The prevalence of non-O157:H7 Shiga toxin-producing Escherichia coli *(STEC) in Namibian game meat*

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Summary
Large game animals play an important role as carriers and transmitters of O157:H7 and non-O157:H7 Shiga toxin-producing *Escherichia coli* (STEC) in nature. Fresh meat obtained from game animals has been identified as an important source of food-borne STEC infections. The aim of this study was to evaluate the incidence of the top 6 non-O157 STEC strains (serogroups O26, O45, O103, O111, O121, and O145) in Namibian game meat based on testing for *stx*, *eae*, and O-group-specific genes. Meat samples from gemsboks (*Oryx gazella*) (n = 75), springboks (*Antidorcas marsupialis*) (n = 41), greater kudus (*Tragelaphus strepsiceros*) (n = 5), and wildebeests (*Connochaetes taurinus*) (n = 5) were collected from 2 Namibian abattoirs and tested for STEC using real-time PCR techniques. Both Shiga toxin (*stx*) and intimin (*eae*) virulence genes were detected in 94 out of 126 samples (74.6%). Five of the top 6 STEC serogroup-specific genes were also detected in samples that were positive for both the *stx* and *eae* genes. The results of this study show a high incidence of non-O157 STEC O-group genes in Namibian game meat, which suggests that further scrutiny and testing may be necessary to avoid foodborne outbreaks.

Prevalenza dei ceppi di Escherichia coli *non-O157:H7 producenti shigatossine (STEC) nella carne di selvaggina in Namibia*

Parole chiave
Escherichia coli,
Selvaggina,
Carne,
Namibia,
STEC.

Riassunto
La carne di animali selvatici svolge un ruolo importante perché trasporta e trasmette i sierogruppi O157: H7 e non-O157: H7 di *Shiga toxin-producing E. coli* (STEC); la carne fresca di questi animali, inoltre, è una causa importante di infezioni da STEC trasmesse da alimenti. Scopo di questo studio è stato quello di valutare l'incidenza dei 6 maggiori ceppi STEC non-O157 (sierogruppi O26, O45, O103, O111, O121 e O145) in Namibia con test per i geni *Shiga toxin* (*stx*), intimina (*eae*) e geni specifici O-group. Da due macelli namibiani sono stati raccolti 75 campioni di carne di orice gazella (*Oryx gazella*), 41 di springbok (*Antidorcas marsupialis*), 5 di kudu grande (*Tragelaphus strepsiceros*) e 5 di gnu (*Connochaetes taurinus*) che sono stati testati per STEC, utilizzando tecniche di real-time PCR. In 94 dei 126 campioni testati è stata rilevata la presenza di *stx* e *eae* (74,6%). Nei campioni positivi per *stx* e *eae* sono stati rilevati anche cinque dei maggiori 6 geni specifici del sierogruppo STEC. I risultati di questo studio mostrano un'alta prevalenza di geni dei gruppi non-O157 STEC-O nella carne di selvaggina della Namibia e suggerisce la necessità di ulteriori controlli e analisi per evitare focolai di origine alimentare.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are considered an important group of food-borne zoonotic pathogens. They cause diarrhoea, hemorrhagic colitis (HC), and life-threatening hemolytic uremic syndrome (HUS) in humans (Hussein 2007). *E. coli* O157:H7 is the STEC strain usually associated with the most severe forms of disease (Rivero *et al.* 2010). More recently, it has become evident that non-O157 STEC, particularly STEC serogroups O26, O45, O103, O111, O121, and O145 (referred to as the 'top 6 non-O157 STEC') cause illnesses similar to those caused by *E. coli* O157:H7 (Gould *et al.* 2010).

Domestic ruminants, especially cattle, are considered to be the major reservoir of STEC (Karch *et al.* 2005). Large game animals are also recognised for playing an important role as carriers and transmitters of O157:H7 and non-O157:H7 STEC in the field (Sanchez *et al.* 2009). Fresh meat obtained from game animals has been identified as an important source of food-borne STEC infections (Magwedere *et al.* 2013, Rounds *et al.* 2012). Meat from wildlife ruminants containing STEC strains has been found in Belgium, Germany, Spain, USA, Japan, and others countries (Miko *et al.* 2009). Game meat and its products are not currently subjected to any official regulation concerning microbiological contamination levels, and the data available concerning the microbiological quality of game meat for some pathogens are limited (Díaz-Sánchez *et al.* 2012). Problems related to traditional livestock farming, as well as the growth of the wildlife meat industry and the tourism sector in Namibia have led to the proliferation of game-farming units on private farmlands, including some in rural areas.

Between 16,000 to 26,000 tons of game meat are produced annually in Namibian farmlands for regional and international export markets, local supply, and personal consumption. It is estimated that there are 2 million different food-producing wildlife species other than fish and forest-dwelling invertebrates in Namibia (Van Schalkwyk *et al.* 2010). The majority of Namibian game meat from gemsboks (*Oryx gazella*), greater kudus (*Tragelaphus strepsiceros*), springboks (*Antidorcas marsupialis*), and hartebeests (*Alcelaphus buselaphus*) is exported to international markets as de-boned meat (Magwedere *et al.* 2012). Concurrent with the expansion of wildlife in Namibia and a decline in domestic animal farming (particularly sheep and cattle due to a long period of drought), some export abattoirs have started to process game meat in their underutilised processing facilities during the wildlife hunting season. In the last years the demand from the international market for game meat, in particular for springboks, gemsboks, blesboks, elands, wildebeests, and kudus increased constantly. The aim of this study was to evaluate the prevalence of the top 6 non-O157 STEC strains in Namibian game meat based on testing for *stx*, *eae*, and O-group-specific genes.

Materials and methods

Between May and July 2016, 126 samples of game meat were collected from 2 Namibian abattoirs. The samples consisted of game meat from gemsboks (n = 75), springboks (n = 41), greater kudus (n = 5), and wildebeest (n = 5). Each sample of 325 ± 32.5 g of meat trim was homogenised 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 minutes, and then incubated at 42 °C for 18 hours. Twenty microlitres of enrichment broth were added to 200 µl of prepared BAX® System lysis reagent in cluster tubes. Lysis was performed by heating the tubes for 20 minutes at 37 °C and 10 minutes at 95 °C, and then cooling tubes at 4 °C for at least 5 minutes. Thirty microlitres of lysate were used to hydrate tablets in polymerase chain reaction (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 analysed using the BAX® System Q7 software version 3.6.

Enrichment broths were screened for the *stx* (Shiga toxin) and *eae* (intimin) genes using the BAX® System real-time PCR screening assay (Du Pont, Wilmington, USA). Only samples positive for both virulence genes were tested using the BAX® real-time PCR STEC Suite Panel 1 (O26, O111, O121) and Panel 2 (O45, O103, O145) to determine the presence of the top 6 non-O157 STEC serogroups.

Results

Among the 126 samples tested, the presence of both Shiga toxin (*stx*) and intimin (*eae*) virulence genes was detected in 94 (74.6%) 95% Confidence Interval (CI) = 66.33-81.39) (95% Confidence Interval (CI) 66.33-81.39) samples (Table I).

Of the 94 samples positive for both *stx* and *eae*, 1 (0.8%) (95% CI 0.19-4.18) was positive for O45; 21 (16.7%) (95% CI 11.19-24.16) for O103, 7 (5.6%) (95% CI 2.76-11.03) for O121, 1 (0.8%) (95% CI 0.19-4.18) for O145, 4 (3.2%) (95% CI 1.29-7.87) for both O45 and O103, 19 (15.1%) (95% CI 9.89-22.37) for O103 and O121, 3 (2.4%) (95% CI 0.86-6.75) for O103 and O145, 4 (3.2%) (95% CI 1.29-7.87) for both O121 and O145, 1 (0.8%) (95% CI 0.19-4.18) for O26, O45 and O145, 1 (0.8%) (95% CI 0.19-4.18) for O45, O121 and

Table I. *Incidences of* stx *(Shiga toxin) and* eae *(intimin) virulence genes in Namibian game meat.*

Sample origin	Number of samples collected	Positive samples (*stx* and *eae*)	
		Number	Percentage
Springbok	41	32	78%
Gemsbok	75	52	69.3%
Greater Kudu	5	5	100%
Wildebeest	5	5	100%
Total samples	126	94	74.6%

O145, and 7 (5.6%) (95% CI 2.76-11.03) for O103, O121 and O145 (Table II). No samples tested positive for the O111 O-group-specific gene.

A total of 32 springbok samples (78%) (95% CI 63.19-87.95) tested positive for both *stx* and *eae* genes. Out of 75 gemsbok samples, 52 (69.3%) (95% CI 58.13-78.61) were contaminated: 1 sample tested positive for O45; 15 samples for O103; 1 sample for O145; 4 samples for both O45 and O103; 13 samples for both O103 and O121; 3 samples for both O103 and O145; and 1 sample exhibited the presence of O26, O45, and O145. For greater kudus, 5 samples were detected positive for the *stx* and *eae* virulence genes (100%) (95% CI 60.70-100.00). Of these, 2 samples tested positive for both O103 and O121, and 3 samples tested positive for O103, O121, and O145. Five wildebeest samples tested positive (100%) (95% CI 60.70-100.00) for O-groups O103 (Tables I and II).

Discussion

These results provide the first report of non-O157:H7 Shiga toxin-producing *Escherichia coli* genes in game meat from different species of wild animals in Namibia using commercial BAX® system assays. The BAX® kits for non-O157 STEC have been evaluated by Fratamico and colleagues (Fratamico *et al.* 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. A previous study conducted on Namibian springbok carcass samples using the PCR method described by Paton and Paton (Paton and Paton 1998) did not show the presence of STEC (Magwedere *et al.* 2013).

The use of molecular methods that do not isolate STEC strains or determine if all of the target genes (top 6 O-group, *eae* and *stx*) are present in a single bacterium, is a limitation for the detection and confirmation of the presence of these pathogens. Samples that tested positive for STEC screening using PCR (*stx*/*eae*) methods and were positive for 1 of top 6 O-group genes can only be reported as 'potentially positive' because *stx* and *eae* toxine can be present in bacteria other than the top 6 STEC.

Table II. *Incidences of non-O157 Shiga toxin-producing* Escherichia coli *serogroups-specific genes in Namibian game meat.*

Targets present	Total number of samples (% total)	Sample origin			
		Springbok	Gemsbok	Greater kudu	Wildebeest
O26	0 (0%)	-	-	-	-
O45	1 (0.8%)	-	1	-	-
O103	21 (16.7%)	1	15	-	5
O121	7 (5.6 %)	7	-	-	-
O145	1 (0.8%)	-	1	-	-
O45, O103	4 (3.2%)	-	4	-	-
O103, O121	19 (15.1%)	4	13	2	-
O103, O145	3 (2.4%)	-	3	-	-
O121, O145	4 (3.2%)	4	-	-	-
O26, O45, O145	1 (0.8%)	-	1	-	-
O45, O121, O145	1 (0.8%)	1	-	-	-
O103, O121, O145	7 (5.6 %)	4	-	3	-

Moreover the detection of *stx* toxin gene in meat samples can be a strong indicator of the presence of STEC in the meat. This, in turn, can be a threat for public health (Scheutz *et al.* 2001).

At present, little is known about the characteristics of STEC strains other than O157 from wildlife meat (Miko *et al.* 2009). STEC strains were detected in game meat in Belgium, USA, and Germany with prevalence rates of 9-14.8% (Lehmann *et al.* 2007, Magwedere *et al.* 2013). In the current study, the samples collected from 2 Namibian abattoirs showed a prevalence rate of 74.6%, and, except for serogroup O111, all the top 6 non-O157 STEC serogroup-specific genes were detected. The most commonly occurring serogroups were O103 and O121.

The information obtained from this study shows a high prevalence of genes related to non-O157 STEC O-groups in Namibian game meat, suggesting that the meat could be contaminated by STEC strains that can cause human illness. Thus, changes in the way the animals are slaughtered and handled in the field and in the abattoirs are advisable. Furthermore, regular scrutiny and testing of the meat for the top 6 non-O157 STEC strains may be necessary to avoid outbreaks of foodborne diseases.

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