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# *E. coli* O157 and *Salmonella* spp. in White-tailed Deer and Livestock

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## Abstract

*Escherichia coli* O157 and *Salmonella* spp. are among the leading causes of food-borne illness in the United States and bacteria have been isolated from numerous ruminant animal sources. The objective of this study was to assess the incidence of *E. coli* O157 and *Salmonella* spp. in white-tailed deer (*Odocoileus virginianus*) and livestock simultaneously grazing the same rangeland. *Escherichia coli* O157 was found in 1.25% of cattle, 1.22% of sheep, and 5.00% of water all from samples taken in September; however, no *E. coli* O157 was found in other sampled months or any species. *Salmonella* spp. were found in the highest quantities in deer and sheep, 7.69% and 7.32%, respectively. *Salmonella* spp. were also found in sampled water troughs, goats, and cattle (5.00%, 3.70%, and 1.25%, respectively). Further research examining pathogen distribution is needed to determine if white-tailed deer are a natural reservoir for these bacteria.

## Introduction

Since its recognition in 1982 (Riley *et al.*, 1983), *Escherichia coli* O157:H7 has been implicated in numerous bouts of illness and multiple deaths (McClure, 2000). Contamination of foods occurs most often during the harvest of domestic livestock, when the contents of the digestive tract or material from soiled hides contaminate the carcass (Elder *et al.*, 2000). Because of the high incidence of contaminated ground beef, investigations have identified cattle as a reservoir of the bacteria (Zhao *et al.*, 1995; Bielaszewska *et al.*, 2000). Sheep as well as other ruminant animals have also been identified as a reservoir of *E. coli* O157:H7 (Wallace, 1999; Hussein *et al.*, 2000; Renter *et al.*, 2001).

Not all meat products are produced in regulated processing plants; carcasses from deer and other sport animals are often processed in unregulated plants, or under less-than-ideal field conditions. *E. coli* O157:H7 from wildlife species have caused enterohemorrhagic

colitis infections in humans (Keene *et al.*, 1997; Rabatsky-Ehr *et al.*, 2002). These repeated outbreaks have prompted research into the possible carriage of *E. coli* O157:H7 by wildlife, and possible relationships between livestock and wild animals co-grazing rangeland (Rice *et al.*, 1995; Sargeant *et al.*, 1999; Rice *et al.*, 2003). The National Animal Health Monitoring Service (NAHMS, 1996) suggested that on-farm control of both *Salmonella* spp. and *E. coli* O157:H7 can be improved by removing known carrier animals from the herd. While this strategy may prove effective in confined settings such as dairies or feedlots, selective animal removal is not likely to be feasible in rangeland settings. The present study was designed to assess the presence of *E. coli* O157 and compare serotypes of *Salmonella* spp. found white-tailed deer (*Odocoileus virginianus*) and livestock simultaneously grazing rangeland.

## Results and discussion

A total of over 1,015,000 individual hunting licenses were distributed by the Texas Parks and Wildlife service in the 2002-2003 hunting season (Jim Rivers-Texas Parks and Wildlife Service, personal communication). The processing of wild game is often performed by untrained personnel, compromising the health of these hunters and their family and friends when eating undercooked meat contaminated with *E. coli* or *Salmonella*. Several human enterohemorrhagic *Escherichia coli* infections have been traced to meat products from contaminated deer (Keene *et al.*, 1997). Genetically indistinguishable *E. coli* O157:H7 isolates were collected from jerky, patient stool samples, uncooked meat from the deer, the hides, as well as from a saw used to process the black-tailed deer carcasses (Keene *et al.*, 1997). More recently, a case of *E. coli* O157:H7 infection in a young child due to consumption of contaminated white-tailed deer meat has been reported (Rabatsky-Ehr *et al.*, 2002). Since deer and other ruminants can be an important reservoir of *E. coli* O157, and because this pathogen is spread via a fecal-oral route it is likely that other ruminants can become infected with food-borne pathogenic bacteria through exposure to contaminated feces.

As shown in Table 1, September was the only month that livestock samples produced any *E. coli* O157 isolates. Three out of 66 total samples (4.55%;  $P < 0.05$ ) were positive for *E. coli* O157. The absence of positive samples in the months of October, November, and December is likely to be due to the bacteria's well-documented seasonal shedding pattern. *E. coli* O157:H7 abundance is positively correlated with warmer weather (Kudva *et al.*, 1996; LeJeune *et al.*, 2001b). Of the three positive samples, cattle, sheep, and water samples each made up 33.3% of the positive sources (Table 2). Cattle results indicated that 1.25% of the cattle population was positive for *E. coli* O157 (Table 2). These results were

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Table 1. Frequency distribution of positive *E. coli* O157 and *Salmonella* spp. from four different livestock sampling dates in 2003.

	<i>E. coli</i> O157		<i>Salmonella</i> spp.	
	Frequency	Percentage	Frequency	Percentage
September	3	4.55 <sup>a</sup>	6	9.09 <sup>a</sup>
October	0	0.00	4	5.88
November	0	0.00	0	0.00
December	0	0.00	1	1.54

<sup>a</sup>values within a column or row with the same or no superscript, do not differ ( $P > 0.05$ ).

similar to an 11 month, range cattle study reporting 1.01% of cattle testing positive for the O157 bacteria (Renter *et al.*, 2003).

One of 82 sheep (1.22%) sampled in our study was positive for *E. coli* O157 (Table 2). Chapman *et al.* (1997) reported a slightly higher percentage of 2.2%. Another study looking at sheep on rangelands reported that the incidence ranged from 31% in June, to 5.7% in August, and finally dropping to 0.0% in November (Kudva *et al.*, 1996). One of 20 (5.0%) water samples tested positive (Table 1). This was higher than a previous finding of 0.34% of water sources testing positive (Renter *et al.*, 2003). This difference is thought to be influenced by this study's small sample size.

While this study did not detect *Escherichia coli* O157 in any of our goat samples, goats have shown to harbor *E. coli* O157:H7 (Dontorou *et al.*, 2004; Pritchard *et al.*, 2000). It is important to point out that in our study, sampled cattle and sheep were grazed together in a rotational grazing system and shared water troughs; however, the goats were grazed at a different location with entirely separate water sources (Fig. 1). At no point in the study did goats interact with either the sheep or the cattle, or have access to their water or food supplies or their grazing land.

*Salmonella* spp. is another food-borne pathogen that is commonly found in the intestinal tract of birds, reptiles, fish and mammals (Ewart *et al.*, 2001) and is the most common food borne illness world-wide (Deshpande, 2002). *Salmonella* contamination has become a major concern of many food processing plants. Although *Salmonella* requires more cells for infection than *E. coli* O157:H7, only a few cells are needed to cause illness (D'Acoust *et al.*, 1985). Lamb meat has been implicated in at least two *Salmonella* outbreaks (Synnott *et al.*, 1993; Evans *et al.*, 1999). Birds are a major carrier of *Salmonella* spp. due to high population density and potentially contaminated feed ingredients like meat and bone meal (Salyers and Whitt, 1994). The present study found white-tailed deer can be carriers of *Salmonella oranienburg* in the rumen (Table 3).

All sampling months produced intestinal samples containing *Salmonella* spp. (Table 1). Our data suggest a potential seasonal shedding pattern for *Salmonella* spp. with the greatest prevalence in the warmer months. In the present study, 6 of 82 (7.32%) sheep samples and three of 81 goats (3.70%) tested positive for *Salmonella* spp. (Table 4). Duffy *et al.* (2001) found 1.9% of fall lamb carcasses tested positive for the bacteria. In a USDA survey on feedlot cattle, 5.5% sampled tested positive (USDA-APHIS-VS, 1995) which is substantially higher than the 1.25% *Salmonella* positive cattle found in this study (Table 4). One of 20 (5.00%) water samples also tested positive for *Salmonella* (Table 4). This is much higher than the 0.8% found in a previous water trough study (LeJeune *et al.*, 2001b), but it is important to note the relatively small sample size of this study.

The incidence of the *E. coli* O157 positive sheep and cow samples, as well as the *S. Munchen* isolated from sheep and water trough samples (Table 3), demonstrates the possibility that the bacterium was transferred to or between domestic animals via the positive water trough. McGee *et al.* (2002) found *Escherichia coli* O157:H7 could survive in field water troughs for 14 d at temperatures over 15°C. LeJeune *et al.* (2001a) documented that, previously non-positive calves became colonized with *E. coli* O157 after drinking from water sources which were fecally contaminated as many as 183 d earlier. It was also found that previously uninfected calves that drank from those contaminated troughs contracted the bacterium and shed it for up to 87 d after the trial (LeJeune *et al.*, 2001a). Wildlife that consumes water from cattle troughs could potentially become infected with the bacteria, or conversely could spread the bacteria to livestock and other wildlife.

The small sample size of deer in the present study was affected by the high frequency of perforated GI tracts during harvest and field dressing of the sample deer. Deer with punctured GI tracts were eliminated from the study to prevent cross-contamination of samples. Neither November nor December sampling dates of deer

Table 2. Frequency distribution of positive *E. coli* O157 from sampled sources.

	n	Frequency	Species %	% of Total Positives
Cattle	80	1	1.25	33.33
Sheep	82	1	1.22	33.33
Goats	81	0	0.00	0.00
Deer, Fecal	26	0	0.00	0.00
Deer, Rumen	26	0	0.00	0.00
Water	20	1	5.00	33.33

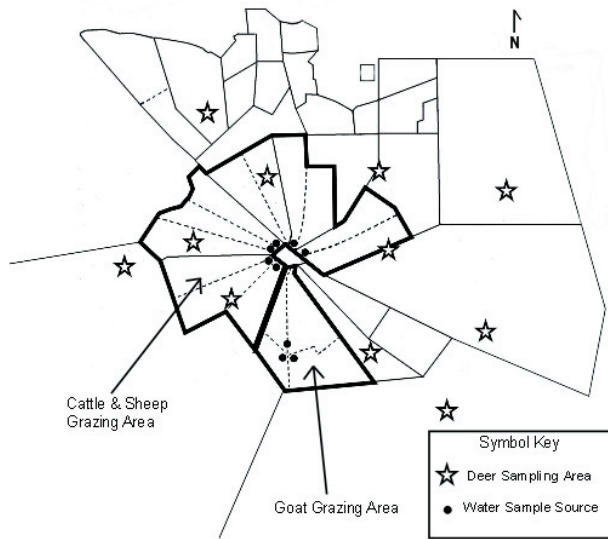


Fig. 1. Map of livestock grazing areas, water sample sources, and deer sampling locations located on the Angelo State University Ranch.

Table 3. *Salmonella* serotypes isolated from different sources.

Sample Source	Serotype (frequency within source)
Cattle	Denver (1)
Sheep	Anatum (1)
	Muenchen (1)
	Urban (1)
	Urbana (2)
	Untypeable (2)
Goat	4, 12: i :-B (1)
	Untypeable (2)
Deer, Rumen	Oranienburg (2)
Water	Muenchen (1)

produced any positive *E. coli* O157 samples, which is consistent with the seasonal shedding pattern of *E. coli* O157. Neither rumen nor fecal contents of the harvested deer were positive for *E. coli* O157 (Table 2). In a large scale study of 609 harvested deer 0.5% of samples tested positive for *E. coli* O157:H7 in feces (Fisher *et al.*, 2001). In a study of white-tailed deer that shared rangeland with cattle, 2.4% of deer fecal samples tested positive (Sargeant *et al.*, 1999). Sampling deer in prime fecal shedding months of the summer and spring could possibly produce more O157 positive animals. Because

hunting season of white-tailed deer is limited to fall and winter months, the threat of food-borne illness due to contaminated venison appears to be somewhat limited to those months. However, with the increasing popularity of harvesting exotic ruminants year-round the threat of food-borne illness from contaminated wild game cannot be discounted.

The present study found no indication of *E. coli* O157 presence in the population of white-tailed deer which graze on the Angelo State University rangeland. However, testing during the peak incidence spring and summer months could produce vastly different results. The occurrence of the bacteria in sheep, cattle, and their shared water source, indicated the organism could potentially be transferred from one animal to another via water supplies. The presence of *Salmonella* spp. in all four animals and the water source indicates that all the ruminant animals can be carriers of the organisms. Results showing a relationship between deer and livestock inhabiting the same rangeland and both testing positive for *E. coli* O157 could have a major impact on on-farm management of the organism. Control of the organism in livestock could prove to be impossible if reinoculation or circulation from wildlife vectors occur.

**Experimental procedures**

Fecal and water samples were collected once each month from livestock grazing on Angelo State University rangeland in September, October, November, and December (Fig. 1). This rangeland supports around 270 ewes, 100 nannies, and 62 cows. Fecal samples were collected via direct rectal grab from 20 Rambouillet ewes (*Ovis aries*), 20 Boer nannies (*Capra hircus*), and 20 Angus cows (*Bos tarus*). Samples were aseptically collected and placed in sterile conical vials. Water samples were collected from water troughs utilized by livestock. All samples were refrigerated at 4°C and transferred on ice to the Food and Feed Safety Research Unit of the United States Department of Agriculture – Agricultural Research Service (USDA-ARS) in College Station, TX within 24 h of collection.

White-tailed deer (*Odocoileus virginianus*) were hunter-harvested over five days in the regular hunting season from Angelo State University rangeland shared between cattle, sheep, and goats. The shared rangeland covers about 2,400 ha, with 40% covered by brush. Over the five days, 25 deer of various ages and both sexes were harvested and brought in from the field to be field dressed by trained technicians to prevent contamination from perforated GI tracts. Digesta samples were

Table 4. Frequency distribution of positive *Salmonella* spp. from sampled sources.

	n	Frequency	Species %	% of Total Positives
Cattle	80	1	1.25	7.69 <sup>c</sup>
Sheep	82	6	7.32	46.15 <sup>a</sup>
Goats	81	3	3.70	23.08 <sup>b</sup>
Deer, Fecal	26	0	0.00	0.00 <sup>c</sup>
Deer, Rumen	26	2	7.69	15.38 <sup>c</sup>
Water	20	1	5.00	7.69 <sup>c</sup>

a, b, c values within a column with the same or no superscript, do not differ ( $P > 0.05$ ).

aseptically removed from the rumen of all deer and were placed in individual sterile conical vials. Fecal samples were collected aseptically from the rectum and placed in individual sterile conical vials. Deer samples were immediately refrigerated and transferred to the USDA-ARS lab in College Station, TX on ice within 48 h of collection.

#### *Escherichia coli* O157 isolation procedure

Fecal and rumen samples (10 g) were enriched in a Gram-Negative (GN) Hajna broth (Difco, Detroit, MI, USA) supplemented with cefsulodin (10 µg/ml), vancomycin (8 µg/ml), and cefixime (1.42 µg/ml) at a 1:10 ratio (Elder *et al.*, 2000). Each sample was incubated at 37°C for 6 h and then quickly refrigerated to 4°C for overnight storage. Samples were specifically enriched for *E. coli* O157 using Anti *E. coli* O157 Immunomagnetic beads (Neogen Corp., E. Lansing, MI, USA) and were plated on CHROMagar® selective medium (DRG Intl, Mountainside, NJ) supplemented with potassium tellurite (0.5 µg/ml). Plates were incubated at 39°C for 24 ± 3 h. Suspect *E. coli* O157 colonies were picked and resuspended in sterile buffer. Resuspended colonies were placed onto *E. coli* O157 Reveal Sticks® (Neogen Corp., E. Lansing, MI, USA). Colonies which activated a positive response from the Reveal Sticks® were reported as *E. coli* O157.

Water samples were processed using the same protocol except Brilliant Green Bile broth was used for enrichment of *E. coli* O157 in place of supplemented GN Hajna.

#### *Salmonella* spp. isolation procedures

*Salmonella* was isolated according to the methods described in the FDA Biological Analytical Manual (FDA-BAM, 2001). Two to five g of fecal or ruminal contents were placed in iodine activated Tetrathionate (Tet) broth (Difco, Detroit, MI, USA) and vortexed. The broth was incubated at 37°C for 24 h. A total volume of 200 µl of Tet enrichment was transferred to five mls of Rappaport-Vassiliadis (RV) broth (Difco, Detroit, MI, USA) and incubated at 42°C for 24 h. Samples from the RV broth were then streaked for isolation on Brilliant Green agar (BGA; Difco, Detroit, MI, USA) plates supplemented with Novobiocin (20 µl/ml) and were incubated at 39°C for 24 h. All *Salmonella* isolates were stored in glycerol and Tryptic Soy Broth at -80°C until serotype confirmation was performed by the National Veterinary Services Laboratory (NVSL) in Ames, IA.

#### Statistical analysis

Frequency distributions for positive and negative results were conducted using the PROC FREQ function of SAS (SAS Inst., Inc.) and pairwise comparisons of percentages were made at a significance level  $P < 0.05$  (Ott, 1988).

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