PREVALENCE AND PATTERNS OF FAECAL SHEDDING OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* BY CATTLE AT A COMMERCIAL FEEDLOT IN SOUTH AFRICA

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ABSTRACT

Healthy colonized cattle are the major reservoir of Shiga toxin-producing *Escherichia coli* (STEC) and play a key role in the entry point of the pathogen into the beef chain. Excretion rates and the concentration of the pathogen in faeces influence the epidemiology and transmission of the pathogen within herds and to humans.

This study evaluated the prevalence and dynamics of faecal shedding of STEC by cattle in a commercial feedlot in Gauteng, South Africa. An initial cross-sectional survey was conducted; faecal samples were obtained from 106 randomly selected weaned beef calves on arrival at the feedlot. Using polymerase chain reaction (PCR) to screen by detecting *stx*$_1$ and *stx*$_2$ genes. Subsequently, a longitudinal study was conducted, 15 STEC-positive and 11 STEC-negative cattle were sampled monthly and followed to slaughter. STEC O157 and non-O157 were enumerated in samples using commercial chromogenic agar. Initial prevalence of STEC shedding was 27% (29/106; 95% CI: 19-37%). All 26 cattle shed STEC intermittently or continuously during the study period, all except one were super-shedders (≥4 $\log_{10}$ CFU/g) at one or more samplings, and 19 (73%) were persistent or intermittent super-shedders. Of the 38 STEC isolates recovered, 15 (39%) were serotypeable, representing 11 non-O157 serogroups, including O101, O168, O178 and O68. The most frequent virulence combination profile was *stx*$_1$+*eaeA*+*ehxA* (n=12; 32%).

This study confirms the occurrence and variability of STEC super-shedding in feedlot cattle and highlights that super-shedding is not limited to STEC O157. It also shows their public health significance.

Keywords: Cattle, O157 and non-O157 STEC, Public health, Shiga toxin-producing *Escherichia coli*, South Africa, super-shedder

Impacts:

- Non-O157 STEC was shed more frequently and at greater concentrations than STEC O157.
- Variations in STEC shedding pattern over time were observed in the feedlot studied, i.e. cattle that were initially STEC negative became positive over time, and *vice-versa*.
- Most of the cattle (73%) positive for STEC were either persistent or intermittent super-shedders of STEC O157 and non-O157.

1. INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) has emerged as an important foodborne pathogen globally and has significantly impacted the beef industry and public health (Callaway et al., 2013). Healthy colonized cattle, among other ruminants, are the major reservoir of STEC (Munns et al., 2015), and they play a key role in the entry point of the pathogen into the beef chain (Heiman et al., 2015). Beef and beef products are frequently identified as a major risk factor for STEC infections and are thus of public health importance, although other sources such as food crops, water and environment contribute to infection (Heiman et al., 2015).

Majowicz et al. (2014) estimated that STEC (O157 and non-O157) is responsible for 2,801,000 acute illnesses annually, resulting in 3,890 cases of haemolytic uraemic syndrome (HUS), 270 cases of end stage renal disease (ESRD) and 230 deaths globally. *E. coli* O157:H7 has been recognised as the serotype most commonly associated with severe infections in both large and sporadic outbreaks (Munns et al., 2015). However, non-O157 STEC has been increasingly linked to human disease worldwide, with an increase of 3,573 confirmed cases in

2009 to 6,073 in 2017 in Europe (Kim et al., 2020). In South Africa, non-O157 STEC (87%; 33/38) were linked with human disease outbreaks from 2006 - 2013 (Karama et al., 2019).

Cattle carriage of STEC is heterogeneous, with a considerable degree of variability in prevalence and concentration of pathogen over time as demonstrated in longitudinal studies of STEC serogroups in herds over time (Dixon et al., 2020). The capacity of the organism to survive in feed, water, soil, and manure contributes to its persistence in cattle herds and contamination of water supplies and crops (van Overbeek et al., 2020). Furthermore, recirculation of STEC could originate from environmental contamination within a geographic region (van Overbeek et al., 2020). Excretion rates of the pathogen and the concentration of the pathogen in faeces influence the epidemiology and transmission of the pathogen within herds and in humans (Collins, 2009; Dixon et al., 2020).

Generally, cattle shed STEC in faeces at concentrations <2 $\log_{10}$ CFU/g (Chase-Topping et al., 2008) and some transiently shed ≥4 $\log_{10}$ CFU/g. Those in the latter category have been described as super-shedders and are known to have a substantial impact on the environmental prevalence and transmission of STEC (McCabe et al., 2019). McCabe et al., (2019) showed that 2.13% (28/1,317) of STEC O157 and 0.23% (3/1,317) *E. coli* O26 cattle determined to be "super-shedders" posed a greater risk of the environmental dissemination of STEC.

Although there are numerous published reports from Europe, Australia and the United States (Smith et al., 2010; Lammers et al., 2015; Jaakkonen et al., 2019) emphasizing the risk of carriage of STEC by cattle, particularly super-shedders in the beef chain. There is a general paucity of published information regarding the prevalence and concentration of both O157 and non-O157 STEC in pre-harvest stages and the key role they play in the entry point of the pathogen into the beef chain in South Africa. Furthermore, foods of bovine origin such as beef products are

frequently identified as a major risk factor for zoonotic diseases and are thus of public health importance. Data are non-existent on the dynamics and relationship of pre-harvest shedding of STEC and post-harvest contamination of beef in the country. To our knowledge, there is no published information on active super-shedding of STEC on cattle farms in South Africa. This study aimed to determine the prevalence of carriage of STEC by cattle and the shedding patterns of O157 and non-O157 STEC in a beef feedlot in South Africa, and to characterize the serogroups and the presence of the virulence genes *stx*$_1$, *stx*$_2$, *eaeA*, and *ehxA* amongst the isolates identified.

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).

2.2 Study design

A longitudinal investigation of cattle in a commercial beef feedlot in Gauteng, conducted between September 2016 to February 2017. The study consisted of three main phases of sample collection: phase 1 was an initial cross-sectional survey (September, 2016), phase 2 was a monthly follow-up longitudinal study on the farm (October to December, 2016), and phase 3 was a follow-up longitudinal study in the abattoir (February 2017), as shown in table 1.

Table 1: Study design for monthly feedlot visits and abattoir follow-up sampling visits in Gauteng, South Africa

	Study type	Month and year	Sample type	Observations
Feedlot	Cross-sectional	September 2016	Cattle faeces	106
	Longitudinal	October 2016	Cattle faeces	26
	Longitudinal	November 2016	Cattle faeces	19
	Longitudinal		Environmental (faeces)	5
			Environmental (feed)	5
			Environmental (water)	7
		December 2016	Cattle faeces	22
			Environmental (faeces)	5
			Environmental (feed)	5
			Environmental (water)	8
Abattoir	Longitudinal	February 2017	Cattle faeces	11
			Carcass: perineum hide swab	9
			Carcass: pre-evisceration swab	12
			Carcass: post-evisceration swab	12
			Carcass: rinsate	11
			Carcass: post-wash swab	19
Total				282

2.3 Source of study cattle

The selected feedlot with a herd size of approximately 12,000 operated a mechanized abattoir that slaughtered approximately 120 head of cattle daily. The feedlot received weaned beef calves mainly from cow-calf farms in and around Gauteng province. On arrival, the calves were aged 7-8 months and weighed 256-338 kg. Calves were sorted by weight into five pens and were fed a total mixed ration of roughage with increasing amounts of concentrates. Most calves spent approximately four months in the feedlot prior to slaughter. The feedlot was selected because of willingness to participate in the study and the presence of an on-farm abattoir which allowed longitudinal follow-up of study cattle to the abattoir.

2.4 Sampling design and methods

In an initial cross-sectional survey, 106 cattle were randomly selected and tagged during two visits. Based on PCR screening results (only 29/106 STEC positive) and for logistical reasons, only 26 cattle were selected for longitudinal follow-up study (15 STEC-positives and 11 negatives). Faecal grab samples were collected monthly to coincide with the farm's routine medical treatments and body weight records. At the abattoir, faecal grab samples were collected at the lairage just pre-slaughter. Only 12 of the 26 study cattle were available for sequential sampling at critical processing stages from pre-slaughter to the dressed carcasses, and seven post wash swab carcass samples were also obtained (Table 1).

A minimum of 50 g of fresh faecal grab sample was collected using a new rectal glove for each animal. Environmental samples were obtained from the farm only during the November and December visits, and included pooled freshly voided faecal samples on the pen floors, five pooled feed samples and approximately 70 ml of water samples from the troughs in pens that harboured selected animals were pooled from the different troughs. All samples were aseptically collected with gloved hands, transferred into a sterile 100 ml specimen container. The overall number of samples collected are as follows: 106 - cross-sectional survey, 78 faecal grab samples- longitudinal follow-up study, 35 environmental, 63 carcass samples - abattoir follow-up study, providing 282 samples in total (Table 1). All samples were stored in cooler boxes containing ice packs and transported immediately to the laboratory where they were stored at 0-4ºC until processed within 1-14 days (Bach et al., 2005; Wang et al., 1996).

Carcass sampling at the abattoir 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). Accordingly, swab samples were obtained from a 100 cm^2 area using

a sterile square metal template that was sterilized between sample collections from each of four selected anatomical sites (4x100 cm^2 areas): rump, flank, brisket and neck using a swab rinse kit (SRK) (Copan Diagnostics, Inc., UK). In the laboratory, the four SRK samples were pooled into a 50 ml centrifuge tube for processing.

2.5 Processing of faecal samples

Briefly, 25 g of faeces was added to 225 ml of Buffered Peptone Water (BPW) modified with Pyruvate (HiMedia Laboratories, India), containing 1 ml of the ACV supplement: Acriflavin 10 mg, Cefsulodin 10 mg, Vancomycin 8 mg, and homogenized in a sterile Seward 400 Laboratory Stomacher bag (Seward, UK). The enrichment control strain used was *E. coli* ATCC 43888 (O157:H7). One ml of homogenate was removed for enumeration, and the rest incubated for 24 h at 37ºC, for subsequent PCR screening.

2.5.1 Enumeration

One ml of 10-fold serial dilutions was plated each on duplicate plates of two different selective media known to target *E. coli* O157 and all STEC serogroups including non-O157 STEC, namely; CHROMagar™ O157 supplemented with potassium tellurite 2.5 mg/l and cefixime 0.05 mg/l for STEC O157 and CHROMagar™ STEC (CHROMagar, Paris, France). Both types of plates were incubated for 24 h at 37ºC, after which characteristic mauve colonies were selected and biochemically screened using the Indole reagent Kovacs' (Remel, USA) for total STEC, and O157 Rapid latex agglutination test (Welcolex® *E. coli*, Remel, UK) for STEC O157. Enrichment Control Strain –*E. coli* ATCC 43888 (O157:H7) was also inoculated for phenotypic control and assessment. Enumeration was performed by viable plate count method and expressed as CFU/g.

To determine the detection limits of the enumeration, 25 g of sterile cattle faeces was spiked with Control Strain –*E. coli* ATCC 43888 (O157:H7) and 225 ml broth added and procedure for processing faecal samples above was followed. Thereafter, 10 $\log_{10}$ CFU/g *E. coli* ATCC 43888 per gram measured using Beckman Coulter Microscan Turbidity Meter (High Wycombe, UK), serially diluted to 10^{10} cells per ml and the procedure for faecal samples above was followed. Detection limit of enumeration of STEC O157 using this methodology was 1.3 $\log_{10}$ CFU/g.

2.5.2 Multiplex PCR to identify virulence markers

DNA Template from the broth enriched samples was prepared using QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany*)* according to manufacturer's instructions. The DNA templates were investigated for the presence of *stx*$_1$, *stx*$_2$, *eaeA* and *ehxA* genes using a multiplex PCR (mPCR) method (Paton and Paton, 1998), conducted as previously described (Onyeka et al., 2020).

2.5.3 Bacteriological isolation and conventional serotyping

Representative colonies were selected and sub-cultured on CHROMagar O157 and on CHROMagar STEC (CHROMagar, Paris, France). Plates were incubated for 24-30 h at 37ºC. Suspect colonies were streaked on nutrient agar plates and were confirmed with mPCR to identify virulence markers *stx*$_1$, *stx*$_2$, *eaeA* and *ehxA* genes. Serotyping was done using conventional serotyping method (Ørskov and Ørskov, 1984; Onyeka et al., 2020).

2.6 Statistical analysis

For the cross-sectional survey, a sample was considered STEC-positive when it was positive for either *stx*$_1$ or *stx*$_2$ genes. For enumeration on CHROMagar^{TM} STEC, total STEC was considered to

include O157 and non-O157 STEC. Prevalence of STEC contamination in the 106 samples was calculated with 95% exact binomial confidence limits. For the longitudinal study, a super-shedder was defined as an animal that sheds ≥4 $\log_{10}$ CFU/g (Chase-Topping et al., 2008) and a high-density shedder as one shedding 3-4 $\log_{10}$ CFU/g (Arthur et al., 2009). Accordingly, results were categorized by shedding level amongst cattle: super-shedders, high-density shedders, shedders (<3 $\log_{10}$ CFU/g) and non-shedders (no growth), as enumerated by direct faecal culture. In addition, shedders were classified into two categories; 'persistent shedders', if they shed O157 and/or non-O157 STEC (total STEC) for three or more consecutive months (Lim et al., 2007; Carlson et al., 2009) and intermittent super-shedders or high density shedders if they showed at least one shedding of ≥4 $\log_{10}$ CFU/g or 3-4 $\log_{10}$ CFU/g respectively.

The individual shedding levels amongst cattle (CFU/g) were tested for normality, and the data were log-transformed prior to analyses, as assumptions for normality were not met. The significance of the differences between the medians of STEC O157 and total STEC faecal shedding levels was tested using Wilcoxon signed-rank test. Relationships between faecal super-shedding of serogroups of O157 and total STEC were examined using McNemar's χ^2 tests. Agreement between PCR screening and culture on solid media was assessed using the Kappa statistic. All P-values reported were two-sided and $P < 0.05$ was considered statistically significant. All the statistical analyses were performed using Stata 14 (StataCorp, College Station, TX, U.S.A.).

3. RESULTS

Of the faecal samples collected from weaned beef calves as they entered the feedlot in September 2016, 27% (29/106; 95% CI: 19-37%) were STEC-positive by PCR. (Table 2).

Detection of combinations of STEC virulence genes in broth samples was as follows: *stx*$_2$ + *ehxA* (6 samples) and *stx*$_1$ + *stx*$_2$ (5 samples), *stx*$_1$ + *stx*$_2$ + *eaeA* + *ehxA* (1 sample) and *stx*$_2$ + *eaeA* (1 sample) as shown in Table 2.

Table 2: Prevalence of Shiga toxin-producing *Escherichia coli* associated genes profiles from weaned calves upon arrival at a feedlot and 38 isolates from feedlot cattle faeces and follow up abattoir carcass in Gauteng, South Africa

Virulence genes	No. positive	%
106 faecal field broth samples		
stx$_1$	18	17
stx$_2$	20	19
eaeA	12	11
ehxA	23	22
STEC	29	27
29 STEC-positive broth samples		
stx$_1$	7	24
stx$_2$	4	14
stx$_1$ + *stx*$_2$	5	17
stx$_1$ + *stx*$_2$ + *ehxA*	3	10
stx$_1$ + *ehxA*	2	7
stx$_1$ + *stx*$_2$ + *eaeA* + *ehxA*	1	3
stx$_2$ + *eaeA*	1	3
stx$_2$ + *ehxA*	6	21
38 STEC isolates from feedlot and abattoir visits		
stx$_1$	6	16
stx$_1$+*eaeA*+*ehxA*	12	32
stx$_1$+*ehxA*	1	3
stx$_1$+*stx*$_2$+*ehxA*	4	11
stx$_1$+*stx*$_2$+*eaeA*+*ehxA*	2	5
stx$_1$+*stx*$_2$	4	11
stx$_2$+*eaeA*+*ehxA*	4	11
stx$_2$	5	13

The overall prevalence of super-shedding cattle in the longitudinal study samples at the feedlot and abattoir lairage follow-up was 73% (57/78; 95% CI: 62-82) for total STEC super-

shedding and 6% (5/78; 95% CI: 3-120) for O157 super-shedding (Table 3). The median and maximum faecal shedding count was 4.8 and 6.6 $\log_{10}$ CFU/g for total STEC, and 3.4 and 5.1 $\log_{10}$ CFU/g for O157 STEC ($P < 0.001$). Monthly super-shedding of total STEC by cattle was significantly more frequent than that of O157 STEC (Table 4).

Table 3: **Prevalence of Shiga toxin-producing *Escherichia coli* (STEC) for the Oct to Dec monthly feedlot visit and the Feb abattoir follow up sampling visits in Gauteng, South Africa**

Source	Variables	Observation	Frequency	% positive for STEC
Feedlot/Abattoir lairage				
	STEC super-shedder		57	73
	STEC high-density shedder		14	18
	STEC non-shedder		7	9
	Total	78		
	O157_super-shedder		5	6
	O157 high-density shedder		8	10
	O157 shedder		1	82
	O157 non-shedder		64	1
	Total	78		
Abattoir slaughter hall				
	Perineum hide swab	9	2	22
	Pre-evisceration	12	2	17
	Post-evisceration	12	3	25
	Rinsate	11	0	0
	Post-wash	19	2	11
	Total	63		

Table 4: Proportion of cattle that were super-shedders of Shiga toxin-producing *Escherichia coli* (STEC) during each feedlot visit in Gauteng, South Africa

		STEC count ≥4 $\log_{10}$ CFU/g		
Month	n	Total STEC (%)	O157 (%)	P-value*
Oct	26	92	19	< 0.0001
Nov	19	95	11	< 0.0001
Dec	22	91	0	< 0.0001
Feb	11	91	36	0.031
Total	78	92	14	< 0.0001

* McNemar's chi-squared test

The number of cattle available for follow-up varied by month (Table 4). Four cattle were persistent STEC super-shedders throughout the study period and 5 cattle were STEC super-shedders for 3 consecutive months (Figure 1). The overall prevalence of STEC in pooled environmental samples was 80% (8/10) in voided faeces on pen floors, 10% (1/10) in feed and 47% (7/15) in water in pen troughs (P = 0.739).

Of the 78 faecal samples enumerated for total STEC and STEC O157 in the longitudinal study, the results for 50% (39/78) of the broth cultures screened using PCR, did not correspond with the enumeration results by culture, i.e. broth samples were PCR-negative but culture- positive. The agreement between PCR and culture was poor for STEC O157 (Kappa = 0) and for STEC (Kappa = 0.03).

Animal	Pen	Feedlot: Arrival (PCR)	STEC O157				Total STEC				Abbatoir (PCR)
		Sept	October	November	December	February	October	November	December	February	
1	1	Positive	0	0	0		3	3	3		
2	1	Positive	0	0	0		3	2	2		
3	1	Positive	0	0	0		3	3	2		
4	2	Positive	0				3				
5	2	Positive	0	0	0	3	3	3	3	3	Negative
6	2	Positive	3		0	0	3		2	0	Positive
7	2	Positive	0		0		0		3		Negative
8	3	Positive	2	0	0	0	3	3	3	3	Negative
9	3	Positive	2	0			3	3			Negative
10	3	Positive	0	0	0	2	3	3	3	3	Positive
11	3	Positive	1	3	0	3	3	3	3	3	Negative
12	4	Positive	2	0	2	0	3		3	2	Positive
13	4	Positive	0	2	2		0	3	3		
14	5	Positive	0	0	0		3	3	3		Negative
15	5	Positive	0		0	0	3		2	3	Positive
16	2	Negative	0		0	0	2		3	2	Negative
17	4	Negative	0	0	0	0	3	3	2		Negative
18	4	Negative	0	0	0		3	3	3		Negative
19	4	Negative	0	0		0	3	3		3	Positive
20	4	Negative	0	0	0		3	3	3		
21	4	Negative	0	0	0		3	3	0		Negative
22	4	Negative	0	0	0		3	3	3		
23	4	Negative	0	0	0		3	3	0		Negative
24	4	Negative	0	2	0	0	2	3	0	2	Positive
25	4	Negative	0		0	3	2		2	3	Negative
26	5	Negative	0				2				Negative

Figure 1. Patterns of Shiga toxin-producing *Escherichia coli* shedding by feedlot calves in Gauteng, South Africa. 0 = non-shedder; 1 = shedder (<3 log10 CFU/g); 2 = high-density shedder (3-4 $\log_{10}$ CFU/g); 3 = super shedder (≥4 $\log_{10}$ CFU/g); Empty cells: no data

At the abattoir, the overall prevalence of STEC in carcass swabs was 32% (6/19; 95% CI: 13-57), and STEC prevalence at the different stages of carcass processing is shown in Table 3. There was no association between super-shedding status just before slaughter and STEC O157 (P = 0.061) or total STEC (P = 0.348) carcass contamination. Likewise, there was no association between super-shedding status just before slaughter and perineum hide swab STEC contamination for either O157 (P = 0.714) or total STEC (P = 0.143).

Only 30 field samples yielded isolates (n = 38) because subculture of other samples were negative; 10 samples were from the cross-sectional survey and 20 from the follow-up longitudinal study. Of the 38 isolates, only 15 (39%) were serotypeable and belonged to a diverse range of 11 non-O157 serogroups (Table 5). Isolates of the same serogroup harboured different virulence gene profiles; for example, serogroups O108 (2 isolates) and O168 (3 isolates) each displayed two different virulence gene profiles. It is noteworthy to mention that serogroups O108 and O68 harboured *stx*$_2$+*eaeA*+*ehxA* virulence genes (Table 5).

Table 5: Virulence characteristics of 15 serotypeable non-O157 Shiga toxin-producing *Escherichia coli* (STEC) isolates

Sample_type	Sample_id	Serotype	*stx*$_1$	*stx*$_2$	*eaeA*	*ehxA*
Faeces	CX 104(3)	O108	-	+	+	+
Faeces	FAF 85(1)	O168	-	+	-	-
Faeces environment	FA3 9B	O178	-	+	-	-
Faeces	LS2 66(2)	O178	-	+	-	-
Faeces	CX 102(1)	O68	+	+	+	+
Faeces	FAF 51(1)	O108	+	+	-	+
Faeces	CX 57(1)	O182	+	+	-	-
Faeces environment	FA2 10C	O88	+	+	-	-
Faeces	LS2 93(3)	O101	+	-	+	+
Faeces	LS2 90e	O168	+	-	+	+
Faeces	LS2 93(1)	O168	+	-	+	+
Faeces	CX 14(1)	O10	+	-	-	-
Faeces	CX 33(2)	O132	+	-	-	-
Faeces	CX 12(2)	O140	+	-	-	-
Post-evisceration carcass swab	FA 77	O36	+	-	-	-

The distribution of virulence genes of the 38 STEC isolates is shown in Table 2. The *eaeA* gene was present in 47% (18/38) of the isolates. The most frequent virulence combination profiles were *stx*$_1$+*eaeA*+*ehxA* (n = 12; 32%), followed by *stx*$_1$+*stx*$_2$+*ehxA*, *stx*$_2$+*eaeA*+*ehxA* and *stx*$_1$+*stx*$_2$ (n =4; 11% each). Two isolates (5%) had a full complement of the targeted virulence genes (*stx*$_1$+*stx*$_2$+*eaeA*+*ehxA*).

4. DISCUSSION

To our knowledge this is the first study to investigate active super-shedding of STEC (O157 and non-O157) in beef feedlot in southern Africa. The finding that the overall prevalence of STEC in calves on their arrival at the feedlot was 27% with 19% and 11% being positive for *stx*$_2$ and *eaeA* genes respectively, demonstrates that STEC carriage in cattle is of potential public health significance, given that the presence of both *stx*$_2$ and *eaeA* has been associated with bloody diarrhoea in Swedish patients infected with STEC (Bai et al., 2018).

In addition to the demonstration of STEC shedding in the beef feedlot, we found that super-shedding by cattle was not limited to STEC O157, but that non-O157 was shed in faeces more frequently and at greater concentrations. These findings agree with the reports of super-shedding of STEC O26 in Ireland (Murphy et al., 2016; McCabe et al., 2019) and STEC non-O157 in Germany (Menrath et al., 2010). The high prevalence and concentration of both O157 and non-O157 STEC highlight the contamination pressure within the feedlot via faeces. Generally, feedlots provide a more conducive environment for between-animal transmission than extensive management systems, because of higher stocking density and resultant higher levels of environmental contamination, including feed and water. Studies conducted in the feedlot have

demonstrated increased probability of shedding by animals housed with a super-shedder (Cobbold et al., 2007; Arthur et al., 2009; Cernicchiaro et al., 2010).

The higher prevalence of non-O157 STEC compared with O157 STEC is of public health significance. There have been a growing number of cases in which non-O157 STEC strains were linked to human disease worldwide; with the highest number of reported HUS cases (37%) caused by non-O157 STEC from 2005 to 2009 worldwide (Valilis et al., 2018). Likewise, non-O157 STEC have been increasingly linked to numerous cases of diarrhoea in children and adults between 2006 and 2013 in South Africa, in which a diverse range of STEC serogroups (O4, O5, O21, O26, O84, O111, O113, O117 and O157) were implicated (Karama et al., 2019, Smith et al., 2011). Furthermore, there have been reports of clinical cases of HUS in humans in South Africa associated with STEC O26 (Smith et al., 2017). None of these reported serogroups was found in our study, although four serogroups (O101, O168, O178 and O68) considered as emerging (CDC, 2016) were isolated. To our knowledge these four serogroups were documented for the first time in beef cattle in South Africa, arguing for further studies and surveillance of these non-O157 serogroups in their bovine reservoir host and in humans to assess their importance in human disease in the country.

The presence of the *eaeA* gene with stx_2 is highly correlated with severe infections (HC and HUS) and outbreaks in humans, whereas a combination of *eaeA* and stx_1 causes less severe infections (FAO and WHO, 2018). It is therefore significant that we detected *eaeA* gene in 47% of isolates and that 32% of the isolates harboured a virulence combination of stx_1+*eaeA*+*ehxA,* stx_2+*eaeA*+*ehxA* (11%) or stx_1+stx_2+*eaeA*+*ehxA*.

It was also of interest to have detected that four cattle were concurrent shedders of both STEC O157 and non-O157, a finding similarly documented by others (Blanco et al., 1996; Cernicchiaro et al., 2014), in which different serogroups of STEC colonized one animal. The

results of our study suggest that non-O157 STEC may have been under-reported in humans in South Africa and that the high-density shedders, including super-shedders, may pose an increased risk of contamination along the beef production chain, which may enter the human food chain.

Our detection of variations in STEC shedding pattern over time, i.e. cattle that were initially negative became positive over time, and *vice-versa*, is consistent with the reported heterogeneity in the epidemiology of STEC shedding patterns within the host (Munns et al., 2015). The factors responsible for variation in super-shedding are largely unknown; however, several factors related to the host and farm management practices could be responsible for epidemiological heterogeneity (Munns et al., 2015: Karmali, 2016). The description of an animal as a "super-shedder" may also be influenced by frequency of sampling, because super-shedding status can change from day to day in the same animal over time (Munns et al., 2015; Stein and Katz, 2017). Even with the limited data in our study, almost all of the animals positive for STEC were super-shedders, which supports the theoretical model that all cattle are transient super-shedders (Lammers et al., 2016; Munns et al., 2015; Stein and Katz, 2017).

Our study failed to detect any significant association between STEC super shedding status of cattle pre-slaughter and detection of STEC on contamination of hides and carcass at the abattoir, a finding at variance with Arthur et al. (2009) who demonstrated a relationship between super-shedding and prevalence on hides and/or carcass contamination at the abattoir. The processing delays in the laboratory, as well as the small sample size in our study, may have resulted in insufficient power to detect an association. However, in addition to transfer from the animal's own hide or gut content, carcass contamination may also result from cross-contamination among carcasses and from the abattoir environment.

The lack of agreement between bacteriological culture and PCR to detect STEC has been previously reported (Noll et al., 2015; Fan et al., 2019) and may be explained, in part, by isolation and PCR-related factors. The low frequency of isolation and failure to recover STEC O157, despite the high sensitivity of CHROMagar ^{TM} O157 for the serotype (Church et al., 2007), suggests the possibility of the loss of *stx* genes during sub-culturing or long-term storage as reported by others (Karch et al., 1991; Joris et al., 2011). Bovine faeces are known to contain PCR inhibitors, which can affect the quality and quantity of the DNA obtained, thereby decreasing the sensitivity of the assay (Rapp, 2010; Verhaegen et al., 2016). To mitigate this factor, we attempted to optimise our methods to provide high levels of DNA quantity and purity from bovine faeces (Inglis et al., 2004; Cook and Britt 2007), based on others' findings that demonstrated the survival and detection of STEC up to 10 weeks of storage at 4-5ºC (Bach et al., 2005) using enrichment procedures to support survival and detection of STEC for both low and high inoculums (Wang et al., 1996).

The main limitations of this study were the small sample size and attrition of study subjects which affected our ability to accurately describe the dynamics of non-O157 STEC super-shedding and to identify potential risk factors from the environmental samples and the abattoir follow-up study (Gustavson et al., 2012). Additionally, assessment of seasonal and geographic patterns of STEC prevalence could not be undertaken due to the limited time period in which this study was done.

In conclusion, our study demonstrated persistent and intermittent super-shedding of STEC O157 and non-O157 in cattle in a feedlot and at the abattoir just before slaughter in Gauteng, South Africa. This resulted in continual environmental contamination and risk of re-circulation of the pathogen in the herd, leading to potential contamination along the food chain. The high counts of non-O157 STEC and the diversity of serogroups show that super-shedding is not limited to STEC

O157. Thus, this study confirms that cattle are a major reservoir of STEC potentially zoonotic for humans. There is need for further investigation of the dynamics of super-shedding in cattle in South Africa and to devise mitigation strategies to reduce the risk of contamination of the food chain or the environment and in turn reduce the risk of human infection.

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