Running title: *E. coli* O157 in Organically- or Naturally-Raised Cattle

Prevalence of *Escherichia coli* O157:H7 in Organically- and Naturally-Raised Beef Cattle†

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ABSTRACT

We determined the prevalence of *Escherichia coli* O157:H7 in organically- and naturally-raised beef cattle at slaughter and compared antibiotic susceptibility profiles of the isolates to those from conventionally-raised beef cattle. Prevalence of *E. coli* O157:H7 were 14.8 and 14.2% for organically- and naturally-raised cattle, respectively. No major difference in antibiotic susceptibility patterns was observed between the isolates.

Many cattle producers have adopted production methods, termed niche marketing, to meet consumer demand for safe and healthy beef. The two main niches for beef cattle producers are organic and natural production (4). Organic beef cattle production, regulated by the United States Department of Agriculture, requires feeding of certified organic feed (17) and raising cattle without the use of antibiotics, hormones and other veterinary products (4). Guidelines for producers to label the product as “natural” differ between natural beef programs, and such programs are administered and regulated by the company or organization that owns the brand name rather than the USDA (12). Natural production guidelines often include a complete restriction on the use of antibiotics and growth-promoting hormones, but unlike organic production, feed from non-organic sources is allowed (12). *Escherichia coli* O157:H7 is a major food-borne pathogen that causes outbreaks of hemorrhagic enteritis, which often leads to hemolytic uremic syndrome in children and the elderly (11). Cattle are major reservoirs of *E. coli* O157:H7 where it colonizes the hindgut, specifically the rectoanal mucosal region. Cattle feces are the major source of food and water contamination (11). Organic production methods impact on prevalence of food-borne pathogens, including *E. coli* O157:H7 and *Campylobacter* in dairy cattle (8, 15) and *Campylobacter* and *Salmonella* in chickens (7, 19), have been studied.
However, there is no published study on the prevalence of *E. coli* O157:H7 in organically- and naturally-raised beef cattle. Additionally, nothing is known regarding the effects of these production methods on antibiotic susceptibilities of *E. coli* O157:H7 in beef cattle. Our objectives were to determine the fecal prevalence of *E. coli* O157:H7 in organically and naturally raised beef cattle at slaughter and compare antibiotic susceptibilities of isolates from organically-, naturally-, and conventionally-raised beef cattle production systems.

Cattle sampled in this study were from three types of production systems, organic, natural, or conventional. Organically-raised beef cattle were from farms that were certified by the National Organic Program (1). The naturally-raised beef cattle were from farms that were certified by the All Natural Source Verified Beef Program (1). Sample collection occurred in an abattoir. Conventionally-raised cattle from two feedlots were sampled in a different abattoir so antibiotic susceptibilities of their isolates could be compared with those from organically and naturally raised cattle. Fecal samples were accessed by spooning contents after cutting open the rectum. The mucosa of the rectum was then rinsed with water until free of visible fecal material and swabbed with a sterile foam-tipped applicator (5). Isolation and identification of *E. coli* O157 and PCR detection of major virulence genes (*eae, stx1, stx2, hlyA, and fliC*) were as described by Reinstein et al. (14). A subset of 60 isolates (20 [10 fecal and 10 RAMS] from each production system) was randomly chosen to determine the antibiotic susceptibility patterns by broth microdilution method (10). Antibiotics (all from Sigma-Aldrich) tested were: amikacin, amoxicillin, ampicillin, apramycin, bacitracin, cefoxitin, ceftazidime, ceftriaxone, cephalothin, chloramphenicol, chlortetracycline, ciprofloxacin, enrofloxacin, erythromycin, florfenicol, gentamicin, kanamycin, lincomycin, monensin, naladixic acid, neomycin, norfloxacin, novobiocin, oxytetracycline, penicillin, rifampicin, spectinomycin, and trimethoprim. The MIC
was defined as the lowest concentration of an antibiotic that prevented visible growth of the organism. Each concentration of the antibiotic compound was duplicated in the microtiter plate and MIC determination was repeated with a different inoculum preparation. Logistic regression was performed using PROC GENMOD of SAS (SAS Institute, Cary, NC) to compare prevalence (binomial distribution of outcomes) of *E. coli* O157:H7 detected in fecal samples, RAMS samples, and fecal or RAMS samples (overall animal-level prevalence). The MIC of antibiotics for *E. coli* O157:H7 isolates were analyzed using a non-parametric survival test in PROC LIFETEST of SAS to determine the effects of the production system (natural, organic, or conventional). Data were right-censored when necessary (resistant at the highest concentration evaluated). The Wilcoxon test was utilized to determine the effect of production system on MIC values.

A total of 553, 506, and 322 organically-, naturally-, and conventionally-raised cattle were sampled, respectively. In organically-raised cattle, the prevalence of *E. coli* O157:H7 in fecal samples across sampling days ranged from 0 to 24.4% with an average of 9.3%, and RAMS prevalence ranged from 0 to 30.9% with an average of 8.7% (Figure 1). In naturally-raised cattle, the prevalence of *E. coli* O157:H7 in fecal samples ranged from 0 to 20.3% with an average of 7.2%, and RAMS prevalence ranged from 0 to 23.8%, with an average of 8.9% (Figure 1). In both organically- and naturally-raised cattle, prevalence detected by both sampling methods was greater (*P* < 0.05) than the prevalence (total) detected by either method (Figure 1).

Thirty-six of 322 (11.2%) of conventionally-raised feedlot cattle were culture positive for *E. coli* O157:H7 (either feces or RAMS). Fecal prevalence of *E. coli* O157:H7 was 6.5% and the prevalence by the RAMS sampling method of sampling was 7.1%. Most isolates (66.7% from organically-raised beef cattle and 77.8% from naturally-raised beef cattle) were positive for eae,
The stx2 gene was present in 100% and 95% of isolates from organically-raised cattle, respectively. Prevalence of *E. coli* O157:H7 that we observed in organically-and naturally-raised beef cattle were similar to prevalence reported in conventionally-raised cattle (2). Our study did not include statistical comparison of the prevalence data because of a number of differences in the production systems, particularly diets, between organically-, naturally- and conventionally-raised beef cattle. Organically- and naturally-raised cattle are either required to graze pasture or fed a forage-based diet. Although conflicting data exist (2), studies have shown that cattle fed a forage diet have both higher levels and longer durations of fecal shedding of *E. coli* O157:H7 compared with cattle on a grain diet (18).

None of the isolates tested from the three production systems was susceptible (MIC > 50µg/ml) to bacitracin, lincomycin, monensin, novobiocin, tilmicosin, tylosin, and vancomycin. The MIC values of isolates collected from the production systems were significantly different (*P* < 0.05) for 12 antibiotics (amikacin, apramycin, cefoxitin, ceftriaxone, gentamycin, kanamycin, nalidixic acid, neomycin, rifampicin, streptomycin, and tetracycline). *Escherichia coli* O157:H7 isolates from conventionally-raised cattle had higher (*P* < 0.05) MIC values compared to isolates from naturally- and/or organically-raised cattle for gentamicin and neomycin (Table 1). However, isolates from conventionally-fed cattle had lower (*P* < 0.05) MIC values for amikacin, apramycin, cefoxitin, ceftriaxone, kanamycin, nalidixic acid, penicillin, rifampicin, and tetracycline compared to isolates from naturally- and/or organically-raised cattle (Table 1).

Among the 60 isolates tested for antibiotic susceptibilities, six isolates (10%) were susceptible to all antibiotics included in the study, excluding the seven antibiotics that were resistant to all isolates. Forty-two isolates (70%) were resistant (MIC > 50 µg or IU/ml) to one antibiotic, nine
isolates (15%) were resistant to two antibiotics, and two isolates (3%) were resistant to five antibiotics. One isolate from the organically-raised cattle group was resistant to 10 (amoxicillin, ampicillin, cefoxitin, cephalothin, chloramphenicol, florfenicol, oxytetracycline, penicillin, streptomycin, and tetracycline) of the 26 antibiotics that were inhibitory to other isolates. We have presented data as median MIC values for each production system. In some instances, the median values were same, but the actual MIC data for the type of production system differed. This was because the data were right censored if isolates were not susceptible at 50 µg or IU/ml.

If more isolates are censored for a particular production system compared to another, it may lead to statistical differences. This justifies the use of survival analysis for this type of data. There were differences between MIC values of isolates from organically-raised cattle and conventionally-raised cattle for many antibiotics (cefoxitin, ceftriaxone, gentamicin, nalidixic acid, neomycin, penicillin, rifampicin, and tetracycline). Similarly, there were differences between MIC values of isolates from naturally-raised cattle and conventionally-raised cattle for many antibiotics (amikacin, apramycin, ceftriaxone, kanamycin, nalidixic acid, and rifampicin). For many of these antibiotics, MIC values for isolates from organically-or naturally-raised cattle were greater than for isolates from conventionally-raised cattle. Resistance genes can be transferred from food animals to humans among the enteric pathogen population (9) and it is possible that resistance genes from other bacteria in the gastrointestinal system of cattle could be acquired by *E. coli* O157:H7. In cattle, heavy metals like copper or zinc, which are also antimicrobial are included in diets at concentrations in excess of the nutritional requirements, often to replace conventional antibiotics, to achieve growth promotion (6). Feeding of metals also results in the emergence of bacterial populations resistant to metals (6), which in some instances could lead to resistance to antibiotics. Copper resistance mechanisms to concentrations...
above those normally tolerated by normal cellular processes have been found on plasmids linked
to resistance to antibiotics in some bacteria (6). Therefore, it is possible that isolates from
organically- or naturally-raised cattle that are not exposed to antibiotics still could become
resistant to antibiotics.

Information on prevalence and antibiotic susceptibilities of foodborne pathogens in organic
or natural livestock production systems is limited and variable. In a study of organic and
conventional dairy cattle farms, conventional farms were more likely to have at least one
Salmonella isolate resistant to antibiotics than were organic farms (13). Kuhnert et. al (8)
showed no difference between prevalence of E. coli O157:H7 isolated from organic and
conventional dairy farms. Escherichia coli isolates from conventional dairies had significantly
higher rates of resistance to certain antibiotics than isolates from organic dairies (16). Cho et al.
(3) compared antibiotic susceptibilities of Shiga toxin-producing, O157 and non-O157 isolates
from organic and conventional dairy farms and concluded that that there was no overall
significant difference in resistance between the two production systems.

Although organic and natural beef production systems are becoming popular, little is known
about the effects these production systems on foodborne pathogens. Because safety of the food
supply is crucial, further investigation into these production systems and their potential for
altering risk of human illness is warranted. Our study found that organically and naturally raised
beef cattle have a similar fecal prevalence of E. coli O157:H7 and our prevalence estimates for
these types of production systems are similar to previous reports for conventionally raised
feedlot cattle.

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REFERENCES


TABLE 1. Median and 95% confidence intervals (parentheses) for minimum inhibitory concentrations (MIC) of antimicrobials for *Escherichia coli* O157:H7 isolates from conventionally-, naturally-, or organically-raised beef cattle

<table>
<thead>
<tr>
<th>Antibiotic agents</th>
<th>Conventionally-raised cattle (n=20)</th>
<th>Naturally-raised cattle (n=20)</th>
<th>Organically-raised cattle (n=20)</th>
<th>P-value (Wilcoxon test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>2.5 (2.3-3.1)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9 (3.1-4.7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.7 (2.3-3.1)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Apramycin</td>
<td>9.4 (8.6-9.4)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.5 (9.4-15.6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.3 (6.3-9.4)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>7.8 (6.3-7.8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.8 (6.3-9.4)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.2 (7.8-10.9)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.04 (0.04-0.05)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05 (NE)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05 (NE)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.6 (0.4-0.6)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6 (0.5-0.8)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4 (0.3-0.5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>3.0 (2.3-3.1)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9 (2.7-4.7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3 (2.0-3.1)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>3.1 (3.1-3.9)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7 (3.9-6.3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.7 (3.1-6.3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Neomycin</td>
<td>1.6 (1.2-1.6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.6 (1.2-2.3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0 (0.8-1.2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Penicillin&lt;sup&gt;d&lt;/sup&gt;</td>
<td>50.0 (NE)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.0 (NE)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>50.0 (NE)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>6.3 (5.5-6.3)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.3 (NE)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.3 (6.3-12.5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>9.4 (9.4-12.5)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.4 (9.4-12.5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.8 (6.3-9.4)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>3.1 (NE)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1 (3.1-4.7)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.7 (3.1-4.7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> MIC are significantly different (P < 0.05) as determined by survival analysis (Wilcoxon test).

<sup>c</sup> NE = non-estimable.

<sup>d</sup> Concentrations were µg/ml for all antibiotics except for penicillin, which was in IU/ml.
Figure 1. Prevalence of *Escherichia coli* O157:H7 in organically- and naturally-raised beef cattle at slaughter. Bars within a production system not sharing the same letter are different at \( P < 0.05 \).