Shiga-toxin-producing *Escherichia coli* contamination of raw beef and beef-based ready-to-eat products at retail outlets in Pretoria, South Africa

Libby O. Onyeka[1,2], Abiodun A. Adesiyun[1], Karen H. Keddy[3], Evelyn Madoroba[4,5], Ayanda Manqele[1,4], Peter N. Thompson[1*]

[1]Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

[2]Department of Veterinary Public Health and Preventive Medicine, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State Nigeria.

[3] Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

[4]Agricultural Research Council – Onderstepoort Veterinary Research, Onderstepoort, South Africa

[5]Department of Biochemistry & Microbiology, University of Zululand, Private Bag X1001, KwaDlangezwa 3886, KwaZulu Natal province, South Africa

HIGHLIGHTS

- STEC contamination was assessed in raw beef and RTE beef products in retail outlets
- A high prevalence of serologically diverse non-O157 STEC strains was detected
- Minced beef and boerewors had the highest prevalence of STEC contamination
- The TAPC of 77% of samples exceeded the South African standard for export

* Corresponding author. Tel: +27-12-5298290; Fax: +27-12-5298315; (Peter.Thompson@up.ac.za),

ABSTRACT

A cross-sectional study was conducted to determine the prevalence of, and factors associated with, Shiga-toxin-producing *Escherichia coli* (STEC) in raw beef and ready-to-eat (RTE) beef products sold in 31 retail outlets in Pretoria and environs, South Africa. A total of 463 beef and RTE samples were screened for four STEC virulence genes (*stx*$_1$, *stx*$_2$, *eaeA, HlyA*) and seven O-serogroups (O113, O157, O26, O91, O145, O111 and O103) using multiplex PCR. The total aerobic bacteria count (TAPC) per g was determined. A total of 38 STEC isolates were recovered and characterized by conventional PCR and serotyping. The overall prevalence of STEC in the beef and RTE samples tested was 16.4% (76/463; 95% CI 13-20%). The prevalence of STEC differed by product-type (P<0.0001), with highest prevalence (35%) detected in boerewors (sausage); the prevalence in minced beef, brisket, RTE cold beef and biltong was 18%, 13%, 9% and 5% respectively. The most frequently detected genotype of *stx* was *stx*$_2$ (13%), and STEC serogroups from recovered isolates were detected at the following prevalences: O2 (15%), O8 (12%), O13 (15%), O20 (8%), O24 (3%), O39 (3%), O41 (8%), O71 (3%), O76 (3%) O150 (12%) and O174 (3%), A high proportion (77%) of the samples had TAPC that exceeded the South African microbiological standards for meat export (5.0 $\log_{10}$ CFU/g). This study demonstrates the prevalence of O157 (16%) and a diversity of non-O157 STEC serogroups detected in five common beef-based products from retail outlets in South Africa, suggesting their exposure to multiple contamination sources during carcass processing and/or cutting and handling at retail outlets. This provides a direct estimate of the potential risk posed to consumers of under-cooked contaminated products and indicates the need to improve sanitary practices during slaughter and processing of beef and beef-based RTE products. It may be prudent to institute a risk-based surveillance system for STEC.

Key words: Beef products, Contamination, *Escherichia coli*, Shiga-toxin, Retail outlet, Multiplex PCR

Despite growing international awareness and concerns about food safety, zoonotic foodborne diseases remain a significant risk to global health. It has been estimated that up to 61% of infectious diseases in humans have been associated with zoonotic agents *(5, 25)*. Furthermore, about 75% of new emerging or re-emerging human diseases have been linked to pathogens originating from animals or products of animal origin *(26, 52).* Livestock act as asymptomatic carrier and reservoir host of important enteric foodborne bacterial organisms *(56).*

Shiga-toxin-producing *Escherichia coli* (STEC) is a zoonotic foodborne enteric bacterial pathogen that can cause both sporadic and epidemic disease in human. Globally, STEC has been estimated to cause 2,801,000 acute illnesses, 3890 cases of hemolytic uremic syndrome (HUS), 270 cases of permanent end stage renal disease (ESRD), and 230 deaths annually *(36).* Symptoms range from mild diarrhea to haemorrhagic colitis (HC) with intense bloody diarrhea and in 5-10% of severe cases progresses to haemolytic uremic syndrome (HUS) *(14, 32).* These symptoms are caused mainly by the production of Shiga-toxins, which are phage-encoded AB_5 bacterial protein toxins that comprise two major distinct subgroups, namely the *stx*$_1$ subgroup and the *stx*$_2$ subgroup (*42).*

Not all STEC strains are pathogenic, but STEC O157 and some non-O157 STEC strains are unusually infectious because of their low infectious dose (10-100 cells), which has been attributed to their capacity to survive in acidic environment *(33, 52)*. This coupled with their association with ruminants, particularly cattle as the principal reservoir make the organism an important food safety threat. STEC strains have been associated with human disease through the consumption of undercooked beef or beef-based products *(32,39, 52)*, which have been contaminated by cattle feces during slaughter or processing due to cross-contamination from the hide and to a lesser extent from gut contents *(25, 38).* Non-O157 STEC are gaining relevance and certain serogroups have been frequently associated with sporadic outbreaks, causing illness comparable in severity with O157-induced disease *(20,50)*. In South Africa and other southern African countries, the importance of the pathogen has been highlighted in numerous clinical cases of diarrhea in children and adults between 2006-2013 in which a diverse range of STEC serogroups (O4, O5, O21, O26, O84, O111, O113, O117 and O157) were incriminated *(31, 58)*. A recent example was the report in South Africa in

which STEC O26:H11 was identified in a cluster of HUS cases in 2017 *(57),* thus indicating the growing importance of this pathogen in the region**.**

Meat retail outlets also play an important role in transmission of STEC-contaminated raw beef and ready-to-eat (RTE) beef products *(2)*. This could arise at several stages such as during meat cutting and further processing, for example, minced beef or sausages made from minced beef. A few colonized livestock or contaminated carcasses could in turn contaminate a great quantity of ground beef (*17).*

Few reports have been published on STEC-associated diseases linked to beef in South Africa. However, because of the STEC-associated illness reports in other countries, contamination of beef products may be a significant risk factor in South Africa. The prevalence of STEC, particularly the non-O157 strains, in beef products and the risk they pose to consumers of beef products in South Africa is largely unknown. Therefore, this study was conducted to determine the prevalence and risk factors for STEC contamination in five commonly consumed beef and beef-based products from butcheries and retail outlets (i) to determine the microbial load (total aerobic plate count [TAPC]) and its relationship to STEC prevalence and (ii) to characterize the STEC isolates regarding their serogroups and the presence of virulence genes, with a focus on genes *stx*$_1$, *stx*$_2$, *eaeA,* and *hlyA*.

MATERIALS AND METHODS

Study design and sample size

A cross-sectional study was conducted using a list of red meat retail outlets in the Pretoria area. The sample size for simple random sampling was calculated using the formula *(60)*:

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

where P_{exp} is the expected prevalence (0.5) and d is the desired precision (0.05), giving a required sample size of 385. To account for clustering (5 samples per outlet), this was multiplied by a design

effect of 1.2 (assuming an intra-cluster correlation coefficient of 0.05) to give a total sample size of 462.

Thirty-one retail outlets were selected by simple random sampling, stratified by geographical location (north, south, central, east, and west Pretoria) and by size (large chain supermarkets and butcheries), to ensure a representative sample (Table 1). Each outlet was visited three times in 2016: autumn, winter, and summer. Sample type selection was based on consumer preference *(55)* and included five types of popular beef and beef products in South Africa. Of the five types of beef and beef products tested, three were raw beef: brisket, minced beef, and boerewors, which is a South African fresh sausage always containing beef (90%) and sometimes mutton, pork, or both and with 10% spices. Two of the products were RTE cold beef (cold, cooked, and sliced) and biltong (dried, salted, and spiced). Based on availability, a minimum of 50 g of each product was purchased from each outlet and transported to the laboratory on ice.

Table 1. Red meat retail outlets in Pretoria, South Africa, that were sampled in a 1-year cross-sectional study during autumn, winter, and summer

	Large chain supermarkets		Butcheries	
Pretoria region	No. of outlets	Total samples	No. of outlets	Total samples
Central	2	30	2	24
East	4	60	2	27
North	6	93	5	78
South	3	45	3	39
West	2	21	2	30
Total	17	249	14	198

Determination of TAPC

Tenfold serial dilutions of samples were made in phosphate-buffered saline, and the pour plate method on plate count agar was used to determine TAPC. Aerobic bacteria were

enumerated with the viable plate count method. Any plate with >300 colonies was recorded as too numerous to count at that dilution. Viable counts were expressed as CFU per gram using the procedure described in ISO 4833:2003 ("Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Microorganisms—Colony-Count Technique at 30 degrees C," International Organization for Standardization, Geneva, Switzerland).

Detection of STEC

All broth enrichment cultures were made following the *Bacteriological Analytical Manual* protocol *(23)* with slight modifications. The sample (25 g) was aseptically weighed into a sterile bag (Stomacher 400, Seward, Worthing, UK), 225 mL of buffered peptone water modified with pyruvate (mBPWp; HiMedia Laboratories, Mumbai, India) was added, and the sample was homogenized. The homogenate was incubated at 37 ± 1°C for 5 h as a preenrichment step, 1 mL (per 225 mL of broth) of supplement was added (10 mg of acriflavin, 10 mg of cefsulodin, and 8 mg of vancomycin; HiMedia Laboratories), and this mixture was incubated at 42°C for 18 to 24 h.

Extraction of DNA template from broth cultures

A 1-mL aliquot of inoculated enrichment broth was incubated overnight and then transferred into a 1.5-mL sterile centrifuge tube for DNA extraction using with a Quick-gDNA MiniPrep Kit (Zymo Research, Irvine, CA) according to the manufacturer's instructions. The DNA extracts were stored at −20°C and subsequently used for screening by PCR assay.

Multiplex PCR to detect virulence genes

Extracted DNA samples were analyzed for the presence of *stx*$_1$, *stx*$_2$, *eaeA,* and *hlyA* genes with a multiplex PCR (mPCR) method as previously described *(48)*. The published oligonucleotide primers *stx*$_1$, *stx*$_2$, *eaeA,* and *hlyA* were commercially synthesized (Inqaba Biotechnologies, Pretoria, South Africa) and reconstituted to 100 μM. mPCR was conducted

in a 25-µL volume containing 2 to 4 µL of nucleic acid template (ca. 30 to 50 ng/µL DNA), 12.5 µL of DreamTaq Green PCR Master Mix (2×; Thermo Fisher Scientific, Waltham, MA), and 0.25 µM concentrations of each primer. The primer sequences, amplicon sizes, mPCR thermocycler program, and the electrophoresis gel procedures were the same as previously described *(48)*.

Validation of PCR

The assay conditions were optimized using molecular control strains obtained from NICD-CED, Johannesburg, South Africa (2014-2015 VTEC EQA—*E. coli* RR18-3022 O157, *eaeA*, stx_{1a}, stx_{2a}) and the enrichment control strain *E. coli* ATCC 43888 (O157:H7) stx_1. The mPCR was validated by Sanger sequencing of PCR products. Only broth enrichments that had stx_1 and/or stx_2 were considered positive for STEC and used in molecular serotyping.

Multiplex PCR detection of STEC serogroups

All DNA from samples positive for Shiga toxin–encoding genes (*stx*) based on initial mPCR screening were further evaluated for clinically relevant STEC serogroups, defined as serogroups implicated in foodborne illnesses: O113, O157, O26, O91, O145, O111, and O103 *(50, 62)*. We used a different mPCR assay as previously described *(62)* with slight modifications. The published oligonucleotide primers were commercially synthesized (Inqaba Biotechnologies) and reconstituted to 100 µM. The mPCR was carried out in a 25-µL volume containing 2 to 4 µL of nucleic acid template (ca. 30 to 50 ng/µL DNA), 12.5 µL of Dream*Taq* Green PCR Master Mix (2×), and 0.25 µM concentrations of each primer. The thermocycler program (Mastercycler, Eppendorf Biotech, Hamburg, Germany) and gel electrophoresis were previously described *(48)*.

Isolation of STEC

Only broth enrichment cultures (76 of 463 samples, 16%) for which stx_1 and/or stx_2 were detected by PCR were considered positive for STEC and selected for isolation. To isolate

O157 STEC strains, an immunomagnetic separation protocol was used with anti–*E. coli* O157 Dynabeads (Thermo Fisher Scientific) as recommended by the manufacturer. The immunoconcentrated bacterial suspension was inoculated onto sorbitol MacConkey agar (Oxoid, Basingstoke, UK) supplemented with 2.5 mg/L potassium tellurite and 0.05 mg/L cefixime (HiMedia Laboratories). A 10-µL aliquot of the enriched broth sample was streaked on a chromogenic agar (CHROMagar O157, CHROMagar, Paris, France) also supplemented with 2.5 mg/L potassium tellurite and 0.05 mg/L cefixime. The plates were incubated for 24 to 30 h at 37°C. Representative colonies that exhibited different phenotypic characteristics were selected from each plate and tested by latex agglutination (Remel Wellcolex *E. coli* O157 rapid latex agglutination test, Thermo Fisher Scientific). The enrichment control strain *E. coli* O157:H7 ATCC 43888 was also inoculated for phenotypic control and assessment. All isolates identified as *E. coli* O157 were streaked onto nutrient agar and incubated overnight at 37°C for confirmation by mPCR as described above.

To isolate non-O157 STEC, 10 µL of each enriched broth sample was streaked on MacConkey agar with crystal violet and salt (Merck Millipore, Johannesburg, South Africa) and onto CHROMagar STEC. Plates were incubated for 24 to 30 h, and representative suspect colonies were streaked on nutrient agar plates for further biochemical testing using the Remel Kovacs indole reagent (Thermo Fisher Scientific). For biochemical confirmation, 10 of the 16 nontypeable isolates were randomly selected and confirmed as *E. coli* using the Vitek 2 compact system (bioMérieux, Marcy l'Étoile, France). Indole-positive isolates were further confirmed with the mPCR assay as described above for the broth enriched culture.

Conventional serotyping of isolates

Following PCR confirmation of STEC isolates, serotyping was conducted at the bacteriology laboratory of the NICD-CED using the conventional serotyping method *(47)*. Serotyping was carried out only for the O-surface antigen (lipopolysaccharides) using the complete *E. coli* O-

pool of polyvalent antisera, the AA-WW antisera range from Statens Serum Institute (Copenhagen, Denmark), according to the manufacturer's instruction. 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 nontypeable.

Statistical Analysis

Conventional PCR results of the enrichment broth samples were used to estimate the prevalence of STEC contamination. A sample was considered STEC positive when it was positive for at least one of the *stx*$_1$ or *stx*$_2$ genes. Prevalence was calculated by sample type and serogroup, with 95% exact binomial confidence limits. Association of each potential risk factor with STEC contamination was assessed by cross-tabulation, odds ratios (ORs), and the Fisher's exact test. Factors considered were product type (biltong, boerewors, brisket, cold beef, and minced beef), season (summer, winter, and autumn), geographic location (central, east, north, south, and west Pretoria), and outlet type (large chain supermarkets and butcheries). A multivariable logistic regression model was used to estimate the association of each variable with STEC contamination, adjusting for confounding. The association between TAPC quintiles and STEC contamination was assessed using cross-tabulation and Fisher's exact test. Data were analyzed with Stata 14 (StataCorp, College Station, TX), and the significance level was set at 5%.

RESULTS

Prevalence and risk factors of STEC

Of the 463 broth-enriched samples collected from 31 retail outlets, 16% (95% confidence interval [CI]: 13 to 20%; 76 samples) were positive for one or more *stx* genes with the mPCR assay (Table 2). The highest STEC prevalence (35%) was observed in boerewors and then minced beef (18%), and the lowest prevalence was in biltong (5%) ($P < 0.001$) (Table 2).

Prevalence of contamination differed between beef sample types within the same season (Table 3). Minced beef had the highest STEC prevalence (39%) in autumn, and boerewors had the highest STEC prevalence in summer (47%). However, overall prevalence did not differ among the three seasons (P = 0.369). STEC prevalence also differed by location (P = 0.024); beef products from east Pretoria had the highest prevalence of STEC (27%) of all five locations. Prevalence did not differ significantly between butcheries (14%) and large chain supermarkets (18%) (P = 0.136). Compared with other sample types, boerewors had the highest STEC prevalence in both types of outlets: 37% in butcheries and 33% in large chain supermarkets. In the multivariable logistic regression model (Table 4), the odds of STEC contamination was significantly higher in boerewors (OR = 10; $P < 0.001$) and in minced beef (OR = 4.0; P = 0.007) compared with biltong. Odds of STEC contamination also differed significantly by location; odds were 4.3 times higher in east Pretoria than in central Pretoria (P = 0.008).

Table 2. Overall prevalence and molecular serogroups of Shiga toxin-producing *Escherichia coli* (STEC) detected in broth enriched culture of five beef sample types from retail outlets in Pretoria, South Africa

STEC Prevalence in total sample (N = 463)								
Sample type	*N*	*Freq*	*%*	*95% CI*	*stx_1 (%)*	*stx_2 (%)*	*eaeA (%)*	*hlyA (%)*
Brisket	88	11	13	6-21	10	6	14	5
Boerewors[a]	92	32	35	25-45	22	26	14	20
Mince	123	22	18	12-26	11	16	7	5
Cold beef[b]	68	6	9	3-18	6	6	3	0
Biltong	92	5	5	2-12	1	5	5	2
Total	*463*	*76*	*16*	*6-21*	*10*	*13*	*9*	*6*

Prevalence (%) of molecular serogroups in STEC positive samples (n = 76)								
	n	*O113*	*O157*	*O26*	*O91*	*O145*	*O111*	*O103*
Brisket	11	0	0	0	0	0	0	0
Boerewors[a]	32	19	16	3	3	3	0	0
Mince	22	23	23	9	5	0	9	0
Cold beef[b]	6	0	33	0	0	0	0	0
Biltong	5	40	0	20	0	0	0	0
Total	*76*	*17*	*16*	*5*	*3*	*1*	*3*	*0*

[a]Boerewors – raw south African spiced beef sausage

[b]Cold beef – cold cooked sliced beef

Table 3. Prevalence of Shiga-toxin-producing *Escherichia coli* (STEC) in different seasons in five beef sample types from retail outlets in Pretoria, South Africa

Season	Sample types	N	freq	%	95% CI	*stx*$_1$ *(%)*	*stx*$_2$ *(%)*	*eaeA (%)*	*hlyA (%)*
Autumn	Brisket	25	2	8	1-26	4	8	0	0
	Boerewors[a]	31	9	29	14-48	16	26	0	0
	Mince	31	12	39	22-58	23	39	0	0
	Cold beef[b]	19	0	0	0-0	0	0	0	0
	Biltong	31	4	13	4-30	0	13	0	0
	Total	*137*	*27*	*20*	*13-27*	*9*	*19*	*0*	*0*
Winter	Brisket	38	6	16	6-31	16	5	0	0
	Boerewors[a]	31	9	29	14-48	10	19	10	6
	Mince	62	6	10	4-20	6	10	3	3
	Cold beef[b]	32	6	19	7-36	13	13	0	0
	Biltong	31	0	0	0-0	0	0	0	0
	Total	*194*	*27*	*14*	*9-20*	*9*	*9*	*3*	*3*
Summer	Brisket	25	3	12	3-31	8	4	48	16
	Boerewors[a]	30	14	47	28-66	40	33	33	53
	Mince	30	4	13	4-31	10	7	23	13
	Cold beef[b]	17	0	0	0-0	0	0	12	0
	Biltong	30	1	3	0-17	3	3	13	3
	Total	*132*	*22*	*17*	*11-24*	*14*	*11*	*27*	*19*

[a] Boerewors – raw South African spiced beef sausage

[b] Cold beef – cold, cooked, sliced beef

Table 4. Multivariable logistic regression model of factors associated with Shiga-toxin-producing *Escherichia coli* (STEC) contamination in retail outlets in Pretoria, South Africa

Variable	Level	Odds ratio (OR)	95% CI (OR)	P value
Sample type	Biltong	1*		
	Brisket	2.7	0.9- 8.0	0.086
	Boerewors[a]	10	3.7 – 28	0.001
	Cold beef[b]	1.5	0.4 - 5.0	0.512
	Mince	4.0	1.5 -11.7	0.007
Season	Autumn	1*		
	Summer	0.8	0.4 - 1.5	0.459
	Winter	0.6	0.3 - 1.2	0.173
Location	Central	1*		
	East	4.3	1.5 - 12.1	0.008
	North	1.5	0.5 - 4.3	0.472
	South	2.6	0.9 - 7.9	0.082
	West	1.8	0.5 - 6.0	0.365
Retail outlet	Butchery	1*		
	Supermarket	1.4	0.8 - 2.4	0.240

[a] Boerewors – raw South African spiced beef sausage

[b] Cold beef – cold, cooked, sliced beef

*Reference Level

STEC prevalence and TAPC

Among the 341 samples evaluated for TAPCs, the highest median count was obtained in cold beef (cold, cooked, and sliced; 6.75 log CFU/g) followed by minced beef (6.3 log CFU/g),

and the lowest was obtained in brisket (5.53 log CFU/g). The majority (77%) of meat TAPCs exceeded the South African microbiological standard for meat for export (5.0 log CFU/g) *(59)*. No association was found between TAPCs and STEC contamination for any of the sample types.

Molecular serotyping of STEC

The 76 enrichment broths that were STEC positive by mPCR yielded seven STEC O serogroup–associated genes (Table 2). Only 45% (34 of 76 samples) were positive for the O group markers: serogroup O113 was predominant (17%; 13 samples) closely followed by serogroup O157 (16%; 12 samples) (Table 2). Only one sample type (minced beef) contained a combination of two O group markers (O113 and O157). Serogroup O103 was not detected in any of the 76 samples tested.

Detection of virulence genes

The virulence profiles of the 76 broth-enriched cultures (Table 2) revealed that the most common was *stx*$_2$ (13%) followed by *stx*$_1$ (10%). The prevalence of *eaeA*-positive samples was highest in brisket (14%) and boerewors (14%). The combination *stx*$_2$ and *eaeA* was detected only once, in boerewors, and the combination *stx*$_1$, *stx*$_2$, and *eaeA* was detected only twice, in minced beef. The combination of all four genes was detected only four times: once in biltong and three times in boerewors.

Table 5. Description of molecular characteristics of Shiga-toxin-producing *Escherichia coli* (STEC) isolates and serogroups in Pretoria, South Africa.

Sample type[a]	Season	Location	Source[b]	*stx*$_1$	*stx*$_2$	*eaeA*	*hlyA*	serogroup[c]	freq
Mince	Autumn	East	B	-	+	-	-	ONT	1
Boerewors	Autumn	North	L	-	+	-	-	O20	2
Mince	Autumn	South	L	-	+	-	-	ONT	1
Biltong	Winter	South	B	+	+	-	-	O41	2
Boerewors	Autumn	East	L	-	+	-	-	ONT	1
Boerewors	Autumn	East	L	-	+	-	-	O178	1
Mince	Autumn	East	L	-	+	-	-	O178	2
Mince	Autumn	North	B	+	+	-	-	ONT	1
Mince	Autumn	North	B	+	+	-	-	ONT	1
Mince	Autumn	North	B	-	+	+	-	O174	1
Brisket	Autumn	South	L	+	-	-	-	O13	1
Boerewors	Autumn	North	L	+	+	+	-	O150	4
Boerewors	Autumn	North	L	+	-	-	-	O39	1
Boerewors	Autumn	North	L	+	+	-	-	O150	1
Boerewors	Autumn	North	L	+	+	-	-	ONT	2
Boerewors	Autumn	North	L	+	+	+	-	ONT	1
Biltong	Autumn	South	L	-	+	-	-	O2	1
Mince	Autumn	East	L	-	+	-	-	ONT	4
Mince	Autumn	East	L	-	+	-	-	O71	1
Boerewors	Autumn	North	B	-	+	-	-	ONT	1
Boerewors	Winter	South	B	+	+	-	-	ONT	1
Boerewors	Winter	South	B	+	-	-	-	O76	1
Boerewors	Summer	West	L	-	+	-	+	O8	1
Boerewors	Summer	West	L	-	+	-	+	O24	3
Boerewors	Autumn	North	L	-	+	-	+	ONT	1
Boerewors	Autumn	North	L	+	+	-	-	ONT	1

[a] Sample Types (Boerewors – raw South African spiced beef sausage)

[b] Source (L = large chain supermarket, B = butchery);

[C] Serogroup (ONT – O-antigen not typeable)

Serotyping and virulence profiles of STEC isolates

Of the 76 PCR-positive broth enrichment cultures, only 21% (16 samples) yielded isolates. The 38 isolates were recovered from the following sample types: 50% from boerewors (8 of 16 total samples), 31% from minced beef (5 of 16), 13% from biltong (2 of 16), and 6% from brisket (1 of 16). Only 26 samples (68.4%) were typeable according to previously published methods *(47),* comprising 12 non-O157 O serogroups. Of the typeable isolates, the clinically relevant serogroups detected were O2 (four isolates), O178 (three isolates), O8 (three isolates), and O174 (one isolate). The frequency of *stx* genotypes for both typeable and nontypeable isolates were as follows: 45% for *stx*$_1$ (17 isolates), 84% for *stx*$_2$ (32 isolates), 24% for *stx*$_1$ plus *stx*$_2$ (9 isolates), 8% for the intimin-coding *eaeA* gene (3 isolates), and 13% for the *hylA* gene encoding enterohemolysins (5 isolates). Only three distinct gene combinations were noted in the virulence profiles: *stx*$_1$, *stx*$_2$, and *eaeA* (two isolates), *stx*$_2$ and *eaeA* (one isolate), and *stx*$_2$ and *hlyA* (five isolates) (Table 5).

DISCUSSION

This study provides epidemiological data on the microbial quality of five common beef-based products from butcheries and large chain supermarkets in Pretoria and nearby areas and the STEC prevalence, virulence, and serogroups associated with these products. The molecular analysis of the broth enrichment cultures revealed a 16% prevalence of STEC contamination, which is important for food safety and provides a direct estimate of the potential risk posed to consumers. This STEC prevalence in beef products may be indicative of the prevalence of the pathogen in beef abattoirs where the products originated; contamination of beef carcasses with STEC commonly occurs during hide removal and occasionally from gastrointestinal tract spillage *(38)*. However, contamination with STEC strains could also occur at the retail outlets during processing and handling *(11)*..

The mPCR for detecting STEC and virulence genes in broth cultures may be the best method when isolation is not achievable or lacks sensitivity *(11, 37)*. Our detection of STEC in 16% of samples is comparable to the 15% prevalence reported in a PCR screening survey of retail red meats in New Zealand *(52)*. However, a wide range of prevalences has been reported from other countries: 36.1% in Argentina *(11)*, 8.4% in Italy *(63)*, 11% in France *(51)*, and 0.1 to 54.2% in the United States *(29, 53)*.

The fairly high prevalence of STEC in boerewors, minced beef, and fresh beef cuts could pose a food safety risk to South African consumers given the prevalent *braai* (barbecue) culture *(18)*, where boerewors and fresh beef cuts are very popular *(16, 55)*. Minced beef and boerewors may be prepared from multiple carcasses; thus, a few colonized animals could contaminate a large quantity of minced beef *(17)*. Boerewors contains spices and often mutton, pork, or both, all of which may serve as sources of STEC and other microbial contamination. These other components of boerewors may have contributed to the high STEC prevalence and TAPCs detected in boerewors in this study.

The size of the retail outlets (large chain supermarket versus butcheries) where the products were purchased did not have a significant effect on the prevalence of STEC in the products. This finding is in agreement with that of studies on the prevalence of STEC in products sold at supermarkets and butcheries, in which no significant differences between the two retail sources were found in New Zealand *(10)*, Ireland *(12)*, and Namibia *(44)*. Location differences in STEC prevalence have been reported *(40, 64)*, but the reason for a higher STEC prevalence in east Pretoria than in other locations is unclear because abattoirs that supply the retail outlets in this area get their cattle from multiple locations across Pretoria and nearby areas.

Indicator bacteria on cattle carcasses and beef have been used to assess the hygiene status of the processing area or butchery *(50)* based on multiple factors, including slaughter hygiene, processing, and direct or indirect fecal contamination of beef. The TAPCs in 77% of

the samples in the present study exceeded the maximum acceptable limit for meat for export *(59),* which may indicate a risk with respect to meat hygiene along the beef chain from abattoir to retail outlets. These samples also may have contained foodborne pathogens not evaluated in the present study, and high TAPCs in raw meat and RTE meat has been associated with the presence of pathogenic *Staphylococcus (7, 60), Salmonella (4),* and *Listeria (26, 54)*. Our findings are comparable to counts reported for red meat samples from the deboning room of a high-throughput abattoir (7.2 log CFU/g) in Free State, South Africa *(43)*. Numerous factors have been associated with high microbial loads in beef and beef products sold in retail outlets, including transfer of contamination from the hide surface to the beef carcass during processing at abattoirs *(3)* and contact surface contamination from working surfaces, knives, butchers' hands, etc., during processing *(41)*. The lack of association between TAPC and the prevalence of STEC in our study is an indication that STEC contamination may occur regardless of the presence of indicator bacteria. However, in some studies an association between TAPC and STEC contamination has been found *(3)*. The presence of STEC in commercially sold beef and RTE beef products that are deemed to have met the stipulated microbiological standards in South Africa underscores the need for a risk-based surveillance system specifically for STEC in food samples in South Africa..

The high prevalence of the STEC serogroups O113 (17%) and O157 (16%) in both beef and beef-based products in the present study is of significance because both serogroups have been frequently associated with disease outbreaks and occasional cases of hemolytic uremic syndrome *(8, 49)*. The relatively high prevalence of these two serogroups presents a potential health threat. STEC O157 strains have been reported in retail beef in South Africa *(1),* but to our knowledge this is the first report of serogroup O113 from retail beef, although it has been associated with human disease in this country *(31)*. Serogroup O113 is the most common STEC serogroup found in beef in the United States *(9, 22).*

Of the 38 STEC isolates recovered in our study, only 3 yielded two unique combinations: s*tx*$_1$, s*tx*$_2$, and *eaeA* (2 isolates) in association with serotypes O150 and O nontypeable, and s*tx*$_2$ and *eaeA* (1 isolate) in association with serotype O174. The pathogenicity of other isolates remains unclear because the present study did not include evaluation of an important adherence factor coded by the *aggR* regulatory gene or other putative factors such as *saa, lpfA,* and *ampA (24)*. Not all STEC strains are pathogenic, and a combination of s*tx*$_2$ and an adhesion factor, principally the intimin protein (*eaeA)* or the *aggR* regulatory gene *(6, 24),* has been recommended as a sufficient predictor of health risk or pathogenicity.

In the present study, isolates belonging to non-O157 serogroups were recovered, but none belonged to the frequently isolated non-O157 "big six" (O26, O45, O103, O111, O121, and O145 in the United States and O26, O103, O111, and O145 in Europe) *(50),* which cause illnesses comparable to those caused by O157 strains. Mainga et al. *(35)* found big-six serogroups (O26, O45, O103, O121, O145, and O157) in cattle feces in South Africa; however, none of these serogroups were detected in our study. We identified four non-O157 serogroups of clinical relevance: O8, O174, O178, and O2. Serogroup O8 is ranked among the 20 most commonly isolated serogroups associated with human disease in Europe *(20)* and the United States *(14, 50)*. This serogroup has also been reported in pigs *(27)* and in human stool samples *(30)* in South Africa. Serogroups O174 and O178 detected in minced beef and boerewors in our study have also been documented in the same type of beef products in Argentina *(34)* and have been implicated in human clinical cases in the United States *(14, 50)*. Serogroup O2 has been recovered in pasteurized and raw milk in South Africa *(46)*. Although none of these four non-O157 STEC serogroups detected in our study have been isolated from clinical cases in South Africa, they may be emerging foodborne pathogens, given that STEC serogroups differ across geographic regions *(40)*.

The 38 isolates in this study were recovered from only 16 PCR-positive broth cultures. This low rate of recovery of STEC isolates from food has been reported previously *(11, 45, 63)* and could be owing to several factors, including low numbers of cells and competing microflora in food samples *(51, 53),* cell stress or sublethal cell injury *(21, 28*), and the high sensitivity of the PCR assay, which can detect free *stx*-carrying phages in meat samples *(51, 53)*. Hence, caution is needed when interpreting the low recovery of STEC isolates, including our failure to recover STEC O157. For the isolation of non-O157 STEC, the use of O group–specific immunomagnetic separation beads for serotypes O26, O45, O103, O111, O121, and 145 could have increased our recovery of these big six serogroups. Further testing of the *stx*-negative samples, e.g., by whole genome sequencing, could have improved the sensitivity of detection in this study.

In conclusion, our study revealed a high prevalence of a serologically diverse group of STEC strains in raw beef and RTE beef products sold in retail outlets in the Pretoria area in South Africa. Serogroups O113 and O157 were predominant, indicating a potential food safety risk to consumers. Minced beef and boerewors had the highest risk of being vehicles of STEC transmission to consumers. The evaluated samples were contaminated with 12 serogroups, including 5 that have not previously been reported in cattle or beef in South Africa. Our study demonstrates the need for a risk-based surveillance system that focuses on identifying specific foodborne pathogens in the beef chain *(13).*

ACKNOWLEDGEMENTS

The study was funded in part by Red Meat Research and Development-South Africa (RMRD-SA).

REFERENCES

1. Abong'o, B. O., and M. N. Momba. 2009. Prevalence and characterization of *Escherichia coli* O157: H7 isolates from meat and meat products sold in Amathole District, Eastern Cape Province of South Africa. *Food Microbiol.* 26:173-176.

2. Ahmed, H. A., E. T. MacLeod, R. M. El Bayomi, R. A. Mohsen, and A. H. Nassar. 2017. Molecular Characterization of *Escherichia coli* O157: H7 and non-O157 Shiga-toxin-producing *E. coli* from Retail Meat and Humans. *Zagazig Vet. J.* 45

3. Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L. Wheeler, M. P. Kent, D. Jaroni, B. Pauling, D. M. Allen, and M. Koohmaraie. 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *J. Food Prot.* 67:658–665.

4. Bantawa, K., K. Rai, D. Subba-Limbu, and H. Khanal. 2018. Foodborne bacterial pathogens in marketed raw meat of Dharan, eastern Nepal. *BMC Res. Notes*. 11:618.

5. Behravesh, C. B., I. T. Williams, and R. V. Tauxe. 2012. Emerging foodborne pathogens and problems: expanding prevention efforts before slaughter or harvest. In Improving food safety through a one health approach: workshop summary. Natl. Acad. Press USA.

6. Beutin, L., and A. Martin. 2012. Outbreak of Shiga-toxin-producing *Escherichia coli* (STEC) O104: H4 infection in Germany causes a paradigm shift with regard to human pathogenicity of STEC strains. *J. Food Prot.* 75:408-418.

7. Birhanu, S., and S. Menda. 2017. Microbial quality of retail raw meat in administrative towns of Gojjam area North-West Ethiopia with special reference of Gram positive cocci species. *Afri. J. Microbiol. Res*. 11:1538-1543.

8. Blanco, M., N. L. Padola, A. Krüger, M. E. Sanz, J. E. Blanco, E. A. González, G. Dahbi, A. Mora, M. I. Bernárdez, A. I. Etcheverría, and G. H. Arroyo. 2004. Virulence genes and intimin types of Shiga-toxin-producing *Escherichia coli* isolated from cattle and beef products in Argentina. *Int. Microbiol*, 7:269-276.

9. Bosilevac, J.M. and M. Koohmaraie. 2011. Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* isolates from commercial ground beef in the United States. *Appl. Environ. Microbiol.*, 77:2103-2112.

10. Brooks, H. J. L., B. D. Mollison, K. A. Bettelheim, K. Matejka, K. A. Paterson, and V. K. Ward. 2001. Occurrence and virulence factors of non-O157 Shiga-toxin-producing *Escherichia coli* in retail meat in Dunedin, New Zealand. *Lett. Appl. Microbiol.* 32:118-122.

11. Brusa, V., V. Aliverti, F. Aliverti, E. E. Ortega, J. H. de la Torre, L. H. Linares, M. Sanz, A. I. Etcheverría, N. L. Padola, L. Galli, and P. Garcia. 2013. Shiga-toxin-producing *Escherichia coli* in beef retail markets from Argentina. *Front Cell. Infect. Microbiol*. 2:171.

12. Cagney, C., H. Crowley, G. Duffy, J. J. Sheridan, S. O'brien, E. Carney, W. Anderson, D. A. McDowell, I. S. Blair, and R. H. Bishop. 2004. Prevalence and numbers of *Escherichia coli* O157: H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. *Food Microbiol.* 21:203-212.

13. Cameron, A. (2012). Manual of Basic Animal Disease Surveillance. African Union-Interafrican Bureau for Animal Resources (IBAR) ISBN 978-9966-1659-1-6., 100.

14. Centers for Disease Control and Prevention (CDC). 2008. Bacterial foodborne and diarrheal disease national case surveillance. Annual Report, 2006. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2008. Available at https://www.cdc.gov/nationalsurveillance/pdfs/combined-report-2006-508c.pdf. Accessed 15 May 2019.

15. Centers for Disease Control and Prevention (CDC). 2011. Vital signs: incidence and trends of infection with pathogens transmitted commonly through food-foodborne diseases active surveillance network, 10 U.S. sites, 1996-2010. *Periodical* 22:749-755.

16. Charimba, G. 2004. The incidence, growth and survival of diarrhoeagenic *Escherichia coli* in South African meat products. Doctoral dissertation, Univ. of the Free State.

17. Duffy, G., E. Cummins, P. Nally, S. O'Brien, and F. Butler. 2006. A review of quantitative microbial risk assessment in the management of *Escherichia coli* O157: H7 on beef. *Meat. Sci.* 74:76-88.

18. Erasmus, S.W., and L.C. Hoffman. 2017. What is meat in South Africa? *Anim. Front.* 7:71-75.

19. European Food Safety Authority (EFSA). 2012. Annual report on EFSA's food and feed safety crisis preparedness and response 2011. Supporting Publications 2012: EN-276. 16. Available online: www.efsa.europa.eu/publications Available at: https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/sp.efsa.2012.EN-276. Accessed 5 May 2019.

20. European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC). 2016. Technical report on multi-country outbreak of Shiga-toxin- producing *Escherichia coli* infection associated with haemolytic uraemic syndrome. EFSA supporting publication 2016: EN-1017. 13. Available at: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2016.EN-1017. Accessed 10 April 2019.

21. Farrokh, C., K. Jordan, F. Auvray, K. Glass, H. Oppegaard, S. Raynaud, D. Thevenot, R. Condron, K. De Reu, and A. Govaris. 2013. Review of Shiga-toxin-producing *Escherichia coli* and their significance in dairy production. *Int. J. Food Microbiol.* 162: 190–212.

22. Feng, P., S. Delannoy, D.W. Lacher, J.M.Bosilevac, and P. Fach. 2017. Characterization and Virulence Potential of Serogroup O113 Shiga Toxin–Producing *Escherichia coli* Strains Isolated from Beef and Cattle in the United States. *J. Food Prot. 80*:383-391.

23. Feng, P., S. D. Weagant, and K. Jinneman. 2011. Bacteriological Analytical Manual, Chapter 4A: Diarrheagenic *Escherichia coli*. available at https://www.fda.gov/food/laboratory-methods-food/bam-diarrheagenic-escherichia-coli. Accessed 20 June 2019

24. Food and Agriculture Organization (FAO) and World Health Organization (WHO). 2018. Shiga-toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and the monitoring the risk. Available at: http://www.fao.org/3/ca0032en/CA0032EN.pdf. Accessed 16 July 2019

25. Fremaux, B., C. Prigent-Combaret, and C.Vernozy-Rozand. 2008. Long-term survival of Shiga-toxin-producing *Escherichia coli* in cattle effluents and environment: an updated review. *Vet. Microbiol.* 132**:**1-18.

26. Hammons, S. R., M. J. Stasiewicz, S. Roof, and H. F. Oliver. 2015. Aerobic plate counts and ATP levels correlate with *Listeria monocytogenes* detection in retail delis. *J. Food Prot.* 78:825-830.

27. Henton, M. M., and M. M. Engelbrecht. 1997. *Escherichia coli* serotypes in pigs in South Africa. *Onderstepoort. J. Vet. Res.* 64:175-187.

28. Hoang Minh, S., E. Kimura, D. Hoang Minh, K. I. Honjoh, and T. Miyamoto. 2015. Virulence characteristics of Shiga-toxin-producing *Escherichia coli* from raw meats and clinical samples. *Microbiol. and immunology*, 59:114-122.

29. Hussein, H. S., and L. M. Bollinger. 2005. Prevalence of Shiga-toxin–producing *Escherichia coli* in beef cattle. *J. Food Prot.* 68:2224-2241.

30. Kalule, J. B. 2017. One Health-One City; the extent of Shiga-toxin-producing *Escherichia coli* in Cape Town. Doctoral dissertation, Univ. of Cape Town.

31. Karama, M., B.T. Cenci-Goga, M. Malahlela, A.M. Smith, K.H Keddy, S. El-Ashram, L.M Kabiru, and A. Kalake. 2019. Virulence Characteristics and Antimicrobial Resistance Profiles of Shiga-toxin-producing *Escherichia coli* Isolates from Humans in South Africa: 2006–2013. *Toxins*, 11:424.

32. Karmali, M. A., V. Gannon, and J. M. Sargeant. 2010. Verocytotoxin-producing *Escherichia coli* (VTEC). *Vet. Microbiol.* 140:360-370.

33. Large, T.M., S.T. Walk, and T.S. Whittam. 2005. Variation in acid resistance among Shiga toxin-producing clones of pathogenic *Escherichia coli*. *Appl. Environ. Microbiol.*, *71*:2493-2500.

34. Llorente, P., L. Barnech, K. Irino, M. V. Rumi, and A. Bentancor. 2014. Characterization of Shiga-toxin-producing *Escherichia coli* isolated from ground beef collected in different socioeconomic strata markets in Buenos Aires, Argentina. *BioMed. Res. Inter. 2014.*

35. Mainga, A.O., B.T Cenci-Goga., Malahlela, M.N., Tshuma, T., Kalake, A. and M. Karama. 2018. Occurrence and characterization of seven major Shiga-toxin-producing *Escherichia coli* serotypes from healthy cattle on cow–calf operations in South Africa. *Zoonoses Public Health 65*:777-789.

36. Majowicz, S.E., E. Scallan, A. Jones-Bitton, J. M. Sargeant, J. Stapleton, F. J. Angulo, D. H. Yeung, and M. D. Kirk. 2014. Global incidence of human Shiga-toxin–producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. *Foodborne Pathog. Dis.* 11:447-455.

37. Martínez-Castillo, A., and M. Muniesa. 2014. Implications of free Shiga-toxin-converting bacteriophages occurring outside bacteria for the evolution and the detection of Shiga toxin-producing *Escherichia coli*. *Front. Cell. Infect. Microbiol.* 4:46.

38. McEvoy, J. M., A. M. Doherty, J. J. Sheridan, F. M. Thomson-Carter, P. Garvey, L. McGuire, I. S. Blair, and D. A. McDowell. 2003. The prevalence and spread of *Escherichia coli* O157: H7 at a commercial beef abattoir. *J. Appl. Microbiol.* 95:256-266.

39. Mead, P. S., and P. M. Griffin. 1998. *Escherichia coli*O157: H7. *Lancet* 352**:** 1207-1212.

40. Mellor, G.E., N. Fegan, K. S. Gobius, H. V. Smith, A. V. Jennison, B. A. D'Astek, M. Rivas, S. Shringi, K. N. Baker, and T. E. Besser. 2015. Geographically distinct *Escherichia coli* O157 isolates differ by lineage, Shiga-toxin genotype, and total Shiga-toxin-production. *J. Clin. Microbiol.* 53:579-586.

41. Melngaile, A., E. Ciekure, and O. Valcina. 2014. Microbiological quality of meat preparations and meat products. *Proc. Baltic Confer. Food Sci. Tech. "FOODBALT",* Jelgava, Latvia (pp. 61-65).

42. Melton-Celsa, A. R. 2014. Shiga-toxin *(Stx)* classification, structure, and function. *Microbiol. Spectr.* 2(2): EHEC-0024-2013.

43. Nel, S., J. F. R. Lues, E. M. Buys, and P. Venter. 2004. Bacterial populations associated with meat from the deboning room of a high throughput red meat abattoir. *Meat. Sci.* 66:667-674.

44. Nkandi, J. 2017. Bacteriological quality and safety of raw beef from selected outlets in Windhoek. Namibia. Doctoral dissertation, University of Namibia.

45. Nobili, G., I. Franconieri, G. La Bella, M. G. Basanisi, and G. La Salandra. 2017. Prevalence of Verocytoxigenic *Escherichia coli* strains isolated from raw beef in southern Italy. *Int. J. Food Microbiol.* 257: 201–205.

46. Ntuli, V. 2017. Shiga-toxin-producing *Escherichia coli* O157 and non-O157 serotypes in producer-distributor bulk milk. Doctoral dissertation, University of Pretoria.

47. Ørskov, F. and Ørskov, I., 1984. 2 Serotyping of *Escherichia coli*. Methods in microbiology (14:43-112). Academic Press.

48. Paton, A. W., M. C. Woodrow, R. M. Doyle, J. A. Lanser, and J. C. Paton. 1999. Molecular Characterization of a Shiga-toxigenic *Escherichia coli* O113: H21 Strain Lacking *eae* Responsible for a Cluster of Cases of Hemolytic-Uremic Syndrome. *J. Clin. Microbiol.* 37:3357-3361.

49. Paton, A.W. and J.C. Paton, 1998. Detection and Characterization of Shiga toxigenic *Escherichia coli* by Using Multiplex PCR Assays *for* stx_1, stx_2, *eaeA,* Enterohemorrhagic *E. coli hlyA*, *rfb* O111, and *rfb* O157. *J. Clin. Microbiol.* 36:598-602.

50. Pihkala, N., N. Bauer, D. Eblen, P. Evans, R. Johnson, J. Webb, and C. Williams. 2012. Risk profile for pathogenic non-O157 Shiga-toxin-producing *Escherichia coli* (non-O157 STEC). US Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Office of Policy and Program Development.1-64. Available at: https://www.fsis.usda.gov/shared/PDF/Non_O157_STEC_Risk_Profile_May2012.pdf. Accessed 20 June 2019.

51. Pradel, N., V. Livrelli, C. De Champs, J. B. Palcoux, A. Reynaud, F. Scheutz, J. Sirot, B. Joly, and C. Forestier. 2000. Prevalence and characterization of Shiga-toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. *J. Clin. Microbiol.* 38:1023-1031.

52. Rivas, L., R. Lake, P. J. Cressey, N. J. King, B. Horn, and B. Gilpin. 2014. Risk profile (update): Shiga-toxin-producing *Escherichia coli* in red meat and meat products. Report Institute of Environmental Science and Research Limited ("ESR") ISBN No: 978-0-908334-15-5 (online). Available at: https://www.mpi.govt.nz/dmsdocument/7272-risk-profile-update-shiga-toxin-producing-escherichia-coli-in-red-meat-and-meat-products. Accessed 15 May 2019.

53. Samadpour, M., M. W. Barbour, T. Nguyen, T. M. Cao, F. Buck, G. A. Depavia, E. Mazengia, P. Yang, D. Alfi, M. Lopes, and J. D. Stopforth. 2006. Incidence of enterohemorrhagic *Escherichia coli, Escherichia coli* O157, *Salmonella*, and *Listeria monocytogenes* in retail fresh ground beef, sprouts, and mushrooms. *J. Food Prot.* 69:441-443.

54. Serrano, N. S., C. Zweifel, S. Corti, and R. Stephan. 2018. Microbiological quality and presence of foodborne pathogens in raw milk cheeses and raw meat products marketed at farm level in Switzerland. *Italian J. Food Saf.* 2:7.

55. Shongwe, M. A., A. Jooste, A. Hugo, Z. G. Alemu, and A. Pelser. 2007. Will consumers pay for less fat on beef cuts? The case in Bloemfontein, South Africa. *Agrekon*, 46:.475-493.

56. Slingenbergh, J. 2013. World Livestock 2013: changing disease landscapes. Food and Agriculture Organization of the United Nations.

57. Smith, A.M., K.J. Bosco, Nicol, M.P., Kleynhans, J., McCulloch, M., Duze, S.T., A. Ismail, M. Allam, N.P.Tau, and K.H. Keddy. 2017. Genome sequence for Shiga-toxin-producing *Escherichia coli* O26: H11, associated with a cluster of hemolytic-uremic syndrome cases in South Africa, 2017. *Genome Announc.*, 5:00989-17.

58. Smith, A.M., N.P. Tau, A. Sooka, K.H Keddy, and Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA, 2011. Surveillance for enterohaemorrhagic *Escherichia coli* associated with human diarrhoea in South Africa, 2006–2009. *J. Med. Microbiol.* 60:.681-683.

59. South Africa Department of Agriculture, Forestry and Fisheries. 2012. Standard for microbiological monitoring of meat, process hygiene, cleaning and Trichinella control. Veterinary procedural notices. Available at: https://www.nda.agric.za/vetweb/VPN%20&%20SOP/VPN15-Standard%20or%20microbiological%20monitoring%20of%20meat%2031-01-2014.pdf. Accessed 12 April 2019.

60. Syne, S. M., A. Ramsubhag, and A. A. Adesiyun. 2013. Microbiological hazard analysis of ready-to-eat meats processed at a food plant in Trinidad, West Indies. *Infect. Ecol. Epidemiol.* 3:20450.

61. Thrusfield, M. 2013. Veterinary Epidemiology. John Wiley and Sons.

62. Valadez, A. M., C. Debroy, E. Dudley, and C. N. Cutter. 2011. Multiplex PCR detection of Shiga-toxin-producing *Escherichia coli* strains belonging to serogroups O157, O103, O91, O113, O145, O111, and O26 experimentally inoculated in beef carcass swabs, beef trim, and ground beef. *J. Food Prot.* 74:228-239.

63. Varcasia, B., F. Tomassetti, L. De Santis, F. Di Giamberardino, S. Lovari, S. Bilei, and P. De Santis. 2018. Presence of Shiga-toxin-producing *Escherichia coli* (STEC) in Fresh Beef Marketed in 13 Regions of Italy (2017). *Microorgan.* 6:126.

64. Whitworth, J., Y. Zhang, J. Bono, E. Pleydell, N. French, and T. Besser, 2010. Diverse genetic markers concordantly identify bovine origin *Escherichia coli* O157 genotypes underrepresented in human disease. *Appl. Environ. Microbiol.* 76: 361-365.