Prevalence and Concentration of *Escherichia coli* O157:H7 in Cattle, Products, and the Environment in the United States of America: A Meta-Analysis Study

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**Abstract**

Shiga toxin-producing *Escherichia coli* O157:H7 are bacterial pathogens that cause foodborne infections in humans. The objectives of this study were to find the pooled prevalence and concentration of *Escherichia coli* O157:H7 in cattle, hides, carcass, and the environment in the United States of America using meta-analysis. The PRISMA and MOOSE research protocols were employed in the methodology. Weighted effect size was calculated using MetaXL software. A total of 1737 publications were screened, out of which 53 were selected for the final analysis. The pooled prevalence in feedlot cattle was 10.96% (95% CI: 4.2-18.8%). In dairy cattle a pooled prevalence of 1.5% (95% CI: 0.11-3.5%) was observed. The prevalence between feedlot and dairy cattle was significantly different (p<0.05). The herd prevalence in combined feedlot and dairy cattle was 31.7% (95% CI: 10.2-55.5%). Hide and carcass samples' pooled prevalences were 54.7% (95% CI: 41.7-67.5%) and 21.3% (95% CI: 9.7-34.2%), respectively. Prevalence of environmental samples was 8.1% for produce (95% CI: 0-29.6%), 4.6% for watershed and sediment samples (95% CI: 0-12.2%), and 2.4% for water taken from troughs (95% CI: 0.39-5.1%). Significant difference was observed in individual, herd, and environment prevalence between feedlot cattle and dairy cattle (p<0.05). The prevalence in feedlot cattle was 10.96% (95% CI: 4.2-18.8%). In dairy cattle a pooled prevalence of 1.5% (95% CI: 0.11-3.5%) was observed. The prevalence between feedlot and dairy cattle was significantly different (p<0.05). The herd prevalence in combined feedlot and dairy cattle was 31.7% (95% CI: 10.2-55.5%). Hide and carcass samples' pooled prevalences were 54.7% (95% CI: 41.7-67.5%) and 21.3% (95% CI: 9.7-34.2%), respectively. Prevalence of environmental samples was 8.1% for produce (95% CI: 0-29.6%), 4.6% for watershed and sediment samples (95% CI: 0-12.2%), and 2.4% for water taken from troughs (95% CI: 0.39-5.1%). Significant difference was observed in individual, herd, and environment prevalence between regions (χ² =903.14, p=0.0000; χ² =11.06, p=0.0039; χ² =13.59, p=0.0004, respectively). *E. coli* O157:H7 concentrations were highest in feces (990-300,000 cfu/g), followed by hides (5-9,800 cfu/100 square cm), and carcass (1-189 cfu/100 square cm). At least one supershedder exists in a herd. The findings in this study showed that *Escherichia coli* O157:H7 serotype is widespread in feedlots, hides, herds, and carcass in the United States of America necessitating appropriate measures to prevent human illnesses. Improving management practices in cattle herds, reduction of environmental contamination, and hygienic slaughter practices are targets of intervention.

**Keywords:** *Escherichia coli* O157: H7; Prevalence; Concentration; Cattle; Feces; Hide; Carcass; Environment; Meta-analysis; United States of America

**Introduction**

Shiga toxin-producing *Escherichia coli* (STEC) are bacterial foodborne pathogens producing disease in humans characterized by diarrhea, Hemorrhagic Colitis (HC), and Hemolytic Uremic Syndrome (HUS) [1-3]. From about 500 O-serotypes of Shiga toxin-producing *Escherichia coli* isolated so far from humans with disease, only seven serogroups (O26, O45, O103, O111, O121, O145, and O157:H7) are associated with severe clinical illness [2]. One of these serotypes, *Escherichia coli* O157:H7 (*E. coli* O157:H7), is studied extensively.

*E. coli* O157:H7, responsible for the majority of human enterohemorrhagic diseases, has a worldwide distribution [4]. Outbreaks of illnesses associated with *E. coli* O157:H7 have been reported throughout the northern hemisphere, most frequently in Canada, Japan, the United Kingdom, and the United States of America [5]. The pathogens most often implicated in outbreaks caused by consumption of fruits and vegetables from 2009 to 2010 were norovirus, *Salmonella* species and *E. coli* O157:H7 [6]. The predominant serotype isolated from patients among Enterohemorrhagic *E. coli* (EHEC) group was *E. coli* O157:H7 [1]. *E. coli* O157:H7 infection alone is responsible for 73,480 illnesses, 2,168 hospitalizations, and 61 deaths annually in the United States [7]. Besides causing foodborne illnesses, *E. coli* O157:H7 is associated with economic losses. Since the 1980s, more than $2 billion have been spent by the cattle industry to combat *E. coli* O157:H7 and STEC in processing plants [8].

Cattle are natural reservoirs of STEC serotypes including *E. coli* O157:H7 [9,10]. However, except less than three days old neonatal calves [11], cattle do not suffer disease as they lack vascular receptors
in their tissues [12]. *E. coli* O157:H7 colonizes the terminal colon specifically the Rectoanal junction (RAJ) mucosa [13-16]. In colonized cattle, a unique class of cattle known as “supershedders” are responsible for most of the contamination of the population [17,18]. Supershedders are defined as animals with *E. coli* O157:H7 concentrations of at least 10^9 Colony Forming Units (cfu) per gram of feces [3,15,19]. Feces are the major source of contamination to beef and produce [20]. In Scotland clustering of human infections was associated with regions with high cattle to people ratio [21-23]. A direct link between cattle and human infection has been established by phage typing and Pulsed Field Gel Electrophoresis (PFGE) [24,25].

Important sources of STEC O157 contamination in the United States of America are food [7,26,27], water [28,29], pen floor [28], processing plant lairage [30]; and unpasteurized apple juice, spinach and salami [31,32]. Sixty-five percent (65%) of STEC O157 outbreaks were transmitted primarily through consumption of food (beef and produce); the rest through animal contact (10%), person-to-person (10%), waterborne (4%), and other or unknown medium (11%) [33]. According to different studies, cattle hides and beef carcass contaminations are common particularly during the slaughter process. *E. coli* O157:H7 prevalence was 20.3% on hides and 6.7% on carcasses [34]. The prevalence of *E. coli* O157:H7 on hides was 50.3% when cattle were loaded onto a transporter [30]. Natural transmission of *E. coli* O157 between cattle is thought to be largely by the fecal-oral route, although transmission may be indirect through an environmental reservoir [35].

Many publications on the prevalence of *E. coli* O157:H7 are available in the United States of America; however, an overall single quantitative estimate of this specific serotype in individual cattle, products, and the environment is lacking. We, thus, conducted a meta-analysis study of *E. coli* O157:H7 in the United States of America to determine (a) a pooled prevalence in cattle, hides, carcass, and environmental samples, and (b) summarize concentrations of the serotype in cattle feces, hides, and carcass.

**Methods**

Meta-analysis, a statistical analysis of a large collection of analyses results from individual studies for the purpose of integrating the findings [36], was the method adopted in this study. The PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) [37] and the Moose Statement (Proposal for Reporting Meta-analysis of Observational Studies in Epidemiology) [38] protocols were selected.

**Study area and population**

The study area was the United States of America. All of the cattle in the country constitute the study population. Relevant databases were searched to screen and select publications for the final meta-analysis. Cattle studied in these selected publications were sampled from all the four regions (Northeast, Midwest, West, and South), consisting of 17 states mentioned by name; and “U.S. States”, “Across U.S. States”, “Western U.S.”, “North U.S.”, “South U.S.”, “Midwest”, “West”, “South”, where the states were unnamed (Table 1). The division of the regions into Northeast, South, West, and Midwest was based on the U.S. Census Bureau.

**Search strategy**

Search terms used were, (i) Prevalence study: “Prevalence of *Escherichia coli* O157:H7 in cattle in the United States of America”; (ii) Prevalence of environmental samples study: “Shiga toxin *Escherichia coli* O157:H7 contamination in environment, slurry, fruits, vegetables, pasture, food, and feed in the United States of America”; (iii) Concentration study: “Colony forming units of *Escherichia coli* O157:H7 in the United States of America”. PubMed (www.ncbi.nlm.nih.gov/entrez/query.fcgi), Science Direct (www.sciencedirect.com), Google Scholar (http://scholar.google.com) were the three free database sources used in the study.

**Inclusion and exclusion criteria**

First author conducted the search. Two authors (first and second) screened records using all set criteria and selected publications used in the analysis. Complete agreement was reached by consensus. Methodology was discussed among authors including other persons and suggestions were incorporated. Criteria used to select eligibility of searched publications are listed (Table 2).

**Data extraction**

For all the studies data were extracted based on author(s), year of study, title of article, diagnostic method, production system (beef, dairy), cases (positive results), sample size, type of sample, cfu per gram of sample, cfu/square cm, state, and type of article.

**Regional difference**

Differences in prevalence for individual, herd, and environment categories were analyzed between regions.

**Data analysis**

The inverse variance heterogeneity model (IVhet model) was used in this study. A better performance of the inverse variance heterogeneity model embedded in MetaXL software compared to the fixed effect or random effect models is described [51]. Heterogeneity among studies was determined to see whether there were true differences underlying the results of the studies or the variation in findings was due to chance. We used I² statistic to assess heterogeneity. A better measure of consistency between studies using I² is described [52]. Doi plots [53], which plots effect size against sample size are used to analyze and display publication bias. The overall effect size estimated in this study was prevalence. Methods for the meta-analysis of prevalence and double arc sine transformation are described [54]. Individual prevalence is defined as the number of animals that are positive (shed *E. coli* O157:H7) among animals tested; and herd prevalence is the number of positive herds among total herds tested. A positive herd (or farm) is a herd which has at least one animal shedding *E. coli* O157:H7. Similar epidemiological approach was used to calculate hide, carcass, and environmental samples’ prevalences. In this study, supershedders are defined as animals with *E. coli* O157:H7 concentrations of at least 10^9 colony forming units (cfu) per gram of feces. Sensitivity analysis was done to assess if the overall effect size changes when outlying small or large values are excluded. The absence of significant changes shows that the estimated overall effect size is robust. Meta-regression was conducted to investigate whether particular covariates explain the observed heterogeneity between studies. Year, sample size, and region were extracted from publications eligible for quantitative meta-analysis. Differences between regions were analyzed using Chi square statistic. Test of homogeneity together with post hoc analysis using pair wise comparison method was selected for further analysis. MetaXL software version 5.3 [55] was used for quantitative meta-analyses. R statistical computing software version 4.0.5 (R Core Team, 2020; RStudio Team, 2020) was used for meta-regression analysis and to calculate Chi square values.
### Results

The number of publications selected for the final meta-analysis is illustrated (Figure 1). Out of a total of 1737 publications screened 53 were selected to be used for the final quantitative meta-analysis.

#### Prevalence of *E. coli* O157:H7 in cattle and the environment

The pooled prevalence of *E. coli* O157:H7 in feedlot cattle in the United States of America was 10.96% (95% CI: 4.2-18.8%) (n=23,048) (Table 3) (Figure 2). In dairy cattle, the pooled prevalence was 1.5% (95% CI: 0.11-3.5%) (n=10,188). The pooled herd prevalence was 31.7% (95% CI: 10.2-55.5%) (n=377) in combined beef and dairy cattle herds. The difference in prevalence between beef and dairy cattle was significant (p<0.05). Unweighted individual prevalence ranged from 0.71 to 27.8% in feedlot cattle, 0.3 to 5.5% in dairy cattle, and 7.1 to 100% in herds. The pooled prevalence decreased from 10.96% (~11%) to 10% when four outliers were excluded by sensitivity analysis (Figure 3). Similarly, $I^2$ dropped from 99 to 89. Further exclusion of any outliers didn’t change the pooled prevalence from 10%. Among environmental samples, the highest prevalence was observed in produce (8.1%; 95% CI: 0-29.6%) followed by watershed and sediment samples (4.6%; 95% CI: 0-12.2%). Prevalence of water taken from drinking troughs was low (2.4%; 95% CI: 0.39-5.1%). Unweighted prevalence of environmental samples ranged from 1 to 68%.

#### Hide and carcass contamination

The pooled prevalences of hide and carcass contamination were 54.7% (95% CI: 41.7-67.5%) and 21.3% (95% CI: 9.7-34.2%), respectively. Hide and carcass contamination showed 400% and 100% percent increases, respectively, from individual feedlot prevalence.

#### Concentration of *E. coli* O157:H7

Only seven (7) out of 792 publications screened were selected for final analysis. Due to lack of appropriate statistical model as data were produced based on different scales of measurement, it was not possible
to estimate a concentration weighted effect size. Hence, the records were summarized as presented in the original publications (Table 4). The concentrations on feces (cfu/g), hides (cfu/100 square cm), and carcass surfaces (cfu/100 square cm) ranged from 900-300,000, 5-9,800, and 1-189, respectively. In all of the final records selected, at least one supershedder was found in a herd.

Regional difference

The pooled individual prevalences (beef and dairy combined) were 4.8%, 12.3%, 0.39%, and 0.96% for South, Midwest, West, and Northeast, respectively. Significant difference was observed in individual, herd, and environment prevalence between the regions ($\chi^2=903.14, \ p=0.0000$; $\chi^2=11.06, \ p=0.0039$; $\chi^2=13.59, \ p=0.0004$, respectively). In the individual animal post hoc analysis, each region was different from the other entire region. In the herd and environment prevalence, the Northeast was significantly different from the rest; however, the South, Midwest, and West regions didn’t show significant differences among them.

Discussion

The study was conducted to determine the magnitude of *E. coli* O157:H7 serotype in cattle, products, and the environment with a single collective quantitative estimate. Studies selected covered all the four regions and at least seventeen states out of the fifty. When only states identified by name are considered, sampled cattle represent 59.86% of the study population. We couldn't compute the exact figure as a good number of publications didn’t name states; hence, the true representation is greater.

Prevalence in cattle

A high presence of *E. coli* O157:H7 in cattle was observed. One in ten beef cattle and one in three cattle herd harbored the pathogen in the study area. In some studies, all herds tested were positive. In agreement to the findings of this study, a meta-analysis study from North America reported that the prevalence of O157 was 10.68% in fed feedlot and 1.79% in adult dairy cattle [76]. The design of many publications searched in this study lacked randomization and convenient sampling was used in study animal selection; thus, our result can overestimate (or underestimate) the true population parameter. *E. coli* O157:H7 and other STEC are shed transiently in the feces. As prevalence is a snap-shot of detecting the presence of infection, the true population parameter can be underestimated.

Prevalence was significantly higher in beef than dairy cattle in this study. However, the findings of a good number of studies reviewed showed prevalence was higher in dairy than beef cattle. For STEC O157, a review of global testing of cattle feces showed prevalence
ranges of 0.2-27.8% in beef cattle [77], and 0.2-48.8% in dairy cattle [78]. In Belgium, the highest prevalence of *Escherichia coli* O157 was found on dairy cattle farms (61.2%), followed by mixed dairy and beef (44.4%), beef (22.7%), and veal calf farms (9.1%) [79]. In Canada, an *E. coli* O157:H7 prevalence of 62.1% was reported in a dairy farm [78]. The prevalence was lower (0.6%) in production systems of low animal density than when animals were kept under systems of high animal density (2.5%) [77]. From these reports, authors argue that increased prevalence of *E. coli* O157:H7 in feedlot cattle in the United States of America is related with management programs than animal type. Different management programs which include bedding and pen surfaces handling, manure management, biosecurity, cattle grouping, transportation and lairage, stress, feeding plan, and watering program is reviewed [8]. High deposition of organisms on pen floors, watering troughs, or open pasture facilitates infection particularly in overcrowded animals.

**Hide and carcass prevalence**

More than half of hide samples tested were found to be contaminated with *E. coli* O157:H7 in this study. The level of hide contamination was five times the prevalence, showing a connection between increased hide contamination and the hygiene of slaughter practices. Thus, safe disposal of gut contents and hygiene at slaughtering plants can reduce hide and carcass contamination.

**Environmental contamination**

Samples found contaminated were produce and different water sources (watering troughs, ponds, irrigation, and watersheds). Pathogen survivals in water troughs, pen floors, and in the immediate environment of animals are significant factors for infection. Water troughs and contaminated pen floors appeared to be particularly influential sources driving *E. coli* O157:H7 population dynamics [28,61]. Based on mathematical model assumptions, contaminated drinking water was the most important pathway of *E. coli* O157:H7 transmission to cattle [80]. Water is the major source of contamination for fresh produce [81]. Survival of culturable *E. coli* O157 for at least 245 days in microcosm sediments is reported [82]. The bacterium can remain alive in manure for 100 days [83]; or more than six months if the manure is kept under anaerobic condition at 16°C [84]. A few Restriction Endonuclease Digestion Patterns (REDPS)

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**Figure 1:** PRISMA flow diagram.

\( n_1 \) stands for prevalence of cattle, hide, and carcass contamination publications; \( n_2 \) prevalence of environmental sample publications; \( n_3 \) concentration publications.

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Table 3: Prevalence of Shiga toxin-producing Escherichia coli O157:H7 in individual cattle and herds, environment, hide and carcass using IVhet model.

| Prevalence type | Production system | Sample | Positive | Sample size | Prevalence range* | Pooled prevalence (%) | 95% Confidence Interval | References |
|-----------------|-------------------|--------|----------|-------------|-------------------|-----------------------|------------------------|------------|
| Individual prevalence | Feedlot | Feces | 2,732 | 23,048 | 0.71-28.0 | 10.96 | 4.2-18.8 | [40,41,43-46,48,56-61,63] |
|                   | Dairy  | Feces | 211  | 10,188 | 0.3-5.5  | 1.5  | 0.11-3.5  | [29,42,48,59,62-67]  |
| Herd prevalence   | Beef and dairy | Feces | 127  | 377    | 7.1-100  | 31.7  | 10.2-55.5 | [29,40-42,44,57,60,63,66,67] |
| Hide prevalence   | Beef and dairy farms | Hide | 5864 | 10,700 | 11.0-71.0 | 54.7  | 41.7-67.5 | [30,34,47,49,50,57,68,69] |
| Carcass prevalence| Beef and dairy farms | carcass | 1497 | 6570 | 3.0-43.0 | 21.3  | 9.7-34.2 | [34,47,49,50,57,68,69] |
| Environment prevalence | Beef, dairy, and ranches | Water-trough | 41  | 1631 | 1.7-5.3 | 2.4  | 0.39-5.1 | [29,64,67,70] |
|                   | Ponds, irrigation, and public places | watersheds and water sediment | 101 | 2038 | 4.0-68.2 | 4.6  | 0-12.2 | [70,71] |
| Vegetable farms   | produce | 114 | 1402 | 7.9-25.0 | 8.1 | 0-29.6 | [72,73] |

*Ranges of prevalences from indicated publications reported before pooling.

Figure 2: Forest plot of the prevalence in feedlot cattle.

Persist and dominate over the entire feeding period in feedlot operation highlighting the importance of the farm environment, and not necessarily the incoming cattle, as a source of infection [85]. Water, hence, can be an easy but important environmental target for intervention against E. coli O157:H7 and other STEC transmission.

Concentration

The concentration of E. coli O157:H7 in feces taken directly from the terminal gut ranged from 900 to 300,000 organisms in one gram of feces. The amount is enough to contaminate other animals, hide, carcass, pen floors, and water troughs. At least 10^4 cfu/g of EHEC in cattle feces are associated with contamination of hides, and subsequently, carcasses, and beef [74]. Less than 700 organisms were sufficient for E. coli O157:H7 to establish illness in humans [86]. Authors recommend that a pooled estimate generated using additional data is required to generate a representative concentration value for the country.

Regional difference

The Northeast region is different from the other three regions in all individual, herd, and environment prevalence. Climate, geographic location, or management differences are apparent between the Northeast and other regions. However, a rigorous study is needed to explain the observed difference.

We have learned three lessons from the study. In the estimation of the overall effect, an increased heterogeneity index (I^2) was observed. Results of meta-regression showed region was found significant covariate accounting for 68.25% of heterogeneity (p=0.0002). Year of study and sample size were not significant covariates (p>0.05); however, year of study explained 9.77% of heterogeneity. One study with a large
sample size was found influential. The major drawback of $F$ is its undue sensitivity to large sample sizes. Presence of heterogeneity indicates a difference among the studies pooled. It is advised that only similar studies are pooled and analyzed. The authors experienced a difficulty in finding a good number of publications satisfying assumption of homogeneity of results under set inclusion and exclusion criteria. Long time span of records published from 1980-2019, states, study design, and sample collection (swab and fecal grab), are the sources of heterogeneity. The increased heterogeneity of results observed in this study, in spite of rigorous selection criteria applied, calls for standardization of study designs in future investigation of STEC O157 serotypes. However, publication bias wasn’t observed (Figure 4). The second lesson was that in the United States of America much attention is given to E. coli O157:H7 as a cause of EHEC human illnesses. ACDC report showed that 64% of all STEC infections in the United States are caused by non-O157 STEC [87]. Similarly, the total number of illnesses was higher in non-O157 STEC than E. coli O157:H7 [88-91]. Consequently, the six non-O157 serotypes (O26, O103, O111, O121, O145, and O45) are declared food adulterants [92]. In a study conducted in California, Cooley MB, et al. [70] reported a prevalence of 37.9% non-STE C in cattle, which is five-fold more than O157:H7 (7.1%). The authors used three methods of culture modifications, O-typing ELISA (Enzyme Linked Immunosorbent Assay), Multilocus Variable Number Tandem Repeat Analysis (MLVA), and ompA gene sequencing in their investigations. Hence, the authors recommend extending study to non-O157 STEC epidemiology, shedding, and disease history. The last lesson learned was that most of the studies rarely used epidemiological study designs. To be valid and applicable to the general population, investigators must incorporate a component of randomization in their research methods.

The prevalence outputs obtained from this study are valid estimates closer to the population parameter on account of rigorous inclusion and exclusion criteria set, large sample size, effect model selected, and sensitivity analysis, not withstanding increased $F$. Hence, the outputs can be used for microbiological risk assessment, sample size calculation, economic analysis, and decision analysis for E. coli O157:H7.

Table 4: Concentration of E. coli O157:H7 in cattle feces, hide, carcass, and feedlot surface area.

| Sample type               | Production system          | Sample size* | Concentration (cfu/g or cfu/100 cm²) | References |
|---------------------------|---------------------------|--------------|------------------------------------|------------|
| Feces                     | Beef                      | 122          | 1.6 × 10^2 cfu/g (median)           | [69]       |
|                           | Dairy                     | 16           | 2.0 × 10^2-1.0 × 10^3 cfu/g (range) | [42]       |
|                           | Feedlot                   | 200          | 9.0 × 10^2-3.0 × 10^3 cfu/g (range) | [74]       |
|                           | Dairy                     | 1            | 7.9 × 10^2 cfu/g (maximum)          | [49]       |
| Hide                      | Beef processing plant     | 86           | 9.8 × 10^2 cfu/100 cm² (maximum)    | [69]       |
|                           |                           |              | 8.0 × 10^2 cfu/100 cm² (median)     | [69]       |
|                           | Beef processing plant     | 245          | 4.0 × 10^2-4.0 × 10^3 cfu/100 cm² (range) | [47]       |
|                           | Dairy                     | 1            | 5.0 × 10^2 cfu/100 cm² (maximum)    | [49]       |
| Carcass                   | Beef processing plant     | 40           | 4.6 × 10^2 cfu/100 cm² (maximum)    | [69]       |
|                           |                           |              | 2.0 × 10^2 cfu/100 cm² (median)     | [69]       |
|                           | Beef processing plant     | 40           | 1.0 × 10^2-1.89 × 10^2 cfu/100 cm² (range) | [47]       |
| Feedlot surface area      | Feedlot                   | 40           | 3.6 × 10^2 cfu/g of soil (average)  | [75]       |

*Enumerable sample sizes.
Conclusion

More than one out of ten beef and close to one-third of cattle herds shed *E. coli O157:H7*. In addition, at least one-fifth of carcass samples harbored the pathogen. The risk of contamination of animals, the environment, food, and humans in the United States of America due to *E. coli O157:H7* is clearly evident. Pre-harvest control strategies (antimicrobials, vaccination, treatment with probiotics, administration of bacteriophages, and modification of the diet) are limited in reducing shedding. In both beef and dairy, on-farm management activities geared to achieve hygiene of pen surfaces, bedding, lairage, transportation, water trough, and feed handling are thus recommended for best outcome. Proper manure removal is critical. Avoidance of stress in beef cattle operations reduces colonization of the gut and thence eliminates or minimizes shedding to a minimum. To effectively protect the public from foodborne illnesses caused by *Escherichia coli O157:H7*, all control strategies should target cattle, the most important reservoir host.

Conflict of Interest

There is no conflict of interest.

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