Research Note

Prevalence and Antimicrobial Resistance Profiles of Escherichia coli O157:H7 and Salmonella Isolated from Feedlot Lambs†

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ABSTRACT

The present study examined the incidence of Escherichia coli O157:H7 and Salmonella in feedlot lambs. Fifty-six feedlot lambs from eight sheep farming operations were grouped in a single drylot pen, fed, and managed as is typical in the southwestern United States. Fecal samples were collected on days 0, 46, 87, and 122 of the feeding period via rectal palpation. Wool samples (ventral midline) were collected one time only at the feedlot, immediately prior to shipping to the processing plant, and carcass swabs were collected following slaughter. All samples were cultured for E. coli O157:H7, Salmonella, and fecal coliforms, and select isolates were examined for antimicrobial susceptibility. Overall, the percentages of fecal and wool samples positive for E. coli O157:H7 averaged 9 and 18%, respectively. One carcass swab was E. coli O157:H7 positive. Of the 155 fecal samples collected, 11 (7%) were Salmonella positive. Salmonella was detected in nearly 50% of the wool samples collected prior to slaughter, while none of the carcasses were Salmonella positive 24 h postslaughter. All isolates (E. coli O157:H7, Salmonella, and fecal coliforms) were susceptible to ceftiofur, enrofloxacin, and trimethoprim-sulfamethoxazole. One E. coli O157:H7 isolate cultured from a carcass swab was resistant to seven antibiotics, and seven wool E. coli O157:H7 isolates were multidrug resistant. Results of this research demonstrate that feedlot sheep are naturally colonized with E. coli O157:H7 and Salmonella and wool can be a source of carcass contamination; however, in-plant processing procedures and intervention strategies were largely effective in preventing carcass contamination.

Verocytotoxin-producing Escherichia coli O157:H7 was recognized as a major foodborne pathogen in 1982 following two food-associated outbreaks of unusual gastrointestinal illness (8). This organism is now recognized as an important causative agent of foodborne disease, having been implicated in several outbreaks in the United States, Canada, and the United Kingdom (8). Ruminants are considered to be an important reservoir of E. coli O157:H7, and sheep, like cattle, are naturally colonized by this pathogen (6).

Salmonella is one of the most important of the foodborne pathogens worldwide and is reported to colonize virtually all animals, including humans, livestock, reptiles, birds, and rodents (14). An estimated 1.4 to 3 million cases of salmonellosis occur in the United States each year, with more than 500 deaths (3). Salmonella infections in humans are usually acquired via consumption of contaminated food; however, direct contact with infected animals can result in human infection (1). As they are for E. coli O157:H7, rumenants are reservoirs for Salmonella, often appearing asymptomatic while shedding this pathogen into the environment (11, 15). While sheep have been reported to be carriers of Salmonella, prevalence data in the United States are limited (9). The objectives of the present research were to examine Salmonella and E. coli O157:H7 prevalence in feedlot sheep throughout the feeding period and following slaughter.

MATERIALS AND METHODS

Animals and sample collection. Fifty-six feedlot lambs (fine wool, hair, and crossbred; wethers and ewes) originating from eight different sheep farming operations were utilized in the study. Lambs entered the feedlot at the same time, were maintained together in a single drylot pen, and were fed and managed as is typical for a sheep feedlot in the southwestern United States. Lambs were shipped to slaughter based on their body weight: typical for a sheep feedlot in the southwestern United States. Lambs entered the feedlot at the same time, were maintained together in a single drylot pen, and were fed and managed as is typical for a sheep feedlot in the southwestern United States. Lambs were shipped to slaughter based on their body weight; therefore, slaughter dates are staggered and most lambs were sampled (feces) on multiple occasions.

Fecal samples (approximately 20 g) were collected from all lambs on days 0, 46, 87, and 122 of the feeding period during the summer and fall of 2003 via rectal palpation. Wool samples were collected one time only at the feedlot, immediately prior to shipping to the processing plant. Wool was shorn from an area...
approximately 300 cm² on the ventral midline, and the clippers
were cleaned and disinfected between samples. Following slaugh-
ter, carcass swabs were collected using Speci-sponge bags (Whirl-
Pak, Nasco, Modesto, CA) with each sponge moistened with ster-
ile phosphate-buffered saline (PBS) immediately prior to use.
Three sites (leg, shoulder, and bung/hock region) were swabbed
on each carcass (one sponge per carcass) following 24 h in
the cooler. All samples were shipped overnight to the laboratory in
College Station, TX, for bacterial culture as described below.

**Bacterial culture and isolation.** All samples were cultured for
*E. coli* O157:H7 and *Salmonella* within 24 h of collection. *E.
coli* O157:H7 culture and isolation were conducted using an
immunomagnetic separation technique as described previously (10).
Briefly, 10 g of feces was enriched in 90 ml of gram-negative
broth containing vancomycin (8 μg/ml), cefixime (0.5 μg/ml), and
cefsulodin (10 μg/ml) and incubated (6 h, 37°C). Following in-
cubation, 20 μl of anti-*E. coli* O157:H7 antibody-labeled para-
magnetic beads (Neogen Corp., Lansing, MI) was added to 1-ml
volumes of the above enrichments, mixed, and washed. Fifty mi-
croliters of the resulting suspension was spread plated on
CHROMagar O157 (DRG International, Mountain Side, NJ)
plates (containing 2.5 μg/ml potassium tellurite) and incubated
overnight (37°C). Pink colonies exhibiting typical *E. coli* O157:
H7 morphology were resuspended in PBS (pH 6.5) and confirmed
as *E. coli* O157:H7 using the Reveal microbial screening test ac-
cording to the manufacturer’s instructions (Neogen Corp.). Wool
(approximately 10 g) and carcass swab sponges were enriched in
20 ml of sterile 22% brilliant green bile broth and incubated (6 h, 37°C).
Following incubation, all culture techniques were the
same as described above for fecal samples. *E. coli* O157:H7 iso-
lates were stored (−80°C) using CryoCare bacterial preservers
(Key Scientific Products, Round Rock, TX).

*Salmonella* was cultured by enriching approximately 10 g of
feces in 90 ml of tetrahyionate broth (37°C, 24 h), followed by a
second enrichment in Rappopont-Vassilidis broth (100 ml in 5 ml,
42°C, 24 h). Enrichments were plated on brilliant green agar (Ox-
oid Ltd., Hampshire, UK) supplemented with novobiocin (25 μg/
ml) and incubated (37°C, 24 h). Wool and carcass swabs were
enriched in 20 ml of buffered peptone water and incubated (37°C,
24 h) prior to secondary and tertiary enrichments and plating as
described above for fecal samples. Following incubation, colonies
exhibiting typical *Salmonella* morphology were confirmed bio-
chemically using lysine and triple sugar iron agars. Positive sam-
ples were restreaked on tryptic soy agar with 5% sheep blood
(Becton Dickinson and Company, Franklin Lakes, NJ) for further
confirmation, and serogrouping was conducted using slide agglu-
tination with *Salmonella* antisera (Becton Dickinson and Com-
pany). *Salmonella* isolates were stored (−80°C) as above.

Fecal coliforms were isolated from CHROMagar O157 plates
based on color and resuspended in PBS and frozen as above. Un-
less noted otherwise, all reagents and antibiotics were obtained
from Sigma Chemical Co. (St. Louis, MO).

**Antimicrobial susceptibility testing.** One isolate from each
*E. coli* O157:H7—positive sample (n = 26) and each *Salmonella*
positive sample (n = 35) and one fecal coliform isolate from the
20 fecal samples at each collection (n = 80) were examined for
antimicrobial susceptibility by using the Sensititre automated an-
timicrobial susceptibility system according to the manufacturer’s
directions (Trek Diagnostic Systems, Westlake, OH). Broth mi-
crodilution was used according to the methods described by
the Clinical and Laboratory Standards Institute (5) utilizing the bo-
vine/porcine isolate susceptibility testing panels to determine
MICs for the following antimicrobials: ampicillin, ampramycin, cep-
tiofur, chlorotetracycline, enrofloxacin, florfenicol, gentamicin, neo-
mycin, oxytetracycline, spectinomycin, sulfachloropyridazine, sul-
fadimethoxine, sulfathiazole, and trimethoprim-sulfamethoxazole.
Resistance breakpoints were determined using the NCCLS inter-
pretative standards (5). *E. coli* ATCC 25922, *E. coli* ATCC 35218,
and *Enterococcus faecalis* ATCC 29212 were used as quality con-
rol organisms.

**RESULTS**

Results of the bacterial culture for *E. coli* O157:H7 and
*Salmonella* are grouped across the collection period and
presented by farm in Table 1. Sheep with fecal samples
positive for *E. coli* O157:H7 upon entering the feedlot orig-
nated from two of the eight farms providing sheep for this
study. Subsequently, throughout the feeding period, only
one animal not originally testing positive or from one of
the two farms producing *E. coli* O157:H7—positive sheep
had a culture-positive fecal sample. Overall, the percentage
of fecal samples positive for *E. coli* O157:H7 averaged 9%. Wool
samples positive for *E. coli* O157:H7 were found on
sheep representing all farms but one, averaging 18% posi-
tive overall. Only one carcass swab was *E. coli* O157:H7
positive.

*Salmonella*-positive fecal samples were detected in
sheep from multiple farms of origin. Overall, 11 (7%) of
155 fecal samples were *Salmonella* positive. One lamb not
shedding *Salmonella* upon entering the feedlot was later
detected shedding *Salmonella* in the feces. *Salmonella*
was detected in nearly 50% of the wool samples collected prior
to slaughter, while none of the carcasses were *Salmonella*
positive 24 h postslaughter (Table 1).

Results of the antimicrobial susceptibility screening are
presented in Table 2 by collection date and isolate source
(feces, wool, or carcass). All isolates were susceptible to
ceftiofur, enrofloxacin, and trimethoprim-sulfamethoxazole
(data not shown). Fecal coliforms isolated from sheep upon
entry to the feedlot were susceptible to all antimicrobials
with the exception of chlorotetracycline and oxytetracycline,
for which there were two and three resistant isolates, re-
spectively. Similarly, *E. coli* O157:H7 and *Salmonella* iso-

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### Table 1. Prevalence of *E. coli* O157:H7 and *Salmonella* in fe-
cal, wool, and carcass swab samples from feedlot

<table>
<thead>
<tr>
<th>Farm</th>
<th>No. of lambs</th>
<th>Feces</th>
<th>Wool</th>
<th>Carcass</th>
<th>Fecces</th>
<th>Wool</th>
<th>Carcass</th>
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<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>0/21</td>
<td>1/7</td>
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<td>1/12</td>
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<tr>
<td>Overall</td>
<td>56</td>
<td>15/162</td>
<td>9/50</td>
<td>1/51</td>
<td>11/155</td>
<td>25/51</td>
<td>0/51</td>
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</table>

* One lamb died or was removed from the pen due to poor
performance.
<table>
<thead>
<tr>
<th>Drug and resistance profile</th>
<th>No. of isolates resistant to indicated drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>27 June</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Fecal</td>
</tr>
<tr>
<td>(n = 20)</td>
<td>(n = 9)</td>
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<tr>
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<td>Resistant to:</td>
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<td>&lt;10 antibiotics</td>
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</tr>
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</table>

*FC, fecal coliforms; EHEC, E. coli O157:H7; SALM, Salmonella.*
lates from the first collection showed little resistance to the antimicrobials examined. However, beginning with the second collection and observed in all subsequent collections, the number of antibiotics to which fecal coliforms were resistant increased, with all isolates resistant to at least one antibiotic, and many of the isolates being multiresistant. Resistance patterns in fecal E. coli O157:H7 isolated in later collections were largely similar to those of the first collection. One E. coli O157:H7 isolate that was cultured from a carcass swab in the last collection period was resistant to seven antibiotics. Seven wool E. coli O157:H7 isolates were also collected at this same time, all of which were multidrug resistant. Salmonella isolates from the feces and wool were increasingly antibiotic resistant as the sample collections progressed. Two fecal Salmonella isolates cultured during the last collection were resistant to three and eight antibiotics each, while wool isolates (n = 13) from this same collection ranged in resistance from one to nine antibiotics.

**DISCUSSION**

To our knowledge, this is the first report concerning the prevalence of E. coli O157:H7 and Salmonella in feedlot sheep in the United States. Previous research has documented that sheeps, like cattle, are naturally colonized by E. coli O157:H7 (4, 6, 13); however, these studies utilized grazing animals and/or were conducted outside the United States. In the present research, the prevalence of fecal E. coli O157:H7 (9%) and that of Salmonella (7%), when averaged across collection times, were generally lower than previously reported figures for feedlot cattle (2, 7, 10, 12). In the case of E. coli O157:H7, all the lambs with positive fecal samples originated from one of two farms and only one lamb not shedding upon entering the feedlot was detected shedding this pathogen at a later sample date. Fecal Salmonella was detected in lambs from multiple farms originally, and similar to what was observed for E. coli O157: H7, only one lamb not shedding Salmonella upon entering the feedlot was detected shedding during the feeding period.

The percentage of wool samples collected prior to slaughter and positive for E. coli O157:H7 was higher than for fecal samples, and these positive wool samples were equally distributed among lambs from all farms. The one exception is farm F, which had the highest percentage of lambs with positive fecal samples but no wool samples positive for E. coli O157:H7. Most likely the wool was contaminated with E. coli O157:H7 in the feedlot; however, as we did not collect wool samples from lambs upon their entering the feedlot, it is possible that the wool was infected prior to feedlot entry. Salmonella was detected on a greater percentage of wool samples (49%) than E. coli O157: H7 (18%), although fecal shedding of the two pathogens was similar. At least one lamb representing each farm of origin had a Salmonella-positive wool sample. The reason for the higher percentage of Salmonella-positive wool samples is unknown. Quantitative enumeration of this pathogen may have explained this difference, but only qualitative analysis of the samples was conducted. These findings are not surprising, as hides have been implicated as a major source of potential carcass contamination in feedlot cattle (12). The good news is that while approximately one-half of the lambs entering the slaughter facility were detected with E. coli O157:H7 or Salmonella on the wool, in-plant processing methodology and carcass washing procedures in this facility were effective in preventing cross-contamination, with only one carcass swab found to be positive for E. coli O157:H7.

Similar to prevalence reports of pathogenic bacteria in feedlot sheep, there is a scarcity of information in regard to their antimicrobial resistance profiles. This is not to say that this type of information is not available for sheep; however, the majority of the data relate to isolates from clinical cases and not those cultured from apparently healthy animals as in the current research. Due to the limited number of E. coli O157:H7 and Salmonella isolates, we also cultured and randomly selected 20 fecal coliform isolates for antimicrobial susceptibility screening on each of the collection dates. In general, we observed an increase in the number of isolates showing resistance to one or more antibiotics, as well as the number of multiresistant isolates, as the feeding period progressed. Multidrug resistance was observed in this study; however, as might be expected, the patterns of resistance were to antimicrobials commonly used in veterinary medicine (ampicillin, tetracyclines, and sulfonamides) and not those used in human medicine. All isolates examined were susceptible to enrofloxacin and cefotior, antibiotics that are indicated for the treatment of salmonellosis in humans.

Results of this research demonstrate that feedlot sheep, like cattle, are naturally colonized with the foodborne pathogens E. coli O157:H7 and Salmonella and that the wool could be a significant potential source of carcass contamination. Furthermore, while in-plant processing procedures and intervention strategies are largely effective in preventing carcass contamination, the implementation of preharvest intervention strategies will be an important part of a multihurdle approach to food safety.

**REFERENCES**


