STEC from feedlot to abattoir

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Epidemiology of Shiga toxin-producing Escherichia coli in beef cattle from feedlot through to abattoir

Industry Sector: Cattle and Small Stock

Research Focus Area: Red Meat Safety, Nutritional Value, Consumerism and Consumer Behaviour

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EXECUTIVE SUMMARY

Introduction

Shiga toxin-producing Escherichia coli (STEC) has emerged as an important foodborne pathogen globally with a significant impact on public health. Healthy colonized cattle are major reservoirs of STEC and bovine "supershedders" are considered to play a key role in the entry of STEC into the food chain. The public health relevance is determined by the pathogen's low infectious dose and capacity to survive and be transmitted along different stages in the beef production chain. Of the over 470 different serotypes of STEC detected in humans, the O157:H7 serotype is the most frequently associated with large food and water-borne outbreaks. However, non-O157 STEC have been increasingly isolated from sporadic cases of haemorrhagic colitis and the sometimes fatal haemorrhagic uremic syndrome. In a recent RMRD-funded project a high prevalence of STEC contamination of beef products was detected in retail outlets in Pretoria, suggesting that STEC may pose a real food-borne disease threat and that further investigation of the epidemiology of the pathogen is required. Since the majority of beef consumed passes through the feedlot system, it is essential that we understand the dynamics of shedding of the organism in the feedlot in order to identify control measures to reduce the bacterial challenge resulting in carcass contamination in the abattoir.

Objective Statements

The specific objectives of the study were (i) to determine the frequency and dynamics of shedding of STEC in cattle in a feedlot; (ii) to longitudinally follow tagged study animals to slaughter to determine the frequency of STEC contamination pre-slaughter, during slaughter and post-slaughter; (iii) to characterize STEC isolates with respect to their serotypes and

presence of virulence factors; and (iv) to establish the genetic relatedness of the isolates between feedlot and abattoir.

Project Aims

To determine the prevalence, dynamics and factors associated with shedding of Shiga-toxin producing Escherichia coli (STEC) in feedlot cattle;

To determine the relationship between faecal shedding of STEC in the feedlot and on arrival at the abattoir, and carcass contamination at various steps in the slaughter process.

Results

On arrival at the feedlot, 27% (29/106; 95% CI: 19-37%) of faecal samples were STEC-positive on PCR. Regarding virulence genes, 18 (17%) tested positive for stx1, 20 (19%) were positive for stx2, 12 (11%) were positive for eaeA and 23 (22%) were positive for hlyA. STEC prevalence during the longitudinal study indicated non-O157 STEC shedding in 92% (72/78; 95% CI: 84-97) of samples and non-O157 STEC super shedding (≥4llog10 CFU/g faeces) in 73% (57/78; 95% CI: 62-82) of samples.

The number of cattle available for follow-up varied for the months of October, November, December and February. There were only 16 cattle that were consistently negative and none was consistently positive for STEC O157 for the whole period. There was intermittent shedding of non-O157 STEC for the entire sampling event. There was a significant difference ($P < 0.0001$) between the proportion of non-O157 super shedders (92%) compared with the proportion of O157 super shedders. For the longitudinal study, the median STEC shedding level for non-O157 was 4.8log10 CFU/g, while the median for O157 was 3.4log10 CFU/g. Some cattle were consistent non-O157 STEC shedders throughout the 4-month period. Four cattle were super-shedders of non-O157 STEC persistently throughout the four sampling events and five were non-O157 super shedders for 3 consecutive sampling events. Four cattle were super shedders of both O157 and non-O157 STEC.

Only 8 animals were followed up at the abattoir primarily because of missing tags and sometimes due to the inability to keep up with fast processing lines. Overall, the prevalence of STEC based on the screening of carcass swabs using PCR was 32% (6/19; 95% CI: 13-57%), while STEC prevalence along the different stages of carcass processing was as follows: 22% (2/9), 17% (2/12), 25% (3/12) and 11% (2/19) for perineum hide, pre-evisceration, post-evisceration and post-wash swabs respectively ($P = 0.688$). There was no association between super shedding status (just before slaughter) and STEC carcass contamination for either O157 ($P = 0.061$) or non-O157 ($P = 0.348$). 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 non-O157 ($P = 0.143$) STEC.

The analysis of virulence genes and serotypes included isolates from the previous study together with this one. The distribution of virulence genes was highest in feedlot faecal samples (40%) compared with abattoir (33%) and retail outlets (28%) and this was highly statistically significant. Of the 86 STEC strains tested, the frequency of detection of stx1, stx2 and a combination of both stx1and stx2 was 24%, 17% and 19% respectively. The eaeA gene was detected in 20 (23.3%) isolates in five different combinations; stx2+eaeA (2 isolates), stx1+stx2+eaeA (1 isolate), stx1+eaeA+hlyA (13 isolates), stx2+eaeA+hlyA (3 isolates), stx1+stx2+eaeA+hlyA (1 isolate). Of the 20 isolates carrying the eaeA gene, only two isolates (2/20; 10%) were found in mince beef, 3 isolates from abattoir carcass swabs (3/20;15%), and

the remaining 15 isolates (15/20;75%) were found in feedlot cattle faeces. Of the 86 isolates recovered, only 39 could be serotyped, from which a wide range of serogroups (35) were detected.

On the pulsed field gel electrophoresis (PFGE) analysis, dendrograms of 55 isolates showed a high diversity with 45 distinct PFGE patterns. This diversity of PFGE patterns was observed in some isolates of the same serogroup that did not cluster together; these included serogroup O178 (feedlot environmental sample, feedlot cattle faeces, and supermarket boerewors). Also included were two isolates belonging to serogroup O20 (boerewors) and four serogroup O168 isolates (feedlot cattle faeces samples and abattoir perineum hide swab). Some patterns were observed in the dendrogram with varying band similarity percentages. At 100% banding similarity, eight band similarity patterns were identified. At 97.5% banding similarity, four closely related patterns were identified in eight isolates (7%): i. winter butchery boerewors and summer environmental feedlot faeces, ii. autumn supermarket boerewors and winter butchery boerewors, iii. summer cattle feedlot faeces and autumn butchery mince, iv. autumn brisket and mincemeat from the same supermarket. Analysis of the PFGE patterns at the 84.5% band similarity percentage or greater, revealed that most of the clades (7-clusters) belonged to isolates from different sources.

Conclusion

This study has established the presence of persistent and intermittent super-shedding of STEC O157 and non-O157 in cattle in a feedlot and at the abattoir just before slaughter. This results in continual environmental contamination and risk of re-circulation of the pathogen in the herds, which may lead to contamination along the food chain. In This study has established the presence of persistent and intermittent super-shedding of STEC O157 and non-O157 in cattle in a feedlot and at the abattoir just before slaughter. This results in continual environmental contamination and risk of re-circulation of the pathogen in the herds, which may lead to contamination along the food chain. In Final Report: STEC from feedlot to abattoir Page: 4 Printed: 31/01/2020 addition, the high count of non-O157, and the diversity of serogroups, shows that super-shedding is not limited solely to serogroup O157. We provide evidence of horizontal transmission and STEC strain recirculation along the beef production chain in Gauteng. All serogroups detected in this study have been previously implicated in STEC infections in human, with four considered as emerging serogroups. The high heterogeneity shown by PFGE and the difference in serogroups and virulence genes demonstrate the presence of a diverse but related STEC population in the beef production chain. There is need for further scientific investigation to advance the understanding of the dynamics of super-shedding in cattle, to sample a wider geographic region representing cattle-farming areas of South Africa, to conduct studies over a longer period to assess the impact of changes in climatic conditions and to promote epidemiologic surveillance for the clinically important STEC serogroups in public health laboratories in South Africa.

Popular Article

Title for Popular Article

Shiga toxin-producing Escherichia coli in beef cattle from feedlot through to abattoir

INTRODUCTION

Shiga toxin-producing Escherichia coli (STEC) has emerged as an important foodborne pathogen globally. Healthy colonized cattle are major reservoirs of this pathogen and cattle that shed STEC are considered to play a key role in the entry of the pathogen into the food chain. STEC causes a broad spectrum of disease from mild to intense bloody diarrhoea and in 5-10% of cases, haemolytic uremic syndrome (HUS). Globally Foodborne STEC have caused more than 1 million illnesses and 128 deaths. Of the over 470 different serotypes of STEC detected in humans, the O157:H7 serotype is the most frequently associated with large food and water-borne outbreaks. However, non-O157 STEC have been increasingly isolated from intermittent cases of haemorrhagic colitis and the sometimes fatal HUS.

The importance of the pathogen in South Africa and other southern African countries has recently been highlighted. STEC O157 was isolated in clinical stool specimens of diarrhoeic patients and environmental water samples in Gauteng, and recent reports from the Western Cape suggest STEC may be an environmental contaminant in informal settings and it is associated with diarrhoea in children under five years of age. Furthermore, in South Africa and other southern African countries, numerous clinical cases of diarrhoea in children and adults were reported between 2006-2013, in which a diverse range of STEC serogroups (O4, O5, O21, O26, O84, O111, O113, O117 and O157) were incriminated.

However, the poor surveillance and inadequate diagnostic techniques employed, almost certainly means that the occurrence of STEC-associated disease in humans is under-reported. Since the majority of beef consumed passes through the feedlot system, it is essential that we understand the dynamics of shedding of the organism in the feedlot and the characteristics of the pathogen in the beef production chain. This will help to identify control measures to reduce the bacterial challenge resulting in carcass and beef products contamination.

The present study aimed to determine the prevalence and dynamics associated with shedding of STEC in feedlot cattle and to characterise STEC isolates recovered at every stage along the beef production chain. The specific objectives were (i) to determine the frequency of shedders of STEC in cattle and associated animal factors in a feedlot in Gauteng Province through monthly sampling of a cohort of animals over a 4-month period; (ii) to longitudinally follow tagged study animals to slaughter and to determine the frequency of STEC contamination pre-slaughter, during slaughter and post-slaughter; (iii) to characterize STEC isolates with respect to their serotypes and presence of virulence; and (iv) to use pulse-field gel electrophoresis (PFGE) and serotyping to establish the genetic relatedness of the isolates between feedlot and abattoir.

MATERIALS AND METHODS

A six-month study from Sept 2016 to Feb 2017 was conducted at a commercial cattle feedlot located near Pretoria, Gauteng. The selected feedlot also owned a mechanized abattoir that slaughtered approximately 120 units per day. One hundred and six (106) cattle were randomly selected on arrival and tagged, and a minimum of 50 g of fresh rectal faecal grab sample was collected from each. Subsequently, over a period of 4 months, 26 cattle, including 15 animals identified as shedders and at least 11 non-shedders were selected, and sampled once a month until animals were sent for slaughter at the abattoir. At the abattoir, swab samples of tagged cattle were obtained from a 100 cm^2 area using a sterile square metal template from each of four selected anatomical sites (4 x100 cm^2 areas): rump, flank, brisket and neck, according to a standardized method.

Broth enrichment for processing of faecal samples was carried out and DNA Template from the broth enriched samples was investigated for the presence of stx1, stx2, eaeA and hlyA genes using multiplex PCR. 10-fold serial dilutions were plated on duplicate plates of two selective media known to target E coli O157 and non-O157 serogroups, incubated for 24 h at 37oC, after which typical colonies were selected and biochemically identified. Enumeration was performed by viable plate count method and expressed as CFU/g. Serotyping was conducted at the National Institute for Communicable Diseases (NICD). Pulsed-field gel electrophoresis (PFGE) of 55 isolates (including isolates from a recent study of beef products at retail outlets in Pretoria) was carried out to determine relatedness of strains.

RESULTS AND DISCUSSION

Samples collected on arrival at the feedlot indicated a STEC prevalence of 27% (29/106), with 19% and 11% being positive for stx2 and eaeA genes respectively. The longitudinal study showed that STEC non-O157 was shed at a significantly higher level than STEC O157. These results have several implications. Firstly, it demonstrates the presence of super shedding cattle in a feedlot herd. Secondly it shows that super-shedding is not limited to STEC O157, as described in recent reports from Europe. Thirdly, the higher prevalence of STEC non-O157 compared with O157 STEC is of public health significance, since non-O157 STEC have been increasingly linked to human disease in South Africa. Numerous clinical cases of diarrhoea in children and adults, as well as HUS, have been reported, in which a diverse range of STEC serogroups (O4, O5, O21, O26, O84, O111, O113, O117 and O157) was implicated.

It was also of interest that some cattle were simultaneous shedders of both STEC O157 and non-O157. In addition, several cattle shed ≥10,000 CFU/g non-O157 persistently throughout the study or for 3 consecutive sampling events. These cattle were identified as "super shedders". Non-O157 STEC may be gaining importance in South Africa and such super shedders may pose an increased risk of contamination along the beef production chain, possibly leading to contamination of the food chain for human consumption.

PFGE has been used to track foodborne bacterial pathogens along the food production chain. In this study, PFGE analysis revealed a high diversity of 45 distinct PFGE patterns among 55 non-O157 STEC strains, which provides useful information on the genomic diversity of non-O157 STEC strains in the beef production chain in Gauteng. We observed eight PFGE-related patterns for 16 isolates originating from the same location and source but from different stages of the beef production chain, suggesting that the specific contaminating strain multiplied and spread at that point/stage of entry into the beef chain, such that once a pathogen is established at any production stage (farm, abattoir or retail processing) it may result in within-production-stage transmission. These data suggest evidence of epidemiological lineage, hence horizontal transmission of STEC strains along the beef production chain.

In this study, of the 86 STEC isolates only 17% carried the stx2 gene and 19% carried both stx1 and stx2. Of the virulence combinations, (23.3%) harboured the eaeA combinations. Epidemiologic studies have shown that the presence of stx2+eaeA gene combinations is important in the likelihood of developing HUS and with severe clinicalsymptoms. Of the 86 isolates recovered, only 39 isolates were serotypeable, from which a wide range of serogroups (35) were detected, including seven serovars of clinical relevance, namely O178, O174, O117, O101, O68, O8 and O2, considered to be emerging serogroups.

CONCLUSIONS

This study confirms that multiple different STEC strains are co-circulating in cattle in South Africa and further work needs to be done to establish whether these are clinically relevant in the human population. The high count of non-O157, and the diversity of serogroups, provides further evidence that super-shedding is not limited solely to serogroup O157. Also, we provide evidence of horizontal transmission and STEC strain recirculation along the beef production chain in Gauteng. There is need for active surveillance of STEC both in their reservoir host and in humans, and further studies to investigate effective methods to prevent contamination of the food chain.

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