Research Paper

# Prevalence, Risk Factors and Molecular Characteristics of Shiga toxin-producing *Escherichia coli* in Beef Abattoirs in Gauteng, South Africa

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## HIGHLIGHTS

- STEC strains were detected in apparently healthy slaughtered cattle in SA.
- Hides of cattle were important entry point of STEC into the abattoir and food chain.
- No correlation between detection of indicator bacteria and STEC strains.
- Non-O157 STEC strains were more prevalent than O157 STEC strains.

**ABSTRACT**

A cross-sectional study was conducted to determine the prevalence, risk factors, and molecular characteristics of Shiga toxin-producing *Escherichia coli* (STEC) in 12 beef abattoirs in Gauteng province, South Africa. The relationship between STEC contamination and aerobic bacterial counts on carcasses at various stages of processing was investigated. Multiplex PCR was used to detect virulence genes in broth- enriched samples, to determine O-serogroups in all samples positive for Shiga toxin-encoding genes (*stx*), and to characterize isolates of STEC. The overall prevalence of STEC determined by PCR in 419 selective enrichment broth samples was 35.1% (147/419), and was significantly higher (P = 0.037) in perineum hide swabs (50%) than in 24 h chilled carcass swabs (20%). The maximum total aerobic plate count (TAPC) was 3.8 $\log_{10}$ CFU/100 $cm^2$ for carcass swabs, but was below the South African microbiological standard for meat export at all stages of carcass processing. There was no significant association between TAPC and STEC contamination. Serogroup O113 was the most prevalent serogroup (13.6%; 20/147) detected. Only 33 isolates, all non-O157 STEC, were recovered, amongst which six different genotype combinations were observed. Additionally, the clinically important serogroups O117, O8, and O2 were isolated. Multivariable logistic regression revealed that the odds of STEC contamination was lower in post-wash (OR = 0.42; 95% CI: 0.18-0.98; P = 0.045) and 24 h chilled (OR = 0.33; 95% CI: 0.12-0.91; P = 0.033) carcass swabs compared to pre- and post-evisceration swabs. It was concluded that non-O157 STEC serogroups more frequently colonize beef cattle slaughtered at abattoirs in our study area than O157 STEC, and therefore have a higher potential to enter the food chain during carcass processing, with food safety implications.

**Key words:** STEC strains, non-O157 STEC, cattle carcasses, *stx*, mPCR, abattoir.

## 1. INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) is a zoonotic, food-borne, enteric pathogen that is gaining public health relevance worldwide (Karmali et al., 2010). Clinical complications of STEC infection include haemorrhagic colitis (HC), which occasionally progresses into haemolytic uraemic syndrome (HUS), and thrombotic thrombocytopenic purpura. HUS is a life-threatening disease characterized by haemolytic anaemia, thrombocytopenia and severe renal failure (Banatvala et al., 2001; Fairbrother and Nadeau, 2006). Globally, it was estimated that STEC strains cause 2,801,000 acute illnesses, 3890 cases of HUS, 270 cases of permanent end-stage kidney disease and 230 deaths annually (Majowicz et al., 2014). Approximately 470 serotypes of STEC are known, most of which are not pathogenic (Mora et al*.,* 2011). Of the pathogenic serotypes, STEC O157:H7 has gained notoriety in major outbreaks and sporadic cases worldwide (FAO/WHO 2018). Non-O157 STEC have also gained public health attention in recent years, with reported outbreaks being associated with HUS (FSIS, 2012, Wang et al., 2013). The STEC serogroups frequently linked with severe human diseases are O157, O26, O103, O145 and O111 in Europe, and O157, O26, O45, O103, O111, O121 and O145 in the USA (FSIS, 2012), all of which are carried within the gastrointestinal tract of ruminants including cattle (FSIS, 2012).

The Shiga-toxin genes are classified into two major types, *stx*$_1$ and *stx*$_2$ (Karve and Weiss, 2014), and the production of Shiga-toxin (stx) alone, which is the definitive virulence factor of STEC, appears to be insufficient to cause severe human disease (Friedrich et al*.,* 2002; Persson et al*.,* 2007). A number of other potential virulence factors such as the intimin protein encoded by the *eaeA* gene, known to facilitate adherence of STEC pathogens to intestinal epithelial cells, and the *hylA* gene encoding enterohaemolysin that potentiates the effect of Shiga toxin, have been reported (Croxen et al., 2014).

Epidemiological investigations have revealed that ruminants, particularly cattle, are major reservoirs of *E. coli* O157:H7 and non-O157 STEC which may be shed intermittently in faeces (Bosilevac & Koohmaraie, 2011; Hancock et al., 1998). Shiga toxin-producing *E. coli* are usually linked with raw beef, and could potentially be deposited on the surface of beef carcasses during the slaughtering process as a result of cross-contamination from the bovine hide or gut contents (Koohmaraie *et al.*, 2005). Cattle hide is considered the primary source of carcass contamination in the abattoir (Koohmaraie *et al.*, 2005), and many intervention strategies ranging from antibiotic dipping to hot water wash, have been employed during slaughtering and dressing processes, to reduce carcass pathogen prevalence (Koohmaraie *et al.*, 2005). The presence and concentration of indicator organisms, quantified by total aerobic plate count (TAPC), have also been used to monitor the cleanliness and evaluate the efficacy of intervention procedures in the abattoirs (Feng et al., 2013; Stanford et al., 2013). Several studies from the U.S.A. have shown a direct relationship between indicator bacteria and STEC contamination at various stages of processing at the abattoir (Arthur et al., 2004; Bosilevac et al., 2005), whereas others have found no relationship (Kain et al., 2001; Stanford et al., 2013). Thus, the relationship between indicator bacteria and carcass STEC contamination is somewhat unclear.

In South Africa and other southern African countries, a diverse range of STEC serogroups (O4, O5, O21, O26, O84, O111, O113, O117 and O157) have been incriminated in numerous clinical cases of diarrhoea in children and adults between 2006-2013 (Karama *et al.*, 2019; Smith *et al.,* 2011). However, the dearth of microbiological surveillance data on this pathogen in South Africa makes it difficult to assess the risk posed to animal and human populations by STEC. A few studies have demonstrated the presence of STEC in cattle faeces and beef in South Africa (Ateba et al., 2008; Ateba & Mbewe, 2011; Mainga et al., 2018), but

there are no published reports on the prevalence of STEC and concentration of indicator bacteria sampled sequentially during the slaughtering and dressing processes at the abattoir. Since the abattoir represents an important potential entry point of STEC into the beef food chain, this study aimed to determine the prevalence of STEC contamination of apparently healthy cattle just before slaughter, sequentially during slaughter and dressing of carcasses and in the abattoir environment in Gauteng, South Africa. It also aimed to identify risk factors for carcass contamination, assess the relationship between STEC contamination and counts of aerobic bacteria at various stages of processing, and characterize the STEC isolates based on their serotypes and possession of virulence genes.

## 2. MATERIALS AND METHODS

### 2.1. Ethical approval

Ethical approval for the study was obtained from the University of Pretoria Animal Ethics Committee (S4285-15, V019-15, V019-16). Permission for sampling in abattoirs was obtained from the Department of Veterinary Public Health of the Gauteng Department of Agriculture and Rural Development (GDARD).

### 2.2. Study area and design

Gauteng is the smallest of South Africa's nine provinces, but is also the most populous, with approximately 14.7 million people (25.4%) (Statistics South Africa, 2013). A cross-sectional survey of abattoirs across Gauteng was conducted during summer (November 2015 to February 2016) and winter (June-July 2016). A list of all abattoirs in the province was obtained from the Department of Veterinary Public Health of GDARD, classified by service centre (Gauteng east, Gauteng west and Gauteng north), throughput (high throughput (HT) slaughtering

>20 units per day and low throughput (LT) slaughtering ≤20 units per day) and species slaughtered. Only active abattoirs that slaughtered cattle were selected, giving a sampling frame of 36 abattoirs (22 HT and 14 LT). Twelve abattoirs were randomly selected from the three service centres, proportional to size, resulting in selection of 7 HT and 5 LT abattoirs. The cattle weights ranged from 250 - 450 kg. All abattoirs visited were either fully or partially mechanized with standard infrastructure and skilled manpower, such as meat inspectors and veterinary consultants.

A structured questionnaire with respect to abattoir management practices was also administered and information obtained was used to identify factors associated with STEC prevalence.

### 2.2.1. Sample size calculation

The sample size for simple random sampling was calculated using the formula (Thrusfield, 2013):

$$n = \frac{1.96^2 P_{exp}(1 - P_{exp})}{d^2}$$

where $P_{exp}$ is the expected prevalence (50%), and d is the desired absolute precision (5%). Using a $P_{exp}$ of 0.5 and $d^2$ of $0.05^2$, the required minimum sample size was 385; therefore, a sample size of approximately 400 faecal and carcass samples was used.

### 2.2.2. Sample collection and processing

Individual animals and carcasses were selected by systemic random sampling, tagged, tracked in simple or continuous slaughter lines and samples obtained at different locations in the processing plants. Faecal samples were collected via rectal grab with a rectal glove and transferred into a 100 ml sterile specimen container. Seven carcasses were sampled at each

selected abattoir: four during summer and three during winter. The sampling was conducted mostly during the morning hours, depending on the schedule of operations of the abattoir. Carcass sampling was carried out according to the European Union Decision 2001/471/EC (https://op.europa.eu/en/publication-detail/-/publication/99e23ed5-0cac-44f0-adc5-a511f22c83f9/language-en). Composite swab samples were obtained from a 100 $cm^2$ area using a sterile square metal template from each of four selected anatomical sites (4 x 100 $cm^2$ areas): rump, flank, brisket and neck. At each site, 10 vertical and 10 horizontal streaks were made by applying gentle pressure using a swab rinse kit (SRK) containing 10 ml rinse solution (Copan Diagnostics, Inc., UK). One carcass side was sampled pre-evisceration and the other side post-evisceration, changing gloves for each carcass. The sources and types of samples are described in Table 1. All samples were stored on ice in iceboxes and were transported immediately to the laboratory, where the four SRK samples from each processing stage were pooled into a 50 ml centrifuge tube for processing. Samples were stored at 1-3°C and processed within 2-6 weeks from the time of collection. From each pooled sample, 1 ml was removed for TAPC determination which was done within 24 h of collection, and the rest was stored at 1-3°C for broth culture enrichment, PCR screening and isolation.

### 2.2.3. Total aerobic plate count

The pour plate method was used to determine the TAPC, following 10-fold serial dilutions in phosphate buffered saline (PBS) and plated on Plate Count Agar (PCA). Viable count calculation of CFU per 100 $cm^2$ for carcass surface and CFU per ml for rinsate samples was done using the procedure described in ISO 4833/2003 (International Organization for Standardization, Geneva, Switzerland). Any plate that had more than 300 colonies was recorded as TNTC (too numerous to count) at that dilution.

**TABLE 1:** Description of sample types obtained from 12 beef abattoirs in Gauteng, South Africa

| Sample type[a] | Description |
|---|---|
| Pre-slaughter faeces | Rectal grab at lairage |
| Perineum hide swab | Immediately after slaughter and bleeding, just before hide removal |
| Pre-evisceration | Immediately following hide removal |
| Post-evisceration | After evisceration, trimming and inspection |
| Rinsate | Collected with four 250 ml sterile glass bottles during carcass washing |
| Post-wash | Just before refrigeration |
| 24 h chilled[b] | 24 h overnight carcasses (-4°C to -20°C) |
| Swabs of wall & floor | Composite swab samples of walls/floors of slaughter halls |
| Effluent[c] | Abattoir effluents collected with four 250 ml sterile glass bottles |

[a] Carcass swab samples were collected from rump, flank, brisket and neck areas

[b] Longitudinal follow-up of the same carcass was not possible due to logistical challenges, but carcasses were randomly sampled from the same batch

[c] Abattoir effluents were obtained only from treatment plants linked directly to abattoir

### 2.2.4. Detection of STEC

All broth enrichment procedures followed the Bacteriological Analytical Manual protocol (US-FDA, 2013) with slight modifications based on sample type. Briefly, 25 g of faecal sample was aseptically weighed and 225 ml of Buffered Peptone Water modified with Pyruvate (mBPWp) (HiMedia laboratories, India) was added in a stomacher bag and the sample mixture homogenized in a Stomacher® 400 circulator (Seward Laboratory, UK). For rinsates and effluents, samples were individually pooled, and 200 ml of each sample type was centrifuged at 10,000 g for 10 min (Beckman Coulter, Allegra$^{TM}$ X-22 Series, Germany). The supernatant was

decanted and pellets were re-suspended in 225 ml mBPWp into a sterile Stomacher bag. For swab samples, 10 ml of the swab rinse solution was added to 90 ml mBPWp in a Stomacher bag. Homogenate was incubated at 37°C ± 1°C static for 5 h, after which 1 ml Acriflavin 10 mg - Cefsulodin 10 mg -Vancomycin 8 mg (ACV) Supplement (HiMedia laboratories, India) was added and incubated at 42°C ± 1°C static overnight (18-24 h).

A 1 ml aliquot of enriched broth was transferred into a 1.5 ml sterile centrifuge tube for DNA extraction using Quick-gDNA™ MiniPrep Kit (Zymo-Research Irvine, CA), according to manufacturer's instructions. The multiplex PCR was carried out as previously described (Onyeka et al, 2020). The assay conditions were optimized using molecular control strains obtained from the National Institute for Communicable Diseases (NICD) - Centre for Enteric Diseases (CED), South Africa (2014-2015 VTEC EQA—*E. coli* RR18-3022 O157, *eaeA*, $stx_{1a}$, $stx_{2a}$) and the control strain *E. coli* ATCC 43888 (O157:H7) $stx_1$. The multiplex PCR (mPCR) was validated by Sanger sequencing of PCR products. Only broth-enriched samples that had $stx_1$ and/or $stx_2$ were considered positive for STEC and used in molecular serotyping.

### 2.2.5. Detection of STEC serogroups using mPCR

All DNA extracted from samples positive for *stx* genes based on initial mPCR screening, were investigated for the following *E. coli* O-antigens: O113 (*wzx),* O157 (*rfbE*), O26 (*wzx*), O91(*wbsD*), O145 (*wzx*), O111 (*rfb*) and O103 (*wzx*) using the procedure described by Valadez et al. (2011). Multiplex PCR was carried out in a 25 µl volume containing 2-4 µl nucleic acid template (approximately 30-50 ng/µl of DNA), 12.5 µl DreamTaq Green PCR Master Mix (2X) (Thermo Fisher Scientific, USA) and 0.25 µM of each primer. The primer sequences, amplicon sizes, mPCR thermal cycle programme and electrophoresis gel procedures were carried out as previously described (Valadez et al. 2011).

### 2.2.6. Isolation and conventional serotyping of STEC strains

Only enriched broth cultures that were PCR-positive for *stx*$_1$ or *stx*$_2$ or both were considered positive for STEC and were cultured to isolate STEC strains. To isolate O157 STEC, the procedure consisted of immunomagnetic separation (IMS) assays using Dynabeads® anti-*E. coli* O157 (Thermo Fisher Scientific, USA), as recommended by the manufacturer. The immune-concentrated bacterial suspensions were then inoculated onto sorbitol MacConkey agar (SMAC) supplemented with potassium tellurite 2.5 mg/l and cefixime 0.05 mg/L (Himedia Laboratories Pvt., India). Ten (10) µl of enriched broth sample was streaked on a chromogenic agar, CHROMagar O157 (CHROMagar Microbiology, Paris, France) supplemented with potassium tellurite 2.5 mg/L and cefixime 0.05 mg/L (Himedia Laboratories Pvt. India). The plates were incubated for 24-30 h at 37°C, after which representative suspect colonies with different phenotypes were tested by latex agglutination (Welcolex® *E. coli* O157 Rapid latex agglutination test, Remel, UK). Enriched Control Strain –*E. coli* ATCC 43888 (O157:H7) was also inoculated for phenotypic control and assessment. Presumptive isolates were confirmed by mPCR as described above.

To isolate non-O157 STEC, 10 µl of enriched broth sample was streaked on MacConkey agar containing crystal violet and salt, and onto CHROMagar STEC (CHROMagar Microbiology, Paris, France). The plates were incubated for 24-30 h and representative suspect colonies were streaked on nutrient agar plates for further biochemical testing using Kovacs' indole reagent (Remel, California, USA). Indole positive isolates were confirmed with mPCR, as described above. Approximately 40% of non-typeable isolates were randomly selected for further biochemical confirmation as *E. coli* using the bioMérieux Vitek 2 Compact system (bioMerieux, Marcy l'Etolie, France)

Following PCR confirmation of isolates, serotyping was conducted at NICD-CED, using the conventional serotyping method (Ørskov & Ørskov, 1984). Only the O-surface antigen (lipopolysaccharide) serotyping was done using the complete *E. coli* O pool of polyvalent antisera: AA-WW antisera range from Statens Serum Institute (SSI) (Copenhagen, Denmark). The method used was according to the manufacturer's instruction and CED laboratory standard operating procedure (NIC1050). Saline was used as a negative control and 2014-2015 VTEC EQA—*E. coli* RR18-3022 O157 (*eae, stx$_{1a}$, stx$_{2a}$*) was used as positive control. Isolates were reported as either typeable or non-typeable.

### 2.3. Statistical analysis

A sample was considered STEC-positive when the broth culture tested positive for at least one of *stx$_1$* or *stx$_2$* genes on mPCR. Prevalence of STEC contamination and of each serogroup was estimated for each sample type with exact binomial 95% confidence intervals. Associations of each potential risk factor with STEC contamination was assessed by cross tabulation and the Fisher's exact test. Factors considered were sample type, season (summer/winter), throughput (HT/LT), multiple species slaughtered, water source (borehole/municipal water), concrete barrier separating species (present/absent) and service centre. All the risk factors were then included in a multiple logistic regression model to adjust for confounding.

The association between TAPC and STEC contamination was assessed using the point-biserial correlation coefficient, and only data from carcass swab samples were analysed. To describe the summary statistics of TAPC, data were log transformed, and plate counts which were reported as TNTC were assigned a value of 5, higher than the highest count. All data were analysed with Stata 14 (StataCorp, College Station, TX, U.S.A.); the level of significance was set at 5%.

## 3. RESULTS

### 3.1. Multiplex PCR screening of enriched broth cultures of samples

For a total of 419 samples comprising 376 samples from 60 cattle and 43 samples from abattoir environment, in 12 red meat abattoirs in Gauteng, collected during summer and winter, the overall prevalence of STEC contamination in selective enrichment samples was 35.1% (147/419; 95% CI: 30.5 - 39.7%). The prevalence of STEC differed significantly between sample types (n = 419; P = 0.039), (Table 2). Notably, the prevalence of STEC in faecal samples (27%) was lower than that in perineum hide swab samples (50%) (n = 419; P = 0.039) (Table 2). STEC prevalence was the same at pre-evisceration and post-evisceration (39%). There was no significant difference between STEC prevalence in summer versus winter (n = 419; P = 0.509) (Table 3).

Amongst the 147 PCR *stx*-positive broth samples, *E. coli* O-antigens were detected in only 39 (26.5%). Serogroup O113 was the most frequently detected serogroup, being found in 14.0% (20/147) of samples across the sample types. Nine (23%) of the 39 samples contained a combination of two O-group markers. Of the STEC serogroups tested, serogroup O103 was the only one not detected in any sample (Table 4). Frequencies of detection of STEC virulence genes and their combinations are also listed in Table 4.

### 3.2. Relationship between TAPC and STEC detection

Amongst the 186 carcass swab samples, the median TAPC was 1.83, 1.74, 1.63, 1.91, log10 CFU/100 cm2 for pre-evisceration, post-evisceration and post-wash and 24 h chilled, carcass swabs respectively. There was no significant association between STEC contamination and TAPC (r = 0.022; 95% CI: -0.10 - 0.14; P > 0.05).

**TABLE 2**. Prevalence of virulence genes associated with Shiga toxin-producing *Escherichia coli* in 12 beef abattoirs in Gauteng, South Africa

| Sample type | n | STEC% | 95% CI | *stx*$_1$*(%)* | *stx*$_2$ *(%)* | *eaeA (%)* | *hlyA(%)* |
|---|---|---|---|---|---|---|---|
| Faeces | 56 | 27 | 16-40 | 21 | 18 | 14 | 11 |
| Perineum hide swab | 56 | 50 | 36-64 | 36 | 46 | 32 | 23 |
| Pre-evisceration | 59 | 39 | 27-53 | 25 | 36 | 31 | 17 |
| Post-evisceration | 59 | 39 | 27-53 | 22 | 36 | 17 | 8 |
| Rinsate | 55 | 36 | 24-50 | 20 | 27 | 13 | 5 |
| Post-wash | 56 | 23 | 13-36 | 13 | 16 | 18 | 4 |
| 24 h chilled | 35 | 20 | 8-37 | 11 | 20 | 11 | 0 |
| Swabs of wall & floor | 24 | 38 | 19-59 | 21 | 29 | 21 | 8 |
| Effluent | 19 | 47 | 24-71 | 42 | 42 | 32 | 5 |
| Total | 419 | 35 | 30-40 | 23 | 30 | 21 | 10 |

**TABLE 3.** Prevalence of virulence genes of Shiga toxin-producing *Escherichia coli* by sample type and season in 12 beef abattoirs in Gauteng, South Africa

| | Summer | | | | | | | Winter | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample type | n | % | 95% CI | *stx*$_1$% | *stx*$_2$ (%) | *eaeA* (%) | *hlyA* (%) | n | % | 95% CI | *stx*$_1$% | *stx*$_2$ (%) | *eaeA* (%) | *hlyA* (%) |
| Faeces | 32 | 28 | 14-47 | 25 | 15 | 13 | 19 | 24 | 25 | 10-47 | 17 | 21 | 17 | 0 |
| Perineum hide swab | 31 | 52 | 33-70 | 39 | 52 | 29 | 42 | 25 | 48 | 28-69 | 32 | 40 | 36 | 0 |
| Pre-evisceration | 34 | 38 | 22-56 | 24 | 35 | 26 | 29 | 25 | 40 | 1-61 | 28 | 36 | 36 | 0 |
| Post-evisceration | 34 | 44 | 27-62 | 26 | 38 | 12 | 15 | 25 | 32 | 15-54 | 16 | 32 | 24 | 0 |
| Rinsate | 34 | 44 | 27-62 | 21 | 29 | 9 | 9 | 21 | 24 | 8-47 | 19 | 24 | 19 | 0 |
| Post-wash | 31 | 23 | 10-41 | 10 | 16 | 10 | 6 | 25 | 24 | 9-45 | 16 | 16 | 28 | 0 |
| 24 h chilled | 19 | 21 | 6-46 | 5 | 21 | 16 | 0 | 16 | 19 | 4-46 | 19 | 19 | 6 | 0 |
| Swabs of wall & floor | 12 | 42 | 15-72 | 25 | 25 | 17 | 17 | 12 | 33 | 10-65 | 17 | 33 | 25 | 0 |
| Effluent | 9 | 22 | 3-60 | 22 | 22 | 11 | 11 | 10 | 70 | 35-93 | 60 | 60 | 50 | 0 |
| Total | 236 | 36 | 30-43 | 22 | 30 | 16 | 18 | 183 | 33 | 26-40 | 23 | 30 | 26 | 0 |

**TABLE 4.** Prevalence of Shiga toxin-producing *Escherichia coli* serogroups, virulence genes and combinations identified using PCR-amplification of enriched broth in 12 beef abattoirs in Gauteng, South Africa

| | | Prevalence of STEC genotypes and combinations (%) | | | | | | | | | Serogroups (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample type | n | *stx*$_1$ | *stx*$_2$ | *stx*$_1$-*eaeA* | *stx*$_1$ + *eaeA*+ | *stx1* + *stx*$_2$ | *stx*$_1$ + *stx*$_2$ + *eaeA* | *stx*$_1$ + *stx*$_2$ + *eaeA*+ *hlyA* | *stx*$_2$ + *eaeA* | *stx*$_2$ + *eaeA*+ *hlyA* | O113 | O157 | O26 | O91 | O145 | O111 |
| Faeces | 15 | 20 | 7 | 0 | 13 | 7 | 20 | 0 | 7 | 7 | 7 | 0 | 13 | 0 | 0 | 0 |
| Perineum hide swab | 28 | 4 | 11 | 4 | 0 | 21 | 11 | 18 | 4 | 11 | 25 | 0 | 4 | 0 | 7 | 0 |
| Pre-evisceration | 23 | 4 | 13 | 4 | 0 | 22 | 4 | 13 | 9 | 9 | 17 | 0 | 9 | 9 | 4 | 4 |
| Post-evisceration | 23 | 9 | 30 | 0 | 0 | 22 | 9 | 4 | 9 | 0 | 13 | 0 | 9 | 4 | 9 | 0 |
| Rinsate | 20 | 20 | 35 | 0 | 5 | 15 | 10 | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 5 | 0 |
| Post-wash | 13 | 15 | 46 | 0 | 8 | 15 | 8 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 |
| 24 h chilled | 7 | 0 | 29 | 0 | 0 | 43 | 14 | 0 | 14 | 0 | 29 | 14 | 0 | 0 | 0 | 0 |
| Swabs wall & floor | 9 | 11 | 22 | 0 | 11 | 22 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Effluent | 9 | 11 | 0 | 0 | 0 | 22 | 44 | 11 | 11 | 0 | 11 | 0 | 0 | 0 | 0 | 0 |
| Total | 147 | 10 | 21 | 1 | 3 | 20 | 12 | 7 | 7 | 4 | 14 | 1 | 5 | 2 | 4 | 1 |

### 3.3. Risk factors for STEC contamination

Of the seven variables investigated, four had significant univariate associations with STEC contamination, namely sample type, daily throughput, concrete barrier separating species and location of service centres. In addition, STEC contamination of perineum hide was more frequent in HT (66%) than in LT (29%) abattoirs (n = 147; P = 0.014). In the multivariable logistic regression model (Table 5), the odds of contamination was lower in post-wash carcasses (OR = 0.42; 95% CI: 0.20 - 1; P = 0.045) and 24 h chilled carcasses (OR = 0.33; 95% CI: 0.20 - 0.90; P = 0.033) compared with pre-evisceration (reference level), and no other significant variables were found. The odds of STEC contamination tended to be highest in abattoirs that slaughtered between 120 to 255 units of cattle per day compared to abattoirs that slaughtered 15 units (OR = 5.25; 95% CI: 1 - 28; P = 0.052). Prevalence of STEC tended to be higher in abattoirs in Gauteng west compared to abattoirs in Gauteng north (OR = 4.21; 95% CI: 0.90 - 18; P = 0.061).

**TABLE 5.** Multivariable logistic regression of factors associated with detection of Shiga toxin-producing *Escherichia coli* in samples from 12 beef abattoirs in Gauteng, South Africa.

| Variable | Category | Odds ratio | 95%CI | P-value |
|---|---|---|---|---|
| Sample type | Pre-evisceration | 1[a] | – | – |
| | Post-wash | 0.40 | 0.20-1 | 0.045 |
| | 24 h chilled | 0.30 | 0.20-0.90 | 0.033 |

[a] Reference category

### 3.4. Isolation of STEC

Of the 147 *stx*-positive broth samples, only 25 (17%) samples yielded 33 STEC isolates, totalling 33. They were recovered from the following sample types: faeces (n = 1), perineum

**TABLE 6.** Serogroups and toxin genes detected in 33 isolates of Shiga toxin-producing *Escherichia coli* from recovered from various sample types in 12 beef abattoirs in Gauteng, South Africa

| Sample type | Serogroups | *stx*$_1$ | *stx*$_2$ | *eaeA* | *hlyA* | No. of isolates |
|---|---|---|---|---|---|---|
| Post-evisceration | O10 | + | + | - | + | 1 |
| Pre-evisceration | O10 | + | - | - | - | 2 |
| Perineum hide swab | O102 | - | + | - | + | 1 |
| Post-evisceration | O117 | + | + | - | + | 1 |
| Post-wash | O117 | + | + | - | - | 1 |
| Wall & Floor swab | O16 | + | + | - | + | 1 |
| Post-evisceration | O162 | + | - | - | - | 1 |
| Perineum hide swab | O168 | + | - | - | - | 1 |
| Perineum hide swab | O2 | + | - | - | - | 1 |
| Post-evisceration | O4 | + | - | + | + | 1 |
| Pre-evisceration | O6 | + | - | - | - | 1 |
| Faeces | O8 | - | + | - | + | 1 |
| 24 h chilled carcass swab | O8 | - | + | - | - | 1 |
| Perineum/pre-evisc./ post-evisc./SWF[a] | ONT[b] | + | - | - | - | 7 |
| Perineum/post-evisc./postwash/SWF[a] | ONT | + | + | - | - | 4 |
| Pre-evisc./post-evisc./ SWF[a] | ONT | + | + | - | + | 3 |
| Post-wash | ONT | + | - | + | + | 1 |
| Pre-evisc./post-evisc. | ONT | - | + | - | + | 3 |
| Faeces | ONT | - | + | - | - | 1 |
| Total | | 14 | 11 | 2 | 9 | 33 |

[a] Swabs of wall and floor
[b] O-antigen non-typeable

hide swab (n = 5), pre-evisceration (n = 5) post-evisceration (n = 8), post-wash (n = 3), 24 h chilled (n = 1) and swabs of wall and floor (n = 2). The mPCR results revealed that all the STEC isolates harboured *stx* genes. Despite concentration using immunomagnetic coated beads plated on SMAC, and the use of O157 CHROMagar, *E. coli* O157 was not recovered (Table 6).

Among 33 non-O157 STEC isolates recovered, 14 (42%) isolates were typeable, yielding 10 different serogroups, and 19 (58%) strains were O-serogroup non-typeable (ONT). The most prevalent serogroups were O10 (3 isolates), O117 and O8 (2 isolates each).

Six different genotype combinations were observed; the most frequent was $stx_1$ (13 isolates), followed by *stx*$_1$-*stx*$_2$-*hlyA* (6 isolates), *stx*$_1$-*stx*$_2$ (5 isolates), *stx*$_2$- *hlyA* (5 isolates) and *stx*$_2$ (2 isolates). Also, among the six different genotype combinations, only two isolates harboured the *eaeA* gene combinations: one isolate belonging to serogroup O4 (*stx*$_1$-*eaeA*-*hlyA*) and one ONT isolate (*stx*$_1$-*eaeA*-*hlyA*).

## 4. DISCUSSION

The significantly higher STEC prevalence observed in perineum hide swabs compared to faecal grab samples is consistent with other reports that have demonstrated that the perineum hide swab provides a better estimate of the prevalence of faecal STEC and possibly of hide contamination compared with faecal grab samples (Barkocy-Gallagher et al., 2003; Stephens et al., 2009), potentially representing samples from several defaecations. However, other studies have reported lower prevalence of STEC in hides than on faeces (Elder et al., 2000). In South Africa, a range of 5.4 to 20% was previously reported for STEC prevalence in faecal samples (Ateba et al., 2008), somewhat lower than the 27% observed in our study. The differences in the prevalence of

STEC between both studies may be due to several factors such as laboratory methods and the choice of sampling site, and is likely to vary geographically and temporally.

The higher prevalence of STEC detected in perineum hide samples in HT vs. LT abattoirs suggests the presence of shedders and the potential for hide cross contamination, particularly in HT abattoirs. Consequently, the prevalence of hide contamination may increase with increased cattle density due to faecally contaminated environments (Frank et al., 2008; Fraumax et al., 2008).

We detected the same prevalence of STEC (39%) in pre- and post-evisceration swab samples, suggesting that there was minimal rumen or gut content spillage during evisceration; hence the contamination observed was more likely associated with the dehiding process and possibly cross-contamination of equipment. This is consistent with studies that have demonstrated that hide prevalence is correlated with carcass contamination (Elder et al., 2000), and that it poses more risk of contamination than ruminal and intestinal content spill during evisceration (Arthur et al., 2004; Arthur et al., 2011; Tutenel et al., 2003).

Following evisceration, carcass decontamination measures are employed. The South African decontamination measures are similar to the measures employed in the European Union Decision 2001/471/EC, which permit only trimming and rinsing with cold or hot water. In this study, the prevalence of STEC on carcasses post-wash (23%) was lower than that found in the pre- and post-evisceration carcasses (39%), suggesting partial effectiveness of the washing procedure. However, the post-wash STEC prevalence of 23% was considerably higher than that documented in some European countries, for example, 1.4% in the UK (Chapman et al., 2001), 3.2% in Ireland (McEvoy et al., 2003) and 0.4% in France (Guyon et al., 2001). These findings indicate that other management practices may contribute to carcass contamination. Specifically, none of the abattoirs visited in our study practised hide decontamination before slaughter and it

is not included as a hygiene management procedure in the South African Meat Safety Act 2000 for red meat (http://www.rmaa.co.za/wp-content/uploads/2016/02/Act-40-of-2000-Regulations-Red-Meat.pdf). Hence, these results may reflect a difference in the pre-slaughter level of carcass contamination, determined by the contamination level on cattle as they arrive at the abattoir and whether pre-slaughter hide decontamination is practised (Arthur et al., 2004; Arthur et al., 2011; Tutenel et al., 2003).

Strains of STEC, like other bacteria, have been demonstrated to survive at -20°C with only a 1-2 $\log_{10}$ CFU/g reduction in concentration over a one-year period (Ansay, 1999). However, chilling has the potential to reduce the counts of aerobic bacteria and STEC O157 counts and that this possibility should be considered when comparing findings from different studies (McEvoy et al., 2003; Tutenel et al. 2003). In our study, a relatively high prevalence of STEC (20%) was detected on 24 h chilled carcasses, comparable to the 23% detected on post-wash carcasses ($P = 0.039$). Although McEvoy et al. (2004) have demonstrated that carcass chilling does not have a significant effect on the prevalence and counts of *E. coli* on carcasses, other studies have reported a reduction in STEC O157 contamination and even general aerobic bacteria count on carcasses after a 24 h chilling procedure (Gill et al., 1996; McEvoy et al., 2003; Tutenel et al. 2003).

In our study, no significant association was found between TAPC and the prevalence of STEC contamination. There are conflicting reports on the relationship between TAPC and detection of STEC with some researchers documenting no relationship (Kain et al., 2001; Stanford et al., 2013), consistent with our study, although McEvoy et al. (2000) indicated that a direct relationship may exist. Generally, it has been suggested that indicator bacteria, as measured by the TAPC, could only be used to evaluate hygienic and the effectiveness of intervention procedures to

reduce carcass pathogen contamination in processing plants (FSIS, 2002). The median TAPC for all the sample types was below the South African microbiological standard for meat export which ranges between 3.5 $\log_{10}/cm^2$ to 5.0 $\log_{10}/cm^2$ (VPN/15/2012). These results reflect the good hygiene practices of South African abattoirs (FSIS, 2002), but also suggest that indicator bacteria may not be a reliable measure to predict pathogen contamination (Stanford et al., 2013). Thus, it raises a public health concern, because of the risk of certified but contaminated beef reaching the table of the consumer.

At univariate level, there was a strong association between geographical location and the prevalence of STEC, although this was not significant in the multivariable model. It is known that geographic effect, as a risk factor can be complex, given multiple potential confounding factors such as the source of animals and abattoir management practices (EFSA, 2016). Chapman et al. (1997) have implicated geographic locations with high STEC contamination levels in the U.K.

Only 20% of the broth-enriched STEC-positive samples in this study were PCR-positive for the O-group-specific genes tested. Iguchi, et al. (2015) validated the use of mPCR for serotyping and demonstrated reliability of PCR as a subtyping tool for epidemiological surveillance. Serogroup O113 was the only serogroup found in all sample types except in swabs of walls and floors, suggesting that O113 may be the most prevalent serotype in cattle in Gauteng. Serogroup O113 is considered to be an emerging serogroup; Paton et al. (1999) associated the serogroup with HUS in Australia and it has also been implicated in human illness in Europe (EFSA, 2018), the USA (Feng et al., 2017) and Canada (Karmali et al., 1985). Serogroup O26 was also detected in this study, raising public health and clinical concerns. Recently, Mainga et al. (2018) isolated serogroup O26 from 10% of cattle faecal samples across Gauteng and North West provinces of South Africa, and STEC O26:H11 was implicated in four cases of HUS in children

in South African (Smith et al., 2017). Serogroup O26 has been associated with HUS cases in many countries: in Germany and Austria O26 was implicated in HUS in children from 1997 through 2000 (Gerber et al., 2002), and in outbreaks in Germany (Weber et al., 2002), Australia (Elliot et al., 2001), Argentina (Rivas et al., 2006) and Italy (Tozzi et al., 2003).

Serogroup O157 was detected only once, in one sample type, a finding comparable with the recent report of O157 in only 1.4% of bovine faecal samples from Gauteng and North West provinces (Mainga et al., 2018). These findings suggest that the O157 serogroup may be uncommon in South Africa, as was similarly reported in other countries, such as Australia, where O157 was reported to be rare in a nationwide surveillance of cases that presented HUS symptoms from 1994 to 1998 (Elliot et al., 2001).

It is of public health and clinical relevance that amongst the STEC isolates recovered, 42% harboured the *stx*$_1$ gene, 33% harboured the *stx*$_2$ gene and 15% harboured both *stx* genes. Our findings are consistent with reports that *stx*$_1$ genes were detected at a higher frequency than *stx*$_2$ genes in isolates of bovine origin compared with isolates of human origin (Boerlin et al., 1997; Wieler, 1996). Isolates of bovine origin are unlikely to harbour the intimin (*eae*) and EHEC-hemolysin (*hlyA*) when compared to human isolates (Arthur et al., 2002; Boerlin et al., 1999; Johnson et al., 1996). In our study only two (one non-O157 STEC and one ONT) isolates had the *eaeA* gene (6%), consistent with a French study finding the *eae* gene in only 5% and 0% of STEC isolates from bovine faeces and beef respectively (Pradel et al., 2000). STEC serotypes such as O91:H21, O113:H21 and O104:H4 do not possess the *eae* gene, yet have been reported to cause HUS (Bugarel et al., 2010; Newton et al., 2009). Interestingly, in this study we identified a rare STEC serogroup, O117, which lacked the *eae* gene. A serotype O117:H7 strain also lacking the

*eae* gene was implicated in an outbreak in 2014 in the U.K. (Baker et al., 2018; Simms et al., 2014). To our knowledge, this is the first report of serogroup O117 in cattle South Africa.

It is also noteworthy that we found 27% of the STEC isolates to be positive for the *hlyA* gene, somewhat lower than the 48% and 33% detected in faecal and beef isolates respectively in France (Pradel, et al., 2000). This is important because the presence of the *hlyA* gene has been associated with virulence potential of some STEC strains (Khaitan et al., 2007, Wolfson et al., 2009).

The main limitation of this study is that the one-year cross-sectional survey is limited in extent and may not accurately predict the actual prevalence of STEC in the beef food chain, which may change from year to year and vary among areas of the country. Further surveys performed in different areas may provide a better representation of seasonal and geographic variability in the prevalence of contamination by STEC. In addition, a second limitation of the study which resulted from logistical issues encountered, was the storage of samples pre-processing for 2-6 weeks at 1-3°C. Extended storage of carcasses at chilling temperatures has been documented to reduce microbial populations, including the TAPC and STEC counts (Gill et al., 1996; McEvoy et al., 2003; Tutenel et al. 2003). In addition, only a limited number of virulence genes were assayed in this study, whereas a more comprehensive genetic characterization which would employ whole genome sequencing as an epidemiologic surveillance tool to determine additional virulence genes and antibiotic resistance genes of pathogenic STEC strains, would provide a better assessment of virulence profiles (Njage et al., 2019). Furthermore, our results demonstrated a low isolation rate from *stx*-positive broth cultures. Researchers have documented low isolation rate of STEC in Europe (Ciupescu et al., 2015), the U.S.A. (Woo, and Palavecino, 2013), Argentina (Brusa et al., 2017) and South Africa (Kalule et al., 2018). Apart from the low prevalence, this could possibly

be due to the presence of free *stx*-phages, some of which are infectious (Martínez-Castillo and Muniesa, 2014), or loss of the *stx* gene after subculture (Joris et al., 2011). It is also possible that the initial PCR detects the presence of low levels of STEC which are subsequently missed when testing a small number of colonies by PCR.

## 5. CONCLUSION

Our study has confirmed the presence of STEC in apparently healthy cattle slaughtered in Gauteng, South Africa and in carcasses entering the food chain. The hides of cattle represent an important entry point of STEC into the abattoir and possibly the food chain. Carcass bacterial load of indicator organisms (TAPC) was not correlated with the prevalence of STEC. Non-O157 STEC strains were more prevalent than O157 STEC strains and included the clinically relevant serogroups O2, O8 and O117, posing a potential health risk to consumers of under-cooked beef or beef products.

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