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Detection of non O157:H7 Shiga toxin-producing *Escherichia coli* (STEC) serogroups O26, O45, O103, O111, O121 and O145 from beef trim in Namibia

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Abstract: Many different serotypes of Shiga toxin-producing *Escherichia coli* (STEC) that cause disease in humans have been described. Illnesses range from mild diarrhea to bloody diarrhea to hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). *E. coli* O157:H7 is the STEC strain most often associated with the most severe forms of disease. However, numerous non-O157 STEC isolates have also been linked to illnesses and outbreaks of disease. Several studies showed that majority of non O157:H7 STEC infections are caused by strains from one of six major serogroups, including O26, O45, O103, O111, O121 and O145. 771 Beef trim obtained from a local abattoir in Windhoek were tested for the presence of the top 6 non-O157 STEC serogroups using commercial BAX® System assays. All samples were screened for the presence of Shiga toxin (*stx1* and *stx2*) and intimin (*eae*) virulence genes, which were both present in 136 (17.64%) out of 771 samples. Of the 136 positive samples for both *stx* and *eae* virulence genes, nine were positive for O26, one for O45, thirty-three for O103, one for O111, five for O121 and three for O145. There were also thirty-five samples positive for more than one STEC serogroup.The presence of virulent STEC in beef trim is a public health concern. The use of polymerase chain reaction assay should aid quick detection of this virulent serotypes and help to prevent severe epidemic of human diseases associated with STEC infections.

Keywords: *E.coli*, Beef, PCR, STEC.

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1. Introduction

Enteric infections continue to be one of the foremost public health problems worldwide, with over 1.5 million deaths occurring each year only in developing countries (Gyles 1992, Browning et al., 1990, Anonymous 2014). *Escherichia coli* is a ubiquitous bacterial organism that is found in a wide variety of places, including the human intestine, where it can lead different type of illnesses (Donnenmberg et al., 1992). More than 70 different serotypes of Shiga toxin-producing *Escherichia coli* (STEC) that cause disease in humans have been described (Brooks et al., 2005). Illnesses range from mild diarrhea to bloody diarrhea up to hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS)(Karmali et al., 2010). *E. coli* O157:H7 is the STEC strain usually associated with the most severe forms of disease (Brooks et al., 2005; Rivero et al., 2010; Tozzi et al., 2003). More recently, it has become evident that non-O157 Shiga toxin-producing *E. coli* (STEC), particularly STEC serogroups O26, O45, O103, O111, O121 and O145 (referred to as the top six non-O157 STEC) cause illnesses similar to those caused by *E. coli* O157:H7 (Gould et al., 2013; Johnson et al., 2006). The proportion of these non-O157 STEC serogroups breaks down as follows: 22% O26, 16% O111, 12% O103, 8% O121, 7% O45, and 5% O145. Although it is seldom isolated and reported, it is estimated that non-O157 STEC may cause diarrhea at frequencies similar to those of other important enteric bacterial pathogens, such as *Salmonella* and *Shigella* (Slutsker et al., 1997). Humans often become infected with STEC by ingestion of contaminated food or water or by direct contact with animals, resulting in sporadic cases of disease or in large outbreaks involving up to several thousand individuals (Griffin and Tauxen, 1991; Karmali et al., 1983). Sources of infection include meat (especially undercooked beef hamburgers), ready-to-eat sausages, raw milk, cheese, unpasteurized apple cider and juice, lettuce, cantaloupes, alfalfa sprouts, radish sprouts, drinking water, water for bathing, and contact with animals (Gyles, 1992). Cattle are recognized to be the reservoir of many STEC serotypes (Meichtri et al., 2004; Paton et al., 1996; Ethelberg et al., 2009). In fact, there are ample data on the prevalence of non-O157 STEC in cattle from fed beef through processing (Arthur et al., 2002) and boneless beef trim destined for grinding, but there is a lack of information on the prevalence of non-O157 STEC in finished ground beef. The study of beef trim destined for grinding showed that 10 to 30% of beef trim contained STEC (Bosilevac et al., 2007); however, data on STEC prevalence in the finished product, as a result of blending various lean and fat materials during production of ground beef, are unknown (Bosilevac et al., 2011). STEC O157 has been considered an adulterant in beef produced in the United States for some time. Six other non-O157 STEC O-groups have been included as adulterants by the U.S. Department of Agriculture–Food Safety and Inspection Services and testing for these pathogens began in June 2012 in both domestic and imported beef manufacturing trimmings (Anonymous, 2011). The objective of this study was to

conduct a survey of beef trim collected from a Namibian abattoir using the BAX® system PCR assays to determine the presence of the top six non-O157STEC.

2. Materials and Methods

Beef sample enrichments

Between April and November 2014, in a local abattoir in Windhoek (Namibia) 771 samples were samples and 325 ± 32.5g of beef trim, were homogenized with 975 ± 19.5 ml of pre-warmed (42°C) BAX® System MP enrichment broth (DuPont Nutrition and Health, Wilmington, DE) in Whirlpack filter bags (Nasco, Fort Atkinson, WI), mixed in a Stomacher (Seward Laboratory Systems, Inc., Bohemia, NY) for 2 min, and then incubated at 42°C for 18 h before being tested with the BAX® System assays.

BAX® system assays

The BAX® System PCR assays used in this study were the following: BAX® System real-time PCR assay suite for STEC-Screening (*stx* and *eae*), Panel 1 *E. coli* (O26, O111, and O121), Panel 2 *E. coli* (O45, O103, and O145) (DuPont Nutrition and Health, Wilmington, DE).

Testing of trim beef enrichments

20 µl of enrichment were added to 200 µl of prepared BAX® System lysis reagent in cluster tubes. Lysis was performed by heating the tubes for 20 min at 37°C and 10 min at 95°C, and then cooling tubes at 4°C for at least 5 min. 30 µl of lysate were used to hydrate tablets in PCR tubes. PCR tubes were loaded into the BAX® System Q7 instrument, and a full process was run according to the procedure described in the BAX® System User Guide and analyzed using software version 3.5.

3. Results and Discussion

Beef trim (n = 771) was obtained from a local abattoir in Windhoek and tested for the presence of the top 6 non-O157 STEC serogroups (O26, O45, O103, O111, O121, and O145) using commercial BAX® System assays. The 771 beef trim samples were subjected to enrichment in BAX® System MP enrichment broth and after were tested using the BAX kits for screening for non-O157 STEC. In the current study, only samples *stx*/*eae* positive by the BAX kits for STEC screening were sent for further testing (STEC Panel 1 and STEC Panel 2) to evaluate the presence of the top 6 non-O157 STEC serogroups. The BAX kits for non-O157 STEC have recently been evaluated by Fratamico (2014) and the assays were shown to be highly specific for the

STEC serogroups, the sensitivity of assays for the different PCR targets was ≥ 1.23×10^3 CFU/mL using pure cultures.

Among the 771 samples tested, the presence of Shiga toxin (*stx1* and *stx2*) and intimin (*eae*) virulence genes was detected in 136 (17.64%) (95% CI 15.11 – 20.49) samples (table 1). Previous reports of imported and domestic boneless beef trim used for ground beef in the United States demonstrated that a range between 10% and 30% of samples contained STEC (Bosilevic et al., 2007) and the results of the current study fall within that range. However, it is very difficult to compare different studies as geographical locations, sampling procedures, and detection methods are different and can affect the prevalence data significantly. Only 87 (63.97%) samples out of 136 (95% CI 55.60 -71.56) positive for *stx* and *eae* virulence genes contained one of the top 6 non-O157 STEC serogroups.Although the screening for *stx* and *eae* virulence genes by PCR is not an approved method for determining STEC prevalence, since other species of bacteria can possess them, the detection of *stx* in meat samples is still considered valid as a presumptive diagnosis and can be a strong indicator of the presence of this pathogen in the meat that can be a threat for public health (Scheutzetal., 2001).

Of the 136 positive samples for both *stx* and *eae* virulence genes, 9 were positive for O26, 1 for O45, 33 for O103, 1 for O111, 5 for O121 and 3 for O145. There were also 35 samples positive for more than one STEC serogroup (table 2).

Table 1- Summary of screening and characterization of non-O157 STEC strains from beef trimsamples

No. (%) of samples		
Total	*stx/eae* positive	Containing STEC serogroups
771 (100)	136 (17.64)	87 (63.97)

Table 2 – Summary of non-O157 STEC strains detected from beef trim samples

Strains	Total N° of samples	% positive samples
O26	9	10.34
O45	1	1.15
O103	33	37.93
O111	1	1.15
O121	5	5.75
O145	3	3.45

O26, O45	3	3.45
O26, O103	19	21.84
O26, O121	2	2.30
O45, O103	4	4.60
O45, O145	1	1.15
O103, O145	1	1.15
O121, O103	1	1.15
O26, O103, O145	1	1.15
O26, O103, O121	2	2.30
O26, O111, O121	1	1.15

4. Conclusion

In summary, the top six non-O157STEC serogroups were all detected in Namibian trim beef samples using the BAX®System real-time PCR assays. The use of polymerase chain reaction assay should aid quick detection of non-O157:H7 STEC serotypes and help to prevent severe epidemic of human diseases associated with STEC infections. The presence of more than one top six non-O157 STEC serogroups in same samples indicates the possibility of cross-contamination between meat and the environment that will need further investigation.

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