantis ($n = 1$), *S.* Give ($n = 1$), and *S. salamae* subsp. *salamae* ($n = 3$). PFGE demonstrated identical *Salmonella* pulse-types from hides and slaughtering steps of skinning and evisceration, as well as from animal hides obtained from distinct slaughterhouses. The obtained data indicate a low prevalence of *Salmonella* spp. during bovine slaughtering in selected industries from Minas Gerais state, Brazil, but identified possible routes of contamination of pathogenic serotypes.

1. Introduction

Salmonella spp. is an important foodborne pathogen associated with products of animal origin, such as beef, pork, and poultry (Abbassi-Ghozzi et al., 2012; Dallal et al., 2010; Stevens et al., 2008). This usual association with such products is a result of the autochthonous presence of *Salmonella* in the intestinal microbiota of farm animals, which facilitates the contamination of carcasses during slaughtering, mainly due to improper handling and processing (Abbassi-Ghozzi et al., 2012; Buncic & Sofos, 2012).

Several steps of animal slaughtering and meat processing are considered key points for *Salmonella* contamination, requiring constant monitoring and control. In the context of contamination control in food industries based on Hazard Analysis and Critical Control Points (HACCP), such key points include hide removal (skinning), evisceration, washing before freezing, handling of cuts and meat products, and cross-contamination at several steps between utensils, equipment, and food products (Buncic & Sofos, 2012; Gill, Bryant, & Landers, 2003; Pointon, Kiermeier, & Fegan, 2012).

Considering the potential hazard of *Salmonella* for humans, its contamination during beef processing must be monitored by reliable and proper methodologies, in order to allow the identification of the exact points of contamination by most pathogenic serotypes. With this purpose, associating conventional isolation procedures with molecular methodologies is undoubtedly required to obtain valuable results to properly track the specific origins of *Salmonella* spp. (Amini et al., 2010; Skyberg, Logue, & Nolan, 2006). Based on the relevance of *Salmonella* spp. in the beef processing chain, the present study aimed to identify the origins of contamination of this foodborne pathogen in distinct points of slaughtering of three slaughterhouses, and also in the processing environment in one of them, located in Minas Gerais state, Brazil, using conventional and molecular methodologies.

2. Materials and methods

2.1. Slaughterhouses and sampling

Three slaughterhouses located in Minas Gerais State, Brazil were included in the present study after agreement by the owners and the identification of regular bovine slaughtering in their facilities.

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0956-7135/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2013.12.018

All selected facilities were representative of slaughterhouses located in Minas Gerais state, Brazil, regarding slaughtering frequency, utensils and equipment employed, and all of them have Hazard Analysis and Critical Control Points (HACCP) programs, and their key characteristics are described in the following:

Slaughterhouse 01 (Sl01): daily slaughtering of 130–150 bovines, conducted by approximately 50 employees, and absence of physical division between dirty and clean areas. Sl03 is allowed to export a diversity of beef products, which are also distributed for sale in Brazilian market.

Slaughterhouse 02 (Sl02): daily slaughtering of 150–180 bovines, conducted by approximately 50 employees, and absence of physical division between dirty and clean areas. Sl02 exports only viscera, and beef cuts are distributed for sale in distinct Brazilian states.

Slaughterhouse 03 (Sl03): bovine slaughtering occurs two or three times a week, with 90–100 bovines being processed by approximately 25 employees, and absence of physical division between dirty and clean areas. Sl02 is not allowed to export any beef product, being its production destined exclusively to Brazilian market.

Each slaughterhouse was visited 10 times in a two-year period, with a total of 836 samples obtained by surface swabbing of 209 bovine carcasses (Sl01: 69, Sl02: 70; Sl03: 70) at four steps of slaughtering: I) after bleeding (from the hide), II) after skinning, III) after evisceration, and IV) after end washing (performed with cold water). Surface sampling was conducted by swabbing four sterile sponges (3M Microbiology, St. Paul, MN, USA), each previously moistened with 10 mL of buffered peptone (1% w/v) saline (0.85% w/v) solution (BPS, Oxoid Ltd., Basingstoke, England), in four 100 cm^2 delimited areas of the shoulder and chest (I and II, only the outer surface of the whole carcass; III and IV, the outer and inner surface of both half-carcasses). After sampling, swabs were placed in sterile bags and kept at 4 °C until microbiological analysis.

Surface samples of tables ($n = 39$), knives ($n = 13$), and employees' hands ($n = 37$) were also obtained in the beef processing environment of Sl01, using the same procedure described previously. These samples were obtained before the start of beef processing activities, as well as during beef processing and handling. Finally, beef cuts obtained at the end of beef processing in Sl01 were sampled by surface swabbing, as described previously: shoulder ($n = 32$), rump ($n = 32$), and tenderloin ($n = 30$).

2.2. Salmonella detection

Samples were subjected to *Salmonella* detection according to ISO 6975 (ISO, 2002), with some modifications. Under sterile conditions, each sample set (four sponges) was treated with 160 mL of BPS (Oxoid) and homogenised at 4 °C and 260 rpm (Stomacher 400 circulator, Seward, Worthing, England). Then, 40 mL of the obtained homogenates were centrifuged at 1000× *g* for 15 min, the supernatant was discarded, and the obtained pellet was re-suspended in 10 mL of buffered peptone water at 1% (w/v) (Oxoid); this was followed by incubation at 37 °C for 18 h. Then, the obtained cultures were transferred to Muller-Kauffmann tetrathionate/novobiocin broth and Rappaport-Vassiliadis medium with soya (both from Oxoid), which were incubated at 37 °C for 24 h and 42 °C for 24 h, respectively. The obtained cultures were streaked onto plates containing xylose lysine deoxycholate agar and mannitol lysine crystal violet brilliant green agar (both from Oxoid) and incubated at 37 °C for 24 h. *Salmonella* suspect colonies were transferred to triple sugar iron agar and lysine iron agar slants (both from Oxoid), and incubated at 37 °C for 24 h.

Cultures that presented typical or suspect reactions were subjected to serotyping at Fundação Osvaldo Cruz (Fiocruz, Rio de Janeiro, RJ, Brazil). To confirm the genus identification, cultures of the suspect colonies were subjected to DNA extraction using the Wizard Genomic DNA Purification kit (Promega Corp., Madison, WI, USA) and subjected to PCR reaction as described by Alvarez et al. (2004) in order to detect the *omp*C gene (typical for *Salmonella* spp.).

2.3. Salmonella fingerprinting

Isolates identified by serotyping and PCR as *Salmonella* spp. were subjected to DNA macrorestriction with *Xba*I and pulsed-field gel electrophoresis (PFGE) for fingerprinting, as indicated by the PulseNet (Centers for Disease Control and Prevention, Atlanta, GA, USA) and following the protocol described by Ribot et al. (2006). Briefly, plugs of the isolates were digested with 50 U of *Xba*I (Promega) at 37 °C for 2 h and the macrorestriction products were separated on agarose gels at 1% (w/v) by PFGE, using TBE 0.5× at 4 °C in CHEF-DR II (Bio-Rad Lab., Hercules, CA, USA) with the following parameters: initial switch of 2.2 s, final switch time of 63.8 s, angle of 120°, 6 V/cm, and run time of 19 h. The obtained gels were stained using GelRed (Biotium Inc., Hayward, CA, USA), and the obtained fingerprints were visualised and recorded for analysis.

2.4. Data analysis

The frequencies of positive results for *Salmonella* sp. of bovine carcasses during the slaughtering process in the three slaughterhouses were obtained and compared by the Chi-square test ($p < 0.05$), using the software XLStat 2010.2.03 (AddinSoft, New York, NY, USA). The fingerprints obtained by PFGE were analysed using BioNumerics 6.6 (Applied Maths, Gand, Belgium) with the following parameters: optimisation of 1%; Dice similarity of bands of 5%. Clustering of the fingerprints was obtained by the Unweighted Pair Group Method using Averages (UPGMA).

3. Results and discussion

Salmonella spp. were detected in the hides of six animals, and during slaughtering after skinning (one carcass), after evisceration (two carcasses), and after end washing (three carcasses). The distribution of positive results by sampled step of slaughtering and slaughterhouse is presented in Table 1. It can be seen that only in Sl01 were significant differences between the frequencies of positive results observed when comparing hide contamination with the slaughtering steps ($p < 0.05$); however, the low frequencies of positive results jeopardised a proper comparison between the studied steps. However, comparing the frequencies of contamination in Sl02 and Sl03, and also all compiled data, no significant differences were observed between the assessed slaughtering steps ($p > 0.05$).

Table 1
Frequencies of positive results for *Salmonella* spp. obtained from 209 bovine carcasses at four steps of slaughtering (I: from hide; II: after skinning; III: after evisceration; IV: after end washing) from three slaughterhouses (Sl01, Sl02, Sl03) located in Minas Gerais state, Brazil.

Step	Slaughter house			All
	Sl01	Sl02	Sl03	
I	4/69	1/70	1/70	6/209
II	0/69	1/70	0/70	1/209
III	0/69	2/70	0/70	2/209
IV	0/69	0/70	3/70	3/209
Chi-square	$\chi^2 = 12.176$, $p = 0.007$	$\chi^2 = 2.029$, $p = 0.566$	$\chi^2 = 6.087$, $p = 0.107$	$\chi^2 = 4.735$, $p = 0.192$

The low occurrence of *Salmonella* spp. in bovine carcasses during slaughtering has already been observed in similar studies conducted in other countries (Li, Sherwood, & Logue, 2004; Madden, Espie, Moran, McBride, & Scates, 2001; Rhoades, Duffy, & Koutsoumanis, 2009). Sofos, Kochevar, Reagan, and Smith (1999) and Ruby, Zhu, and Ingham (2007) highlighted the relevance of bovine hide as a reservoir of *Salmonella* spp. for slaughterhouses, as observed in the present study for Sl01 (Table 1). Also, the contamination of bovine carcasses after the end of the slaughtering process (after the end washing) could be a relevant source of the initial contamination of *Salmonella* spp. in the beef processing environment of slaughterhouses (Barkocy-Gallagher et al., 2003; Rivera-Betancourt et al., 2004; Ruby et al., 2007), as observed in Sl03 in the present study (Table 1). However, due to the absence of beef processing in Sl03, it was not possible to verify the impact of *Salmonella* spp. contamination of the carcasses after final washing of the end beef products.

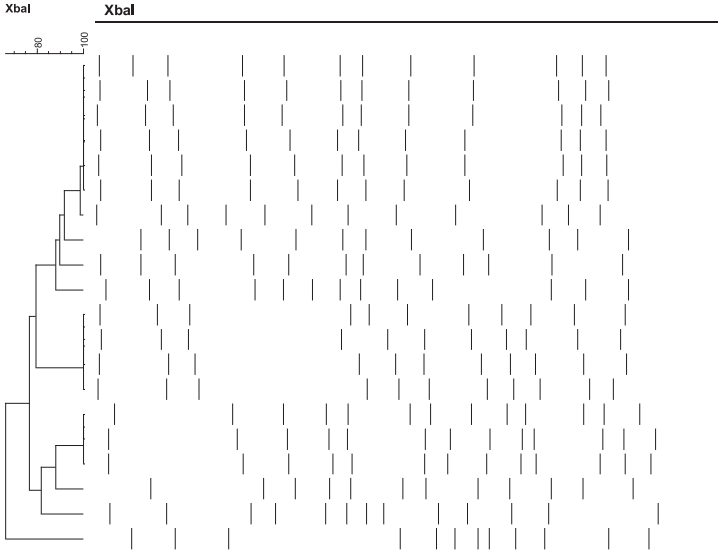
None of the samples tested from the beef processing environment of Sl01 presented a positive result for *Salmonella* spp., and neither did the tested beef cuts. Also, none of the bovine carcasses from Sl01 were positive for *Salmonella* spp. after the final washing, the last step of the slaughtering process (Table 1). These results suggest the poor relevance of bovine carcasses as possible sources of contamination by *Salmonella* spp. in the beef processing environment, an unexpected result considering the data obtained in previous studies (Bosilevac, Guerini, Brichta-Harhay, Arthur, & Koohmaraie, 2007; Ghafir et al., 2005; Wong, Nicol, Cook, & MacDiarmid, 2007). In addition, these results suggest that the hygienic procedures conducted during bovine slaughtering were enough to avoid the contamination by *Salmonella* spp. in the processing environment.

Twenty isolates obtained from positive samples were identified as *Salmonella* spp. by serotyping and PCR (targeting *ompC* gene). These isolates were named S01 to S20; Fig. 1 shows their *Xba*I macrorestriction profiles, as well as their genetic relationship obtained by clustering, their origins (slaughtering step, bovine, and slaughterhouse), and their identified serotypes. The isolates were grouped into 10 pulse-types, presenting genetic similarity varying from 65 to 100%. It could be seen that isolates that presented identical genetic profiles were present in distinct slaughtering steps from Sl02, indicating a route of contamination by this specific *Salmonella* strain in bovine hides (S03 and S04), after skinning (S07), and after evisceration (S09) (Fig. 1). In addition, this specific *Salmonella* strain was detected in carcasses from distinct animals (Fig. 1), highlighting the relevance of cross-contamination of this foodborne pathogen during slaughtering (Stevens et al., 2008). Despite not including beef processing procedures, the presence of positive results for *Salmonella* spp. in the final carcasses from Sl03 must be considered a key contamination point for end-products, as already demonstrated by PFGE in previous studies (Stevens et al., 2008; Yang et al., 2013, 2010).

The genetic profiles of the isolates also allowed the identification of common sources of contamination for distinct slaughterhouses. S03 and S04 were isolated from Sl01, and S05, S06, and S07 were isolated from Sl03; all these isolates presented identical *Xba*I macroresctriction patterns (Fig. 1). The identification of common sources of contamination by *Salmonella* in the beef processing chain, as identified in the present study, highlights the necessity of proper control from the initial steps of production onwards (Stevens et al., 2008).

The isolates were serotyped as *S.* Dublin ($n = 7$), *S.* Derby ($n = 8$), *S.* Infantis ($n = 1$), *S.* Give ($n = 1$), and *S. salamae* subsp. *salamae* ($n = 3$) (Fig. 1). *S.* Dublin is commonly associated with bovines, but rarely associated with salmonellosis cases and outbreaks, despite being known for its high potential for intestinal mucosa invasion (Davis et al., 2007; Litrup et al., 2010). The presence of this serotype in hides ($n = 4$) indicates faecal contamination on the animals, and transfer to bovine carcasses after skinning ($n = 1$) (Fig. 1). In addition, one *S.* Dublin isolate was detected in a bovine carcass after evisceration, indicating failures in this step during slaughtering (Fig. 1).

S. Derby is usually associated with swine slaughtering and pork processing, and the few infections caused by this serotype are caused by the ingestion of contaminated foods (Litrup et al., 2010; Michael, Cardoso, Rabsch, & Schwarz, 2006). The occurrence of this serotype in bovine carcasses may also have been a result of inadequate cleaning procedures in Sl02 and Sl03, as these facilities also conduct swine slaughtering, usually in the period before bovine slaughtering.



Key	Step	Animal	Slaughterhouse	Serotype
S03	I	3	Sl01	Salmonella Dublin
S04	I	3	Sl01	Salmonella Dublin
S05	I	4	Sl03	Salmonella Dublin
S06	I	4	Sl03	Salmonella Dublin
S07	II	5	Sl03	Salmonella Dublin
S09	III	5	Sl03	Salmonella Dublin
S11	III	4	Sl03	Salmonella Derby
S10	III	5	Sl03	Salmonella Derby
S08	III	5	Sl03	Salmonella Dublin
S01	I	1	Sl01	Salmonella Infantis
S13	IV	7	Sl02	Salmonella Derby
S14	IV	7	Sl02	Salmonella Derby
S15	IV	7	Sl02	Salmonella Derby
S16	IV	7	Sl02	Salmonella Derby
S18	I	9	Sl02	Salmonella enterica subp. salamae.
S19	I	9	Sl02	Salmonella enterica subp. salamae
S20	I	9	Sl02	Salmonella enterica subp. salamae
S02	I	2	Sl01	Salmonella Give
S17	IV	8	Sl02	Salmonella Derby
S12	IV	6	Sl02	Salmonella Derby

Fig. 1. Schematic representation of the obtained PFGE pulse types after DNA macrorestriction (*Xba*I) of 20 *Salmonella* spp. isolates obtained at four steps of slaughtering (I: from hide; II: after skinning; III: after evisceration; IV: after end washing) from three slaughterhouses (Sl01, Sl02, Sl03) located in Minas Gerais state, Brazil. Also, identification of their serotypes. Similarities between the identified PFGE pulsetypes were estimated using the Dice coefficient (5% tolerance).

S. Infantis, *S.* Give and *Salmonella enterica* subsp. *salamae* were also identified, but at low frequencies. S. Infantis is often isolated from animals and their foods, and is considered a relevant pathogenic agent for humans that is usually associated with foodborne diseases (Rivoal et al., 2009; Shahada et al., 2006). Despite *S.* Give usually being associated with bovine and swine, this serotype is not a common cause of salmonellosis cases and outbreaks (Girardin, Mezger, Hachler, & Bovier, 2006).

Despite the low frequencies of positive results observed for *Salmonella* spp. during bovine slaughtering and beef processing, the obtained data allowed the identification of cross-contamination of this foodborne pathogen between key steps of the slaughtering process from the selected slaughterhouses in Minas Gerais state, Brazil. These data also demonstrate the presence of pathogenic serotypes at such points, highlighting the significance of the proper control of contamination during bovine slaughtering.

Acknowledgements

The authors are thankful to CNPq, CAPES, and FAPEMIG.

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