Prevalence of *Escherichia coli* O157 in Cattle Fed Distillers Grains in Feedlots and Harvested Through a Commercial Abattoir in Minnesota

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Take Home Message

The prevalence of *E. coli* O157 follows typical seasonal trends (e.g., a stark decrease during the winter early spring months) regardless of the feeding of DGS. In the feedlots the seasonal distribution was confirmed by the odds ratio; however, it was not in the processing plant. This was most likely caused by a prominent spike in positives isolated during a winter sampling. Transportation of the animal and lairage are also factors that might have played an important role in the isolation of positives from the abattoir samples.

In the feedlots, age of the animals was related to increased prevalence of EHEC O157. This difference in age might indicate that the cattle contamination happens mainly horizontally and not from mother to calves. Animals from the packing plant had very little difference in age.

We observed some possible influences on the genotype composition of the isolates by the season, farms source of feed, and type of feed. The highest selective pressure was observed by seasons. Spring seemed to favor the *stx1* and *2* genotype, whereas winter appeared to select for the *stx2*-only. The genotype *stx1*-only was very rare.

No impact of DGS feeding on the prevalence of EHEC O157 was observed. In the processing plant animals that were fed no DGS had no statistically different prevalence compared to animals that were fed DGS.

Introduction

In humans, Shiga-toxin-producing enterohemorrhagic *Escherichia coli* (EHEC) infections can cause hemorrhagic colitis and severe renal dysfunctions, including hemolytic-uremic syndrome [1]. Serotype O157:H7 is the main cause of human infections caused by the Shiga-toxin producing *E. coli* and cattle are considered to be its primary natural reservoir as they asymptomatically carry this pathogen in their gastrointestinal tract (GIT).

The prevalence and persistence of this serogroup in cattle has been measured in different countries and it has been found that more than 10% of animals of typical herds can shed the pathogen in their feces [5]. In general, shedding of the organism is sporadic and of short duration [6,7,8,9,10]. However, the reasons for a sustained prevalence of this pathogen within a herd are still unclear, although some influencing factors, such as age of the animals [8,13,14], seasonality [8,15,16], and geographic location have been identified.

During the last ten years much attention has been put on the composition of the animals feed, starting with the observation that high-grain fed animals had a higher prevalence of EHEC O157 than forage fed cattle [17,18]. More recently, Distillers Grain Solids (DGS), an ethanol production by-product, has been identified as a new potential factor by several different studies. Because of the increase in ethanol demand and the subsequent expansion of its production, DGS has become abundant and, because of its cost to nutrition ratio, extremely valuable as a component of cattle feed.

A link between EHEC O157 prevalence and DGS was first reported in 2003 by Synge *et al.*, in Scottish cattle [19]. The majority of the subsequent studies have been performed on animals under controlled feeding and/or environmental conditions, either naturally infected or artificially inoculated [21,22,23], or on EHEC O157 naturally present or inoculated in fecal slurries [24,25,26]. These studies showed a certain level of correlation between feeding DGS and prevalence of the pathogen, but they did not uncover the mechanism linking the two. Furthermore, a recent study from Jacob *et al.* comparing distillers grains to dried rolled corn failed to find any correlation between DGS and EHEC O157 prevalence [27]. Being a major ethanol producer, the state of Minnesota has seen a large increase in the use of DGS. Interestingly, several major outbreaks of EHEC O157 have originated in this state but very little is known about the prevalence of this pathogen in the cattle and its possible link with the increase in DGS usage.

Here we present a work aimed at surveying the prevalence of *E. coli* O157 in cattle raised in Minnesota and unraveling its relationship with DGS inclusion in the feed.

Methodology

Samples were collected from October 2010 through September 2011. Three farm sites were evaluated, comprising a total one-time capacity of approximately 7,000 cattle. The feedlots evaluated represented a wide variety of facility types (open, enclosed, slatted-floor) and cattle types (calves and yearlings). Multiple pens of cattle within each site were sampled. Fresh fecal samples (n=1,461) were collected randomly from the feedlot floor at 5% of the pen population at least once monthly. Temperature, number of cattle per pen, days on feed, sex, age, and ration composition were recorded upon each sample collection. At the abattoir, fresh fecal samples (n=1,201) were collected randomly once monthly for a year. Samples were collected from the perianal area of the animal prior to hide removal at 30% of the lot population. The total population sampled consisted of 4,166 head of cattle and 59 lots.

E. coli O157:H7 was isolated using a combination of enrichment, immunomagnetic separation, plating onto sorbitol MacConkey plates and confirmation of isolates by agglutination test and virulence factors were determined by multiplex PCR analysis (Figure 1) [37].

Multivariate logistic regression was used to determine which factors best predicted prevalence and to estimate the magnitude of association between each factor and the outcome. We adjusted for other factors in the model using the Glimmix Procedure of SAS.

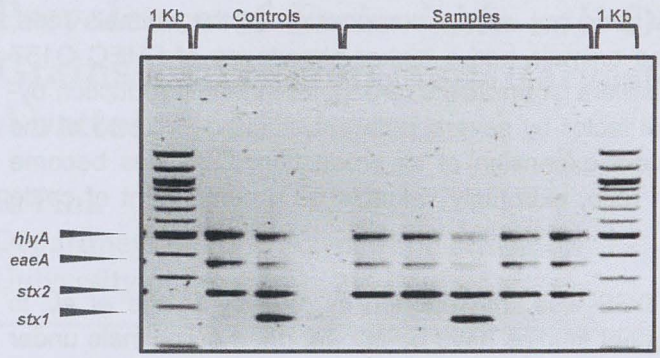

Figure 1. Multiplex PCR of a subset of isolates classified as presumptive *E. coli* O157. The controls consisted in amplification of one strain carrying stx2 only, one strain carrying stx1 and stx2, and simple H2O (negative control). The ladder used in the electrophoretic runs was 1 Kb. The amplification that yielded negative results were repeated three times before being considered negative. All the isolates analyzed yielded amplifications for hlyA and eaeA genes.

Results

Feedlots

The feedlot survey showed that the overall *E. coli* O157:H7 prevalence across the three farm sites was 7.5%. The odds of finding positive samples during summer and winter were the same with an Odds Ratio [OR] of 1.67 and a 95% Confidence Interval [CI] spanning from 0.79 to 3.54. Summer was almost 7 times more likely to yield positive samples than fall with an OR of 6.95 and a 95% CI from 3.71 to 13.04. Winter was more than 4 times more likely to yield positives than fall with an OR of 4.15 and a 95% CI spanning from 2.22 to 7.75. Yearlings were twice more likely to carry E. coli O157 than calves with an OR of 2.33 and a 95% CI spanning from 1.48 to 3.67. Contrary to what is reported in literature, dietary concentration of DGS (>25 vs. ≤ 25%, DM basis) had no effect on the odds of a positive test occurring with an OR of 1.05 and a 95% CI spanning from 0.36 to 3.09 (Table 1).

Distribution of the Genotype Frequencies in the Feedlots

All the isolates were carrying both *eaeA* and *hlyA*. However, there was variation in the presence and combination of the two versions of the shiga toxin genes, *stx1* and *2*. Over the 110 isolates, the majority, 52.4%, carried both the genes, 46.6% carried *stx2* only, and 1% carried *stx1* only. These values were used as expected outcome in the χ^2 test to evaluate the statistical significance of the differences observed. Genotypic frequencies by gender, origin, feed, and source were significantly different from the general distribution observed with p values lower than 0.05. In Farm 1, 23.5% of the isolates had both *stx1* and *2* and 76.5% had only *stx2*. In Farm 2, 66.7% of the isolates had *stx1* and *2*, 31.5% had only *stx2*, and 1.9% had only *stx1*. In Farm 3, 43.8% of the isolates had both *stx1* and *2* and 56.2% had only *stx2* and the difference from the general frequency was not significant.

Seasonal frequencies of the genotypes were all significantly different from the general distribution, with the *stx2*-only genotype reaching a frequency of 90% in winter and the combination *stx1* and *2* 80.8% in spring. The *stx1*-only genotype was present in the fall. The frequencies among male animals followed the general pattern, however, females showed a much higher prevalence of the *stx1* and *2* genotype (76.9%). WDG (Wet Distillers Grain) was the only significant category in the feed groups that was significant, with a higher *stx2*-only frequency (76.5%).

Abattoir

Results from the abattoir surveillance indicated an overall prevalence of 11.7% when averaged across all samples. Distillers grains had no effect on occurrence of positives with an OR of 1.02 and a 95% CI spanning from 0.28 to 3.74. There was no significant effect on occurrence of a positive for

region with an OR=1.27 with a 95% CI spanning from 0.09 to 17.946, or sex with OR=1.58 and a 95% CI spanning from 0.60 to 4.17.

Table 1. Odd ratios for the detection of positives all the other categories except age and season were not significant. The odds ratio for age shows that it is more likely to detect positives in one year old (or older) animals than in calves. Odd ratios for the detection of positives in a specific season, the first column represents the season that is more likely to have positives, the highest likelihood is during summer.

Parameter	Categories		Odds Ratio	Confidence Interval 95%	
				Lower	Upper
DGS Concentration	<25	>25	1.048	0.356	3.086
Age	Yrlg	Calf	2.330*	1.481	3.665
Season	Spring	Fall	1.951*	1.079	3.528
	Winter	Fall	4.147*	2.22	7.747
	Summer	Fall	6.953*	3.707	13.042
	Winter	Spring	2.125*	1.044	4.327
	Summer	Spring	3.564*	2.157	5.887
	Summer	Winter	1.677	0.794	3.542

Table 2. Genotypes for the presence of *stx1/2*, *stx1*, or *stx2*. The χ^2 test was performed by hypothesizing that the overall frequencies were expected to be unchanged in each single categories. Seasons, farm 1 and 2, heifers, WDG (Wet Distillers Grains) were all significantly different from the overall distribution, thus indicating some selective pressure for those categories.

	stx1/2	*stx1*	*stx2*	χ^2
Fall	58.1	3.2	38.7	*
Spring	80.8	0.0	19.2	**
Summer	38.9	0.0	61.1	*
Winter	10.0	0.0	90.0	**
Farm 1	23.5	0.0	76.5	**
Farm 2	66.7	1.9	31.5	**
Farm 3	43.8	0.0	56.3	NS
Bulls/steers	44.2	1.3	54.5	NS
Heifers	76.9	0.0	23.1	**
Calves	59.1	0.0	40.9	NS
Yearlings	50.6	1.2	48.1	NS
Conventional	47.7	1.5	50.8	NS
Natural	60.5	0.0	39.5	NS
Modified Distillers Grain	58.1	1.2	40.7	NS
Wet Distillers Grain	23.5	0.0	76.5	**
≤ 25 DDG	45.0	0.0	55.0	NS
> 25 DDG	54.2	1.2	44.6	NS
Total	52.4	1.0	46.6	

NS = Not significant.
* = $p < 0.05$.
** = $p < 0.01$.

Conversely, seasonality had a significant impact on the likelihood of isolating positive samples. In particular, winter was 58 times more likely to yield positives than summer with an OR of 58.4 and a 95% CI spanning from 4.7 to 730.2, 18 times more likely than spring with an OR of 18.4 and a 95% CI spanning from 5.7 to 59.2 and 3 times more likely than fall with an OR of 3.55 and a 95% CI spanning from 1.109 to 11.372 (Table 3).

Table 3. Odd ratios for the detection of positives in a specific season, the first column represents the season that is more likely to detect positives, the highest likelihood is during winter. There is a discrepancy with the prevalence detected where fall and summer have the highest prevalence, most likely because of the sampling size and the interference of the cattle transportation and lairage. Odd ratios for the detection of positives in all the other categories were not significant.

				Confidence Interval 95%	
Parameter	**Categories**		**Odds Ratio**	**Lower**	**Upper**
DGS	Yes	No	1.023	0.280	3.742
Region	35-40°F	40-45°F	1.270	0.090	17.946
Sex	HFR	STR	1.576	0.596	4.165
Season	Fall	Summer	16.45*	1.355	199.654
	Winter	Summer	58.429*	4.675	730.214
	Spring	Summer	3.178	0.234	43.236
	Fall	Spring	5.176*	1.308	20.487
	Winter	Spring	18.386*	5.706	59.245
	Winter	Fall	3.552*	1.109	11.372

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Notes