JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Brief Original Article

Shiga toxin-producing *E. coli* isolated from sheep in Namibia

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Abstract

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are an important group of emerging zoonotic pathogens carried in the intestinal tracts of ruminants. They can cause mild diarrhea and fatal disease characterized by hemolytic uremic syndrome, especially in children, the elderly, and immune-compromised individuals.

Methodology: The aim of this study was to determine if sheep harbor STEC. Sheep feces (n = 40), brisket wool (n = 40), and 150 meat samples were collected from the flank (n = 35), rump (n = 35), brisket (n = 20), shank (n = 25), diaphragm (n = 10), and neck (n = 25) of slaughter-age sheep at a high-throughput abattoir and tested for STEC using a combination of culture and real-time polymerase chain reaction techniques.

Results: *E. coli* O103 (5/40) and O145 (5/40) strains were isolated from the feces and *E. coli* O157:H7 was isolated from brisket wool (10/40) and flank meat (5/35). The results of this study provide the first report of STEC infections in sheep in Namibia.

Conclusions: The results of this study show that sheep, like cattle, can shed STEC strains in their feces, which can contaminate meat and expose humans to infections.

Key words: STEC; sheep; Namibia.

J Infect Dev Ctries 2016; 10(4):400-403. doi:10.3855/jidc.6903

(Received 22 March 2015 – Accepted 26 October 2015)

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are a group of zoonotic pathogens that cause a wide range of clinical disorders, ranging from mild watery diarrhea to hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), which is fatal, especially in children, the elderly, and immune-compromised patients [1,2]. They can be broadly grouped into O157 STEC and non-O157 STEC strains. The pathogenic capability of STEC is primarily based on the possession of virulence factors including Shiga toxins (Stx1 and Stx2) [2] and genes encoding the attaching and effacing function (*eae*) [2,3]. *E.* O157:H7 is the STEC species commonly associated with foodborne disease in humans. Although non-O157 STEC strains are less associated with human illness, some strains with the potential to cause severe disease are increasingly being isolated. In 2011, *E. coli* O104 strains were identified in cases of HUS in Germany associated with human deaths [4]. In the United States, six non-O157 strains (O26, O45, O103, O111, O121, and O145) are recognized as a cause of foodborne infections in humans, and meat contaminated by these strains is considered adulterated [5].

Human STEC infections have been traced back to many sources, but most infections are associated with the consumption of undercooked contaminated beef and with direct contact with STEC-infected asymptomatic carrier animals, including sheep [6]. Naturally acquired infections and the shedding of STEC have been reported in sheep [7]. Sheep have been reported as key reservoirs of STEC in a number of studies [7-9]. Non-O157 strains O26, O91, O115, O128, and O130 that harbor genes encoding key virulence factors that are commonly found in STEC strains causing foodborne disease have been isolated from sheep [8,9].

Sheep meat is one of the cheaper sources of protein in Namibia. About 750,000 sheep are slaughtered annually for local meat consumption and for exports. Although large numbers of sheep are slaughtered for meat at abattoirs, there is no information available on STEC contamination of sheep in Namibia. In addition, STEC serotypes O113, O43, and O102 have been reported in lamb exported from Namibia. The aim of the study was to determine the sources of STEC contamination so as to recommend appropriate preventive and control measures.

Methodology

Sampling of feces, wool, and meat was done between March and June 2014 in the Kharas Region of Namibia in sheep of at least six months of age. The

study was carried out to quickly investigate the source of STEC contamination in lamb meat after a consignment of meat tested positive for STEC. Fecal samples (n = 40) weighing approximately 40 g each were collected from the rectum of randomly selected slaughter-age sheep after evisceration by milking out the feces into sterile dilution bags. Wool samples (n = 40) were sampled aseptically by clipping wool from a 100 cm^2 area of the brisket of hanged slaughtered sheep just before the start of dressing procedures. The wool was stored in sterile dilution bags. In both cases, 10 samples were pooled from different animals to reduce the costs of testing. A total of 150 sheep meat samples of approximately 25 g each were collected aseptically from the flank (n = 35), rump (n = 35), brisket (n = 20), shank (n = 25), diaphragm (n = 10), and neck (n = 25) of randomly selected carcasses just before the chilling phase. At each sampling session, five samples from different carcasses were pooled. All samples were preserved on ice in an insulated cooler box and dispatched for testing at ASPIRATA Laboratories (Johannesburg, South Africa).

The procedure used to test for and identify STEC serotypes in this study is a modification of the Food Safety and Inspection Services (FSIS) methods described in the Microbiology Laboratory Guidebooks 5B.05 [10] and 5.09 [11].

Pre-enrichment was done at the laboratory by mixing 1 g of feces with 9 mL of modified tryptone soya broth (mTSB) (Oxoid, Hampshire, UK) supplemented with novobiocin (10 mg/mL) (Oxoid, Hampshire, UK); this mixture was then vortexed. Meat and wool samples were also mixed with mTSB at a ratio of 1:9.

Suspensions of feces, meat, and wool in mTSB were incubated overnight at 37°C. After overnight incubation, the enrichment products were directly screened for Shiga toxin (*stx*) and intimin (*eae*) genes using the BAX System Real-time polymerase chain reaction (PCR) screening assay (Du Pont, Wilmington, USA). Samples positive for both pathogenicity genes were identified as presumptive positive, while those that tested negative were reported as negative. Presumptive positive samples were cultured and isolated on modified rainbow agar (mRBA) (BIOLOG, Hayward, California, USA).

For *E. coli* O157:H7 identification, black/grey colonies isolated using mRBA were sub-cultured on sheep blood agar (Oxoid, Hampshire, UK), characterized as *E. coli* using biochemical tests, and the presence of *stx* and *eae* genes was confirmed using the BAX real-time PCR assay.

For non-O157 *E. coli*, colonies on mRBA with specific coloration representing the top six non-O157 STEC strains were subjected to BAX real-time PCR assays (*stx* and *eae* gene identification and the STEC suite panel 1: O26, O111, 0121 and panel 2: O45, O103, O145) to confirm the presence of pathogenic genes and genes within the O-antigen gene cluster that are specific for each serogroup. Biochemical testing was used to confirm *stx*, *eae*, and O-group-positive colonies as *E. coli*.

Results

The results of this study are presented in Table 1. *E. coli* serogroups O103 (5/40) and O145 (5/40) were isolated from sheep feces. *E. coli* O157:H7 was identified on sheep wool (10/40) and dressed carcasses on the flank area (5/40). STEC was not recovered from other areas on sheep carcasses.

Discussion

These results provide the first report of *E. coli* O103, O145, and *E. coli* O157:H7 in sheep in Namibia. In the United States, *E. coli* O103 and O145 are part of the six non-O157 strains that have been identified as potential causes of foodborne disease by the FSIS [10]. Meat contaminated by these strains is considered a risk to human health. In this study, these strains were isolated from sheep feces. Therefore, a risk to human health exists from meat contaminated with these strains, especially if fecal and ingesta contamination of meat is not prevented. Although a prevalence of 12.5% was recorded for both *E. coli* O103 and O145 strains, this prevalence may have been magnified by the pooling of samples prior to testing. Failure to isolate these two STEC strains in wool and meat may be a reflection of the low level of shedding in the feces or the difficulty

Table 1. Shiga toxin-producing *E. coli* (STEC) strains recovered from sheep

Sample type	Number of samples collected	Number of samples positive	STEC serotype isolated
Feces	40	5	O103
		5	O145
Brisket wool	40	10	O157:H7
Meat	150	5	O157:H7

of isolating these strains. *E. coli* O103 and O145 strains isolated in this study possessed Shiga toxin (*stx*) and intimin (*eae*) genes and were therefore strains capable of causing foodborne disease. In several studies, *E. coli* O103 and O145 strains [12,13] and STEC O103:H2 [14,15] have been implicated in foodborne disease. Non-O157 STEC strains have been reported to cause disease with severity that is similar to that of *Salmonella* and *Shigella* infections [16], but with the potential to cause fatal symptoms of HUS [12].

E. coli O157:H7 was isolated from sheep wool and meat. This study confirms that sheep wool can be contaminated with pathogenic *E. coli* O157:H7 and that contaminated wool can act as a source of contamination for meat if hygienic dressing practices such as the regular washing and disinfection of hands and equipment are not practised during carcass dressing. The source of *E. coli* O157:H7 contamination of wool could not be confirmed, but it is well known that this bacterium is an inhabitant of the intestinal tract and that it may be shed in the feces of animals. Wool contamination may occur due to contact with other animals' feces or contaminated wool. *E. coli* O157:H7 is a well-documented cause of foodborne disease in humans and has been isolated in many studies in feces of clinically healthy sheep [17-19]. The isolation of *E. coli* O157:H7 in sheep meat in this study confirms that there is a risk of human infection and HUS in Namibia because sheep meat is a major source of protein and a significant proportion of the human population is immune compromised due to HIV infection. However, the relationship between STEC in sheep and foodborne disease has not been consistently demonstrated [9].

Conclusions

Our results indicate that sheep (feces, wool, and contaminated sheep meat) are reservoirs of STEC strains O103, O145, and *E. coli* O157:H7, which can cause human disease. Sheep meat is therefore a potential source of STEC infections for humans in Namibia. Therefore, sheep abattoirs should take account of STEC in their food safety management systems to prevent the contamination of meat.

Acknowledgements

The author wishes to thank Mr. J. Smith and Mrs. M. Nashiluwa for assisting with sample collection, preparation, and dispatch to the laboratory. Samples were processed at Aspirata Laboratories in South Africa.

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Conflict of interests: No conflict of interests is declared.