Meat Science 86 (2010) 418–421

Contents lists available at ScienceDirect

Meat Science

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

Occurrence of Shiga toxin-producing *E. coli* (STEC) on carcasses and retail beef cuts in the marketing chain of beef in Argentina

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ARTICLE INFO

Article history:
Received 27 November 2009
Received in revised form 16 March 2010
Accepted 14 May 2010

Keywords:
Shiga toxin
STEC
Meat
Carcasses
Contamination

ABSTRACT

Argentina has the highest incidence of HUS in the world. HUS is produced by STEC O157 and non-O157. Cattle's faeces and hides are sources of STEC contamination of carcasses during slaughter. We investigated the presence of STEC in carcasses and cuts of meat in the marketing chain in an agricultural city located in Buenos Aires Province (Argentina). In this study, the detection of the *stx* gene was used as an indicator of carriage of meat with STEC. In carcasses, we detected 12.34% and 18.64% of STEC at the slaughter and sanitary control cabin (place where carcasses arrive from slaughters located outside the city), respectively. These percentages increased at butcheries (24.52%). The 25% of retail beef cuts were STEC-positive with significant differences among the different cuts of meat (chuck: 12.12%, rump roast: 12.12% and minced beef: 40.74%). The *stx2* gene was the predominant gene detected in all samples at different levels of the commercialization meat chain.

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1. Introduction

Many Shiga toxin-producing *E. coli* (STEC) cause foodborne diseases ranging from bloody diarrhoea to haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). HUS is a syndrome characterized for renal failure, haemolytic anemia and thrombocytopenia that affect children less than 5 years old (Voyer, 1996). This damage is produced by the action of cytotoxins: Stx1 and Stx2, although other factors are also involved in pathogenicity such as intimin, by which STEC adheres to intestinal cells, and a haemolysin. Stx1 and Stx2 are encoded by *stx1* and *stx2* genes, respectively. Intimin, encoded by the *eae* gene, has several subtypes associated with distinct STEC serotypes, like *eae*-γ1 expressed by *E. coli* O157:H7 (Adu-Bovie et al., 1998).

The strains carrying *stx2* are potentially more virulent than those carrying *stx1* or even strains carrying both *stx1* and *stx2* (Thorpe, Ritchie & Acheson, 2002). Epidemiological studies indicate that Stx2-producing strains are more frequently related to HUS than those producing Stx1 (Paton & Paton, 2002). Actually, detection of the *stx* gene in samples without performing the corresponding strain isolation is incomplete and is regarded as a presumptive diagnosis, but it is valid for the identification of carriage status (Fernández, Rodríguez, Arroyo, Padola & Parma, 2009; Scheutz, Beutin & Smith, 2001).

Argentina has the highest rates of HUS (400 new cases per year) and the incidence is 13.9/100000 children under 5-years old (Ministry of Health, 2006). In this country, non-O157 STEC serotypes have been isolated from human cases although O157:H7 is yet the prevalent STEC reported serotype (Rivas et al., 2006a). The easy transmission and its very low infectious dose i. e. <10 cells, remark the significance of this bacterium as a foodborne pathogen (Willlshaw et al., 1994). Investigations have demonstrated that human disease outbreaks are often linked to bovine food sources (Armstrong, Hollingswoth & Morris, 1996; Parma et al., 2000; Rivas et al., 2006) and that cattle are the main reservoir of these bacteria (Chinen et al., 2003; Meichtri et al., 2004; Mercado et al., 2004; Padola et al., 2004; Parma et al., 2000; Sanz, Viñas & Parma, 1998). In previous studies, we have detected prevalence ranged from 22 to 67% in different categories of cattle, and 44% in cattle pre-slaughter (Fernández et al., 2009; Padola et al., 2004; Parma et al., 2000; Sanz et al., 1998).

Cattle's faeces and hides are considered to be sources of STEC contamination of carcasses during slaughter and it can occur during

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doi:10.1016/j.meatsci.2010.05.027

removal of the hide or the gastrointestinal tract (Aslam et al., 2003, 2000; Elder et al., 2000; McEvoy et al., 2003). In whole carcasses, the prevalence rates of STEC O157 and non-O157 ranged from 0.01 to 43.4%, and 1.7 to 58.0%, respectively (Hussein, 2007). Slaughter presents numerous opportunities for contamination and cross-contamination that may vary among plants. As expressed by The Committee on the Review of the USDA *E. coli* O157:H7, Farm-to-Table Process Risk Assessment (2002), the variability in contamination and cross-contamination may be originated in factors such as plant size, design, age, equipment, automation, speed of slaughter, and animal holding facilities; geographic location; season of the year; type, lot, and origin of animals; labor shift; and personnel training and turnover. As the hide is separated for removal, contamination may be introduced onto the carcass surface. A single source (one animal or the plant environment and equipment) may contaminate carcasses not only during dehiding but also during later steps. Some operations such as skinning and evisceration are more likely than others to result in carcass contamination, and some carcass areas are more prone than others to exposure to potential contamination or cross-contamination. Contamination of meat with STEC during slaughter is the principal route by which these pathogens enter at the meat supply chain (Meng & Doyle, 1998). Among the numerous retail meat cuts, ground beef posses more risk than intact muscle because it can be contaminated during the grinding operation. The increased risk is associated with the protection of *E. coli* from heat during the cooking process, since the organism may be protected inside the body of reformed meat (Barlow, Gobius & Desmarchelier, 2006). In the conversion of beef carcasses to ground beef and retail cuts, microbial contamination is currently unavoidable (Barlow, Gobius & Desmarchelier, 2006).

Although there is now considerable information on the prevalence of STEC in animals, at slaughters and in ground beef (Acheson, Lincicombe, De Breucker & Keusch, 1996; Chinen et al., 2003; Hussein, 2007; Johnson et al., 1996; Padola et al., 2004; Parma et al., 2000), there is no available information about the prevalence of STEC in retail beef cuts neither in the different levels of marketing chain.

The aim of this study was to investigate the presence of STEC in samples of carcasses and cuts of meat in the marketing chain of these foods in a city from Argentina.

2. Materials and methods

2.1. Sampling plan

Samplings were conducted from July to December of 2007 and were designed based on the beef supply network in the Buenos Aires province and the consumption habits of the population (retail beef cuts bought by people).

Samples were taken from carcasses at different segments of the marketing chain: 2 slaughters located in the city, sanitary control cabin (office where carcasses arrived from 5 slaughters located outside the city and from there they were delivered to butcheries), and 7 butcheries. In addition, in butcheries were taken samples of retail beef cuts: chucks, rump roast and minced beef.

2.2. Sampling of carcasses, minced meat and cut of meat

The samples of carcasses, chucks and rump roast were taken by swabs in concordance with circular No 3496/02 and Anexo II of Circular 3579 of SENASA and international rules. In each carcass we took samples of forequarters (F) and hindquarters (H). Briefly, a surface of 100 cm^2 of each carcass at chuck level (hindquarter) and rump roast (forequarter) was sampled employing first swabs embedded in a sterile solution of NaCl 0.85% and peptone 0.1% and then dry swabs (SENASA, Circular No 3496/02 and Anexo II of Circular 3579). The swabs were carried to laboratory in a sterile solution of NaCl 0.85% added with peptone 0.1% and processed within 24 h. For minced meat samples, 100 g of the product was taken. At butcheries, samples from chucks and rump roast were taken swabbing all external surfaces.

Samples were taken of 81 carcasses at slaughters (81 F and 81 H), 59 carcasses at sanitary control cabin (59 F and 59 H), 52 carcasses at butcheries (52 F and 52 H), 33 chucks, 33 rump roast and 54 minced beef aliquots from butcheries.

2.3. Determination of Shiga toxin-producing E. coli

In this study, the detection of the *stx* gene was used as an indicator of carriage of meat with STEC (O157 and non-O157).

The swabs in a sterile solution of NaCl 0.85% with 0.1% peptone were incubated overnight at 37 °C and then they were grown onto EMB plates at 37 °C 24 h. An aliquot of confluent growth was inoculated into 30 ml of Luria Bertani broth, incubated with shaking at 37 °C for 4 h and processed for DNA extraction as described previously (Padola et al., 2004). Shiga toxin genes (*stx1* and *stx2*) were detected by multiplex PCR. Primer sequences and experimental conditions for *stx1* and *stx2* amplifications were indicated by Paton and Paton (2002). Each positive sample for either *stx1* or *stx2*, or both, was tested for the presence of the *eae*-γ1 gene (Gannon, Rashed, King & Golsteyn Thomas, 1993; Oswald et al., 2000).

One milliliter from each culture was frozen at −70 °C with the addition of 20% glycerol for further isolation of individual *stx*-positive colonies.

2.4. Statistical analysis

The association between STEC-positive carcasses and their origin (slaughters, sanitary control cabin and butcheries), as also between STEC-positive retail beef cuts and sort of retail beef cuts (chucks, rump roast and minced beef) were analyzed by using Chi square test. When one of expected frequencies was below five, Fisher's Exact test was used (confidence 95%). These analyses were made by using the procedure PROC FREQ del Statistical Analysis Systems, Version 9.1.3 (SAS Institute Inc., Cary, North Carolina, USA, 2004).

3. Results

The 12.34% and 18.64% of carcasses were STEC-positive at slaughters and sanitary control cabin, respectively. These percentages increased in carcasses at butcheries (25.00%) (Table 1).

The STEC-positive samples were significantly greater in hindquarters (H 14.57%) than in forequarters (F 5.10%) ($p = 0.0021$). This rate was constant although the differences were not statistically significant for carcasses among the slaughters (H 10.84%; F 3.61%), sanitary control cabin (H 14.75%; F 3.39%) and butcheries (H 20.00%; F 9.26%) ($p = 0.3268$ for H and $p = 0.3162$ for F).

Table 1
STEC in carcasses and retail beef cuts and *stx1*, *stx2*, and *stx1*/*stx2* genes at different segments of the beef marketing chain.

	Samples		Genes		
	STEC-positive	Total	*stx1*	*stx2*	*stx1*/*stx2*
Carcasses					
Slaughter	10	81	2	5	3
Sanitary Cabin Control	11	59	0	7	4
Butcheries	13	52	1	11	1
Retail beef cuts					
Rump beef	4	33	0	3	1
Chuck	4	33	1	3	0
Minced beef	22	54	1	15	6
Total	64	312	5	44	15

The 25% of retail beef cuts were STEC-positive with significant differences among rump roast, chuck and minced meat samples ($p = 0.00469$) (Table 1). In rump roast and chuck it was found with a prevalence of 12.12% each while in minced beef it was found with a prevalence of 40.74% of STEC. It was observed with an increase of STEC-positive samples in the big butcheries (61.76%) and regarding the small ones with (12%).

Among the STEC-positive carcasses at slaughters and sanitary control cabin we detected samples positive only for *stx2* (57.14%), only for *stx1* (9.52%) and for *stx1*/*stx*2 (33.33%). In butcheries, the percentages of positive carcasses for *stx* genes were *stx2* 84.61%, *stx1*/*stx2* 7.69% and *stx1* 7.69%. The same trend was observed in retail beef cuts (chucks, rump roast and minced beef), being 70% for *stx2*, 33.33% for *stx*1/*stx*2 and 9.52% for *stx*1. The *eae*-γ1 gene was not detected in either of the samples.

4. Discussion

The prevalence of STEC at slaughter found in this work is quite different from previous studies reporting mainly *E. coli* O157 prevalence. Chapman, Siddons, Malo and Harkin (1997) in the United Kingdom; Omisakin, MacRae, Ogden and Strchan (2003) in the United Kingdom and Paiba et al. (2002) in Great Britain detected only *E. coli* O157:H7 at slaughter and suggested that less than 10% of cattle are shedders of that particular serotype. In an Irish beef abattoir McEvoy et al. (2003) reported an occurrence of 3.2 % of *E. coli* O157:H7 on carcasses, and a similar occurrence of this pathogen was found by Carney et al. (2006) in a beef slaughter plant in Ireland. In contrast, a survey of *E. coli* O157:H7 in cattle at slaughters in the USA found the prevalence to be 28% overall (Elder et al., 2000) while Terrance, Barkocy-Gallagher, Rivera-Betancourt and Koohmaraie (2002) detected 8.3% of non-O157 STEC from 326 beef carcasses. It is important to consider that the sampling method and methodology used for the processing of samples may influence the variability of results (Johnson et al., 1996).

The presence of *E. coli* O157:H7 is the result of cross-contamination during the slaughter process and/or lairaging–stunning phase (Nastasijevic, Mitrovic & Buncic, 2009). It has been previously reported that this contamination correlates with the presence of STEC in hides and faeces (Elder et al., 2000). The head, as the lowest part of the animal during the early stages of dressing, is at particular risk of contamination regarding other parts of the carcass by splash, draining of surface water and contact with operatives and surfaces (Gill, McGinnis & Jones, 1999). These data do not agree with our findings since we detected STEC in the hindquarters in higher proportion than forequarters.

In this study we found high prevalence of STEC on retail beef cuts at butcheries comparing with data reported previously (Chinen et al., 2003). In beef samples at retail level, prevalence of *E. coli* O157:H7 usually range from 0% to 9% (Brooks et al., 2001; Doyle & Schoeni, 1987; Suthienkul et al., 1990; Tutenel et al., 2003; Vuddhakul et al., 2000) although an exceptionally high prevalence of 36% was also published (Radu et al., 1998). In minced beef at meat processing level an occurrence of 0.12% was reported by Vernozy-Rozand et al. (2002).

Stx2-producing strains are often more related to HUS than Stx1-producing strains (Paton & Paton, 2002). In our study, we found that *stx2* was the predominant gene over *stx1* and the combination of *stx1*/*stx2* in all samples at different levels of the commercialization meat chain in concordance with previous studies of our laboratory (Blanco et al., 2004; Fernández et al., 2009; Padola et al., 2004) and other groups from Argentina (Chinen et al., 2003). The *stx2* positive samples were 5/10 at slaughter, 7/11 at sanitary control cabin, and 32/43 at butcheries. These results agree with the data published in most of the studies performed in the USA, Japan and European countries where it was found that the *stx2* gene was more frequent than *stx1* (Barkocy-Gallagher et al., 2001; Bonardi et al., 1999; Chapman, Cerdan Malo, Ellin, Ashton & Harkin, 2001; Johnsen, Yngvild, Heir, Berget & Herikstad, 2001; Keen & Elder, 2002; Sekiya, 1997; Zhao, Doyle, Shere & Garber, 1995).

In previous studies we found that STEC non-O157 strains are prevalent and frequently involved in cases of HUS in Argentina (Rivas et al., 2006) and other countries. For this reason, in this study we looked for the presence of *stx* as an indicator of carriage of meat with STEC (O157 and non-O157) by using methods that do not exert selection pressure towards any particular serotype. Actually, the identifications of carriage by the detection of *stx* genes in samples without the isolation of strain is accepted worldwide (Fernández et al., 2009; Johnson et al., 1996; Padola et al., 2004).

Interestingly, in January 2002, the American Society for Microbiology's Public and Scientific Affairs Board Committee on Agriculture and Food Microbiology submitted a recommendation to FSIS to include also the testing for STEC non-O157 (Park, Kim & Hixon, 2002).

5. Conclusion

These results show an increase in the number of STEC when the meat is transported from the slaughters until the sale point. This situation represents an increased risk for the consumers and a challenge for those working in the beef sanitary control service.

Acknowledgements

Authors thank María R. Ortiz for her technical assistance. This work was included in the Project of Technological Transference: Public Universities and Municipalities of Buenos Aires Province and was supported by CIC-PBA and SECYT-UNCPBA. A. Kruger is a member of the Research Career of CONICET. A. Taraborelli is a holder of a fellowship from CIC-PBA. A.E. Parma, N.L. Padola and A.I. Etcheverría are members of the Scientific Research Commission Prov. Buenos Aires (CIC).

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