Impact of distillers grain solids (DGS) and seasonality on the prevalence of Escherichia coli O157 at an abattoir in the U. S. Upper Midwest

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ABSTRACT

Enterohaemorrhagic Escherichia coli (EHEC) serotype O157:H7 is carried asymptomatically by cattle gastrointestinal tract and the inclusion of distillers grains solids (DGS) in feed is thought to be a factor in the prevalence and persistence of EHEC O157 in a herd. The present study surveys the faecal prevalence of E. coli O157 in cattle processed at an abattoir in the Upper Midwest and its association with environmental factors and feeding practices. Faecal samples were collected from pre-processing cows during a 1-year period. E. coli O157 prevalence was estimated isolation of putative positives and confirmation of isolates by immunosay and multiplex virulence genes PCR analysis. Overall, E. coli O157 was confirmed in 11.2% of samples. Prevalence during winter was the highest at 14% followed by summer (11.6%) and declined to less than 8% the rest of the year. Winter was the only season that had a statistically significant effect on prevalence. As a category unto itself, DGS feeding before arrival had no significant influence on faecal prevalence. However, we found a significant interaction of DGS feeding and summer. This observation is extremely relevant because it corroborates a previous study and suggests possible feeding practices to abate EHEC O157 presence during harvest.

Introduction

Enterohaemorrhagic Escherichia coli (EHEC) is the causative agent of serious infections in humans (e.g. haemorrhagic colitis and severe renal dysfunctions, including haemolytic-uremic syndrome) (Paton & Paton 1998b). In particular, the Shiga-toxin producing serotype O157:H7 has been at the centre of several foodborne outbreaks. This bacterium is carried asymptomatically by cattle in their gastrointestinal tract (GIT) and bovines are considered one of its natural reservoirs. E. coli O157:H7 can be transmitted to beef during slaughter and can also contaminate other foods through manure.

The prevalence and persistence of this serogroup in cattle has been measured in different countries and several studies have reported that more than 10% of animals of typical herds can shed the pathogen in their faeces (Gansheroff & O'Brien 2000). The shedding is generally sporadic and of short duration (Besser et al. 1997). However, the pathogen can contaminate and survive in the environment spreading to other animals (Conedera et al. 2001; Lahti et al. 2003).

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 (Mechie et al. 1997; Van Donkersgoed et al. 1999; Synge 2000), seasonality (Chapman et al. 1997; Mechie et al. 1997; Garber et al. 1999; Ekong et al. 2015), and geographic location, have been identified for some time now. During the last 15 years, the impact of the composition of the animals feed on EHEC O157 faecal shedding has been actively investigated, starting with the hypothesis that high-grain fed animals could have a higher prevalence of E. coli than forage fed cattle (Diez-Gonzalez et al. 1998; Callaway et al. 2009). More recently, distillers grain solids (DGS), an ethanol production by-product, has been identified as a new potential factor in several 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 Syng & al., in Scottish cattle (Syng et al. 2003). That initial report was followed by similar observations in U.S. feedlots fed with brewers grains, a by-product of the brewing industry (Dewell et al. 2005). The majority of the ensuing studies have been performed on animals under controlled feeding and/or environmental conditions, either naturally infected or artificially inoculated (Jacob et al. 2008, 2010; Wells et al. 2009), or on EHEC O157 naturally present or inoculated in faecal slurries (Varel et al. 2010, 2008; Yang et al. 2010). Those studies observed a certain level of association between feeding DGS and increased prevalence of the pathogen, but they did not uncover the mechanism linking the two. Our group recently reported that DGS inclusion in the feed had no significant impact on EHEC O157 prevalence over a year. However, a seasonal interaction between DGS feeding and prevalence was identified, with summer significantly more likely to yield positive samples when the animals were fed DGS (Fink et al. 2013).
The impact of feeding DGS on the prevalence of EHEC O157 during processing has never been studied. We hypothesized that based on those previous findings, a higher prevalence at the abattoir would be detected when the loads were coming from farms feeding DGS. To address this hypothesis, a study focused on a Midwestern abattoir was undertaken involving the collection of fresh faecal samples randomly collected once a month for a year from the rectal area of the animals prior to hide removal.

**Materials and methods**

**Sample collection**

Samples were collected from a Northern Midwest abattoir from October 2009 to October 2010. The animals sampled were 1211 from a total population of 4138 heads of cattle structured in 59 lots. Faecal samples were collected from cows by perianal swabs and placed into separate plastic bags using separate gloves for each sample. Demographic information (i.e. animal gender, age, place of origin) and herd management practices (i.e. feeding) from each cattle load was collected through a questionnaire. The area served by the abattoir was divided into two different geographical regions roughly following the demarcation isotherm between climate zone 4 and 5 as described in the USDA 2006 Plant Hardiness Map. The reported origin of the animals was used to assign them to a specific zone. Table 1 described the categorization of the samples taken and the total of the animals sampled.

**Isolation of E. coli O157 by immunomagnetic separation**

A 10 g portion of a sample was added to a stomacher bag containing 90 ml of modified EC broth supplemented with novobiocin (final concentration, 20 µg/ml; Sigma Chemical Co., St. Louis, Mo.) and blended through stomaching. Samples were incubated for 2 h at 37°C, duplicates of 1 ml of culture were mixed with 20 µl of magnetic beads coated with antibodies against E. coli O157 (Dynal, Oslo, Norway) for 15 min with constant rotation at room temperature. The samples were placed into a magnetic rack and washed three times with a washing solution containing per litre: 10 g peptone, 5 g NaCl, 3.5 g Na2HPO4, and 1.5 g NaH2PO4, 5 BSA, 0.5% Tween 20, pH 7.2. The immunomagnetic beads were suspended in 200 µl of the washing solution and spread plated were spread plated onto duplicate plates of MacConkey sorbitol agar (Neogen, Inc., Lansing, MI), supplemented with cefixime (50 µg/l; Lederle Laboratories, Pearl River, NY) and potassium tellurite (25 mg/l; Sigma) and incubated at 37°C overnight. Typical clear colonies were tested with an O157:H7 latex agglutination assay (RIM E. coli O157:H7 latex test, Remel, Lenexa, KS).

**Identification of virulence factor genes**

All positive agglutination test isolates were tested by multiplex PCR for the presence of specific virulence genes as explained elsewhere (Paton & Paton 1998a). Briefly, two or three colonies for each agglutination-positive isolate were collected from a selective plate, resuspended in pure water, boiled, centrifuged, and used in a multiplex PCR in an MJ thermal cycler system (Stratagene, Inc., La Jolla, CA). The primers were specific for Shiga-like toxins (stx1 and stx2), E. coli attaching and effacing (eaeA), and enterohaemorrhagic E. coli (hlyA). The PCR primers and conditions are described in Table 2. The amplification was carried out in a total 25-µl reaction volume containing a 2.0 mM concentration of deoxynucleoside triphosphates, a 1 mM concentration of each primer, 5 ml of 10 × PCR buffer, and 1.25 U of Taq DNA polymerase (Promega, Madison, WI). The distribution of the positive isolates among the categories is reported in Table 3.

**Statistical analyses**

Logistic regression was used to model the log odds of a sample testing positive for E. coli O157 as a function of predictor variables: prior history of distillers grains in diet (yes, no, unknown), sex (heifer, mixed, or steer), isothermal region (cooler, warmer), and season (fall, winter, spring, summer). Random effects were incorporated into the model to adjust for variation between producers. Logistic regression modelling with random effects used the Glimmix Procedure of SAS (SAS Inst. Inc., Cary, NC) with the binomial link. Interacting predictor effects were assessed through models suggested by summary tables and stepwise model selection methods. Model results are presented in Table 4 with odds ratios for each effect relative to a specified reference category. Confidence intervals for each estimated odds ratio are also provided to assess the uncertainty of the estimated odds ratio. Estimated odds ratios clearly above or below 1.0 provide evidence the factor level of interest differs statistically from the reference category. Residuals and influence diagnostics were examined during model selection to guide the form of the final model.

**Results and discussion**

A subpopulation of 1211 animals out of a total of 4138 was sampled for a year (September 2009 to August 2010) with the largest number tested in fall (444 heads) and the least in winter (162 heads, Table 1). The distribution between animals from steer- and heifer-only herds was similar with a smaller group of animals from mixed herds. The majority of the animals sampled originated from the cooler isothermal region (940 heads, Region 1) with the largest sample in fall and the smallest in spring. We were not able to determine the area of origin for 49 animals. In spring, no animal was sampled in the warmer isothermal region (Region 2). Similarly to what we previously reported, the use of DGS-containing feed was widespread and for the majority of the animals tested (853 heads) it was confirmed that they were given DGS-containing feed before arriving to the abattoir (Fink et al. 2013). Only a small group was fed no DGS (116 heads) and for 242 cattle the information on the feed composition was not available.

From a total of 1211 samples collected, 136 were positive for E. coli O157 antigen and confirmed by PCR (Table 3) resulting on an overall prevalence of 11.23%. This prevalence is well within...
the broad range of EHEC O157 values reported in literature (from undetectable to 28.2%) (Heuvelink et al. 1998; Elder et al. 2000; Bonardi et al. 2001; Ransom et al. 2002; Minihan et al. 2003; Nastasijevic et al. 2008). This prevalence was not statistically different from 9.7% found in our parallel study conducted in the same Upper Midwest region focused on three feedlots (Fink et al. 2013). In one of the few studies conducted recently in the same U. S. region, Walker et al. (2010) reported an average cattle faecal prevalence of 7.4% during two consecutive summers, but the total positive animals from other intestinal content sites was 20%. In that study, the investigators also observed that the prevalence from cattle originating in Minnesota ranged from 3 to 55%.

The presence of EHEC O157 in a herd is particularly important during transportation, harvesting, and processing of the animals at the abattoir. The prevalence of EHEC O157 in cattle at slaughtering has been reported to follow a broad range from undetectable to 28.2% (Heuvelink et al. 1998; Elder et al. 2000; Bonardi et al. 2001; Ransom et al. 2002; Minihan et al. 2003; Nastasijevic et al. 2008). High levels of the pathogen on the cattle’s hinds and faeces are a major problem for the meat processing industry because it is one of the major sources of animal to animal transmission (McGee et al. 2004) and cross-contamination of the carcasses (Elder et al. 2000; Barkocy-Gallagher et al. 2003). Studies sampling animals pre- and post-harvest showed that higher concentrations of EHEC O157 in faeces or on hides of certain individuals corresponded to detection of the pathogen on the carriscasses (Fegan et al. 2005; Brichta-Harhay et al. 2008), although a link with the so-called phenomenon of super-shedding has not been confirmed (Fox et al. 2008; Dodd et al. 2010; Williams et al. 2014).

By our definition of a positive sample, all the isolates carried the eaeA and hlyA genes. Also, all the E. coli isolated contained the stx2 variant of the Shiga-toxin gene, but only 80 (58.9%) carried the stx1 variant. The frequency of the stx1 we detected is very similar to the estimate we obtained for Minnesota feedlots (e.g. 55.2%) and is also in accordance with literature reporting that this variant is very rarely found alone, particularly in O157 (Galland et al. 2001; Fernández et al. 2009). In a recent study conducted in Belgium that included dairy and beef cattle, 83% of 295 EHEC O157 isolates had only stx2 genes and the remainder had both Shiga-toxin types (Cobbaut et al. 2011).

The isothermal region of origin of the animals did not have a significant effect (p = .60) on the faecal shedding of E. coli O157 (OR = 1.54, 95% [CI] = 0.31–7.73; Table 4). This might indicate that the variation in temperature and precipitation averages between the two climate zones is too small to produce any significant variation on the pathogen prevalence. Another possible explanation could be that temperature itself may not have an impact on EHEC O157 shedding. In contrast, a previous study conducted in Nebraska and Colorado found a greater prevalence in cattle from Central Nebraska than in animals originating from Eastern Colorado (Dewell et al. 2005). That report also observed a six-fold greater prevalence in cattle fed brewers grains. Our analysis only detected the same trend in the summer season.

The purported effect of the temperature alone is rather improbable because the seasonal changes in EHEC O157 prevalence are believed to be most likely linked to the seasonal variation of environmental parameters (Dewell et al. 2005; Stanford et al. 2013). The analysis of the prevalence during seasons showed that after adjusting for other factors, winter and spring were significantly lower in prevalence than fall (p < .01) (Table 4). This is in conflict with a previous study we undertook that focused on Minnesota-raised cattle in feedlots, in that case we found summer and winter were both significantly at higher risk of O157 shedding when compared to the other seasons (Fink et al. 2013). However, we note that our conclusions about season are sensitive to small definitional changes. If observations taken in mid-December were classified as winter instead of fall, we would report increased risk in winter relative to fall, and no evidence of other season differences. In our feedlot study, we found a positive association between DGS levels in the feed and summer season. For this reason, we tested the interaction between these two factors and found that, indeed they were interacting (p < .01). Following this observation, we divided the samples collected during summer into samples from animals fed DGS and not fed DGS. Interestingly, the animals tested in summer and not fed DGS were almost 10 times less likely to shed O157 than animals tested during fall regardless of their diet (p < .01). Also,

| Table 2. Primers employed in the multiplex PCR for the identification of Escherichia coli O157 isolates. |
|---|---|---|---|
| Gene | Primer | Sequence (5′–3′) | Amplicon size (bp) |
| stx1 | stx1 F | ATAAATCGCCATCTGAGTACTC | 180 |
| stx1 R | GAAAGCCCACTGGATCATC | |
| stx2 | stx2 F | GCCAATGCAGAATCATCC | 255 |
| stx2 R | TGCGCATATCTGACATTCTG | |
| eaeA | eaeA F | GACCCGGAGCAACACCTACC | 384 |
| eaeA R | CGACCTGCAACGCAAGAGG | |
| hlyA | hlyA F | GCACTATCGACCTGCTCC | 534 |
| hlyA R | AATGAGCCAAGCTGGTTAAGCT | |

Note: Data show number of sampled animals and the total number of animals in 59 lots in parenthesis included in this study.

*Regions 1 and 2 are the isothermal regions according to the USDA 2006 Plant Hardiness Map. We could not obtain the origin information of 49 animals.

### Table 2. Categories of the animals sampled by delivery to the plant date, gender, feed, and state of origin.

| Gender | Geographical regions | DGS Feeding |
|---|---|---|
| Steer | 1 | Yes | Un-known | Total season |
| Heifer | 2 | No | | |
| MIX | | | | |

By our definition of a positive sample, all the isolates carried the eaeA eaeA eaeA F GACCCGGCACAAGCATAAGC 384 and hlyA hlyA hlyA R ATAAATCGCCATTCGTTGACTAC 180 genes. Also, all the E. coli isolated contained the stx2 variant of the Shiga-toxin gene, but only 80 (58.9%) carried the stx1 variant. The frequency of the stx1 we detected is very similar to the estimate we obtained for Minnesota feedlots (e.g. 55.2%) and is also in accordance with literature reporting that this variant is very rarely found alone, particularly in O157 (Galland et al. 2001; Fernández et al. 2009). In a recent study conducted in Belgium that included dairy and beef cattle, 83% of 295 EHEC O157 isolates had only stx2 genes and the remainder had both Shiga-toxin types (Cobbaut et al. 2011).
animals fed DGS in summer were 1.24 times more likely to shed O157 (p = .79). Although these observations were above the significance threshold (p = .05), they led us to test also the impact of season on the DGS exposure category. Therefore, we decided to divide this category between animals sampled during summer and other seasons. The animals fed DGS during summer were much more likely to shed O157 than animals that were not fed DGS. The odds ratio is not precisely estimated, OR = 158.51, 95% CI = 5.36 to 4689.44, but was significant with a p < .01 (Table 4). Animals fed DGS during any other season were not significantly different in O157 shedding when compared to animals not fed DGS (p = .80). This corroborates and complements the findings from our previous survey in which we found an interaction between summer, type of barns, and O157 shedding (Fink et al. 2013).

The combined effect of the interaction of summer and DGS feeding on EHEC O157 prevalence found in our related study was not as strong as in the present report given the limitation that none of the three feedlots that participated in the survey had any animals that were not fed DGS (Fink et al. 2013). In the present work, a portion of the animals arriving to the feedlot was not fed DGS and then the difference appeared to be more marked. Given the inconsistency of results among previously published work on the relationship of DGS feeding and EHEC O157 and based on the findings based on our observational studies, we speculate that the DGS stimulating effect may be predominantly occurring during summer.

In conclusion, the present work corroborates our previous finding that there is an interaction between summer and DGS feed. To our knowledge, this observation has never been reported in literature before and it can have a great impact on the husbandry practices for cattle finished with a DGS-based diet. In fact, it indicates that a possible way to abate EHEC O157 presence at harvest could be avoiding DGS-containing feed during the summer.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**Table 3.** Faecal prevalence of *Escherichia coli* O157 by season of collection, gender, use of distiller’s grains solids as feed before arrival, and state of origin in beef cattle at a Minnesota abattoir.

| Season          | Gender | Geographical region | DGS feeding |
|-----------------|--------|---------------------|-------------|
|                 | Steer  | Heifer              | MIX         | Yes | No | Unknown | Total season |
| Fall 2009       | 31 (15.3) | 30 (16.1)                | 1 (1.8) | 43 (13.2) | 19 (16.1) | 44 (14.3) | 12 (13.2) | 6 (13.0) | 62 (14.0) |
| Winter 2010     | 4 (7.1) | 12 (11.3)                | NS | 16 (17.20) | 0 (0.00) | 4 (3.3) | 12 (28.6) | 16 (9.8) |
| Spring 2010     | 4 (2.9) | 17 (12.8)                | 0 (0.00) | 21 (7.4) | NS | 7 (9.1) | NS | 4 (4.2) | 21 (7.4) |
| Summer 2010     | 28 (16.7) | 3 (4.9) | 6 (6.6) | 21 (8.9) | 16 (19.0) | 36 (15.0) | 0 (0.00) | 1 (7.7) | 37 (11.6) |
| Total           | 67 (11.9) | 62 (12.8) | 7 (4.3) | 101 (11.8) | 12 (10.3) | 23 (9.5) | 136 (11.2) |

Note: Numbers of animals that tested positive and percent prevalence per each category are shown in parenthesis.

NS: Not Sampled.

Regions 1 and 2 are the isothermal regions according to the USDA 2006 Plant Hardiness Map.

**Table 4.** Model-adjusted associations between characteristics of abattoir respondents and prevalence of *Escherichia coli* O157:H7.

| Predictor variable | Odds ratio | Confidence interval | p-value |
|--------------------|------------|---------------------|---------|
| Geographical region |            | 95%                 |         |
| Warmer             | 1.54       | 0.31                | 7.33    | .60     |
| Cooler             | 1.00       |                     |         |         |
| Prior access to DGS|            |                     |         |
| Yes (not summer)   | 1.33       | 0.15                | 12.09   | .80     |
| Yes (Summer)       | 158.51     | 5.34                | >999.9  | <.01    |
| Unknown            | 11.71      | 0.96                | 142.22  | .50     |
| No                 | 1.00       |                     |         |         |
| Sex                |            |                     |         |
| Steer/bulls        | 0.42       | 0.13                | 1.42    | .16     |
| Mixed              | 0.15       | 0.02                | 0.97    | .05     |
| Heifer             | 1.00       |                     |         |         |
| Season             |            |                     |         |
| Summer (DGS Yes)   | 1.24       | 0.26                | 5.84    | .79     |
| Summer (DGS No)    | 0.01       | 0.00                | 0.16    | <.01    |
| Spring             | 0.03       | 0.01                | 0.15    | <.01    |
| Winter             | 0.09       | 0.02                | 0.45    | <.01    |
| Fall               | 1.00       |                     |         |         |
| Summer × DGS (by DGS) | .013    |                     |         |         |

Regions 1 and 2 are the isothermal regions according to the USDA 2006 Plant Hardiness Map.
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