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"Preharvest" Food Safety for *Escherichia coli* O157 and Other Pathogenic Shiga Toxin-Producing Strains

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ABSTRACT Preharvest food safety refers to the concept of reducing the rates of contamination of unprocessed foods with food-borne disease pathogens in order to reduce human exposure and disease. This article addresses the search for effective preharvest food safety practices for application to live cattle to reduce both contamination of foods of bovine origin and environmental contamination resulting from cattle. Although this research has resulted in several practices that significantly decrease contamination by *Escherichia coli* O157, the effects are limited in magnitude and unlikely to affect the incidence of human disease without much wider application and considerably higher efficacy than is presently apparent. Infection of cattle with *E. coli* O157 is transient and seasonally variable, likely resulting from a complex web of exposures. It is likely that better identification of the true maintenance reservoir of this agent and related Shiga toxin-producing *E. coli* is required to develop more effective control measures for these important food- and waterborne disease agents.

INTRODUCTION

Upton Sinclair's novel, *The Jungle*, which described horrific conditions in historical Chicago meat packing plants, engendered numerous reforms and regulations of the industry, including the Pure Food and Drug Act and the Meat Inspection Act of 1906, which in turn led to vast improvements in the sanitary conditions under which meat and meat products were handled. The massive and highly publicized 1993 outbreak of *Escherichia coli* O157 associated with Jack in the Box had a similar broad impact for the microbiological safety of food, including the classification of this pathogen as an "adulterant" in ground beef, and led to the implementation of the formal Pathogen Reduction and Hazard Analysis and Critical Control Point Program for this bacterium and other food-borne agents in meat processing plants. These changes were credited with significant reduction in the incidence of human infection with *E. coli* O157 in the United States over the subsequent several years; however, this trend did not continue, and in recent years the incidence of disease due to *E. coli* O157 has remained stubbornly stable. Incidence of disease caused by non-O157 Shiga toxin-producing *E. coli* (STEC) has paradoxically steadily increased, although this trend is undoubtedly due in part to increased use of more efficient diagnostic procedures.

The continued occurrence of disease outbreaks from *E. coli* O157 and other pathogenic STEC strains linked

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to ground beef indicates the limitations of postprocessing interventions to completely eliminate risk of human exposure through contaminated meat and meat products. New evidence of disease linked to other sources, including contaminated produce, water, and other environmental exposures including direct animal contacts, indicates that this group of pathogens has a more complex ecology than may have been previously recognized. This article addresses some of the data supporting this complexity to explain why human disease incidence is not declining, discusses the implications of the different genetic lineages of *E. coli* O157 on sources and severity of human infection, and reviews the benefits and limitations of control measures directed toward reducing the prevalence and shedding level of *E. coli* O157 and other pathogenic STEC strains by cattle, otherwise known as preharvest food safety in cattle production.

Twenty Years after the "Jack in the Box" Outbreak, Why Is *E. coli* O157 still a Problem?

Why has the incidence of human infection with *E. coli* O157 and other STEC pathogens remained stubbornly steady despite the implementation of stringent regulations and large investments in improved equipment and processing methods in meat packaging plants? One important factor is seasonal variation, or the marked increase in the numbers of cattle shedding *E. coli* O157 in their feces accompanied by increased contamination of hair coats (hides) during summer months. This seasonal variation results in increased contamination pressure, potentially overwhelming the control measures that are otherwise effective in preventing meat contamination during the rest of the year. The effects of higher contamination of cattle that overwhelm the control measures could be mitigated, at least in part, by adding a final decontamination step such as gamma irradiation for meat products of beef origin. However, in the absence of such a highly effective decontamination step, further reductions in meat-borne exposures to *E. coli* O157 may require interventions that reduce the degree of contamination of cattle sent to slaughter. Over the years, it has become clear that apart from ground beef, there are numerous vehicles for *E. coli* O157 that can result in human exposure, including fresh produce, drinking and recreational water, direct contacts with animals, and other environmental sources and reservoirs. This complex ecology of *E. coli* O157 likely contributes to seasonal infection pressure on cattle as well, and needs to be addressed in order to develop highly effective methods to reduce cattle infections with *E. coli* O157 and other pathogenic STEC strains.

How Do Foods of Bovine Origin Become Contaminated with *E. coli* O157?

There is a strong correlation between *E. coli* O157 prevalence in the feces and on the hair coats of cattle entering slaughter plants and carcass contamination during processing (1). Recent studies have begun to characterize the level of pathogen reduction in cattle feces that may be necessary to significantly reduce the hide and carcass contamination during processing. Woerner et al. showed that fecal pen prevalence exceeding 20% was associated with hide contamination prevalence of 80% or more (2). Similarly, Arthur et al. determined that slaughter cattle from feedlot pens with more than 20% positive fecal pats had both higher hide contamination rates (25.5%) and higher carcass contamination at pre-evisceration (14.3%), post-evisceration (2.9%), and post–final intervention (0.7%) stages (3). Comparative figures for slaughter cattle from feedlot pens with <20% positive fecal pat samples were lower hide contamination (5%) and carcass contamination 6.3%, 0% and 0% at pre-evisceration, post-evisceration, and post–final intervention stages, respectively (3). Overall, these data suggest that 20% fecal pat prevalence may be a functional threshold or marker for predicting groups of feedlot cattle having increased risk of hide or carcass contamination. Management practices that consistently result in fecal pat prevalence of less than 20% may therefore be required to accomplish further progress in preharvest food safety.

Can Live Cattle Be Managed to Reduce or Prevent *E. coli* O157 Infection?

Heavily contaminated cattle entering meat processing plants can apparently overwhelm the best sanitary procedures in practice; therefore, preharvest interventions in cattle rearing, management and husbandry, transport, and lairage that can effectively reduce the frequency of cattle infection with *E. coli* O157 offer the potential to reduce human exposures. In the last 2 decades, the development of preharvest interventions has remained a major focus of the food safety research in the United States. The early emphasis of preharvest food safety research was based on the hypothesis that the emergence of *E. coli* O157 disease in humans resulted from relatively recent changes in cattle management practices that favored this pathogen. Examples of such management practices included increased grain components in cattle feeds (4, 5), the use of antimicrobial drugs and other growth-promoting feed additives (6–11), increased intensity of cattle production, rearing larger herds and increased confinement (12–16), and

the adoption of new methods of manure handling and disposal on farms(17–19). Unfortunately, however, each of these attractive hypotheses has since either been refuted or shown to have only minor influence on cattle infection with *E. coli* O157, as described in several comprehensive recent reviews (20–23). The hypothesis that *E. coli* O157 infection of cattle results from high-grain diets and that feeding hay to the cattle would eliminate the problem merits particular note; while it has not been supported by subsequent research (5, 24), it is still frequently cited as if true in the news media responses to each new *E. coli* O157 disease outbreak, demonstrating a clear disconnect between scientific data and the popular support for an idea.

Unfortunately, with the exception of cattle vaccination against *E. coli* O157, the efforts to identify cattle management practices that consistently result in significant reductions in the frequency of cattle infection with *E. coli* O157 have largely failed (reviewed in references 20–23).

VACCINATION OF CATTLE AGAINST *E. coli* O157: A RAY OF LIGHT

Although certain interventions [for example, probiotics (20, 22)] show some promise for preharvest food safety against *E. coli* O157, vaccines have been the most effective interventions documented to date. Currently, two commercial vaccines against *E. coli* O157 in cattle have been developed and are available in at least some locations: a type III secretion system (T3SS) protein-based (Bioniche Life Sciences Inc., Belleville, Ontario, Canada) and a siderophore receptor and porin (SRP) protein-based (Epitopix, LLC, Wilmar, Minnesota) vaccine.

Cattle Vaccine Mechanisms

These vaccines target different mechanisms to induce immunity against *E. coli* O157 in cattle; T3SS proteins play important roles in bacterial adherence to the bovine intestinal epithelium, whereas SRP proteins are important for iron acquisition and survival of bacteria within the host. The products of T3SS genes such as *eae* and *tir* (intimin and Tir), encoded within the locus of enterocyte effacement (LEE), play key roles in the colonization of bovine intestines by *E. coli* O157 (25–30). Translocation of Tir and other effector proteins into host cells requires the T3SS-secreted EspA protein, which forms filaments connecting the bacteria to the host cell surface, as well as EspB and EspD, which are thought to form a membrane pore [reviewed by Frankel et al. (31) and Caron et al. (32)]. The T3SS protein-based vaccine strategy results in induction of mucosal antibodies capable of blocking adherence and subsequent colonization of the bovine intestinal mucosa by *E. coli* O157. Under low-iron conditions, bacteria produce a high-affinity iron transport system (e.g., SRP proteins) to bring the required nutrient inside the bacterial cell (33). The SRP protein-based vaccine results in induction of antibodies that bind to SRP located on the outer membrane of the bacterial cell, subsequently blocking iron transport into the cell, compromising the bacterial cell iron acquisition. Blocking iron transport by anti-SRP antibodies renders the bacteria at a selective disadvantage in a mixed microbial environment, resulting in reduced colonization. These approaches were recognized over a decade ago, resulting in a number of subsequent vaccine trials using purified T3SS or SRP protein-based vaccines. Although vaccines targeting T3SS proteins and SRP function via two entirely different mechanisms, recent meta-analysis studies suggest that both vaccines are efficacious at reducing the proportion of culture-positive animals (34–37).

Efficacy of Vaccination

Although the effectiveness of current vaccines in terms of reduced carcass contamination and ultimately reduced human illnesses is unknown, if 20% fecal prevalence is considered as a functional threshold marker for significantly reduced hide and carcass contamination, then the current vaccine efficacy would have to effectively reduce pen prevalence to <20%. Ideally, the precise efficacy of each vaccine can be calculated; however, significant variation in the efficacy of current vaccines is reported in different trials, and recent meta-analyses of multiple vaccine studies suggest that the efficacy of current vaccines is largely uncertain (36, 37). Consequently, Vogstad et al. simulated the uncertainty about vaccine efficacy using a log-normal distribution and estimated that the mean efficacy of current T3SS protein-based vaccine is approximately 58% (36). Using this vaccine efficacy, the authors developed a stochastic simulation model to compare distributions of *E. coli* O157 fecal shedding prevalence between cattle vaccinated with T3SS protein vaccine and nonvaccinated cattle.

The model outputs included distributions of fecal pen prevalence of *E. coli* O157 among vaccinated and nonvaccinated summer-fed cattle and nonvaccinated winter-fed cattle. One of the outcomes of this model was a reduction in the percentage of high-prevalence pens among immunized cattle fed in the summer. In this

model, approximately 58% of pens of nonvaccinated, summer-fed cattle showed fecal prevalence of >20%. In contrast, when summer-fed cattle were vaccinated with the T3SS protein-based vaccine, the percentage of pens with >20% fecal prevalence was reduced to approximately 30%. These results suggest that vaccination as an intervention in cattle prior to slaughter may roughly halve the number of pens with fecal prevalence of >20%, a significant improvement but still leaving 30% of pens with fecal prevalence >20%. As already discussed in this article, according to Arthur et al. (3) and Woener et al. (2), pens with >20% fecal prevalence contribute significantly to hide and carcass contamination. In turn, hide and carcass contamination can compromise apparent vaccine efficacy due to cross-contamination of hides during transport to harvest (38) or cross-contamination of carcasses during processing (39). On the basis of a postulated threshold effect involving vaccine-induced reductions in shedding density (reductions in the numbers of animals with fecal shedding exceeding 10^3 CFU/g *E. coli* O157, also known as super-shedders), Matthews et al. recently proposed that cattle vaccination would in fact produce substantially greater reductions in human disease caused by *E. coli* O157 than predicted based solely on effects on cattle shedding prevalence (40). Overall, it is still questionable whether the current vaccines would provide sufficient efficacy to accomplish the goal of controlling or reducing postharvest *E. coli* O157 contamination of cattle-derived food products.

Is It Practical to Vaccinate Cattle?

Recently, Withee et al. (41) combined quantitative risk assessment and marginal economic analysis to estimate the cost-benefit ratio of the "hypothetical O157:H7 vaccine" to prevent human food-borne illness. These authors determined that vaccinating the entire U.S. herd would be an effective intervention for preventing *E. coli* O157 illness in humans; however, the true efficiency of vaccination will primarily depend on three factors: (i) overall efficacy of the vaccine, (ii) herd coverage of immunity, and (iii) the cost of vaccine per unit. For example, the authors estimated that if the vaccine efficacy and coverage for herd immunity were assumed at 100% and the vaccine cost was assumed to be $3.00 per unit, then vaccination will optimally prevent approximately 21,000 human illnesses each year (41), or one-third to one-fifth of the annual burden of disease as estimated by the CDC (42, 43). This level of control would require vaccinating 22 million cattle intended for slaughter each year at a total cost of $66 million. In this scenario, the total benefits expected to accrue as a result of preventing 21,000 human illnesses would be $131 million (21,000 forgone cases times $6,256 per case). In contrast, if the vaccine efficacy was assumed at 50% (close to the estimated efficacy of current vaccines) and required herd coverage for immunity was assumed at 100%, then a $4.00 per unit cost of vaccination will optimally produce approximately 5,000 forgone illnesses (41). Therefore, even the moderate efficacy of current vaccines is predicted to prevent several thousand food-borne illnesses each year; however, there is still clearly significant room for the improvement of the efficacy of current vaccines and vaccination strategies.

Possible Future Directions for Vaccine Development

Given that two current vaccines provide protection by completely unrelated mechanisms, it is possible that simultaneous vaccination with both currently available products could have synergistic effects and result in significantly improved efficacy; however, no published studies in the literature address this possibility. Alternatively, new vaccines may be developed with improved efficacy. Dziva et al. (27) showed that in addition to the genes encoded on LEE-T3SS, *E. coli* O157 colonization in cattle is mediated by numerous other cell surface structures, including fimbriae, outer membrane proteins, O antigens, and other bacterial proteins. These authors have identified a novel fimbrial locus (z2199–z2206; ecs2114–ecs2107/locus 8) required for intestinal colonization in calves, and demonstrated that a deletion mutant is rapidly outcompeted by the parent strain in coinfection studies (27). For another example, Torres et al. (44) described two chromosomal operons (*lpf1* and *lpf2*) in *E. coli* O157 closely related to the long polar fimbrial (*lpf*) operon of *Salmonella enterica* serovar Typhimurium that have been associated with the appearance of long fimbriae that enhance colonization in animal models (reviewed in reference 45). Finally, in studies that used bovine terminal rectal primary epithelial cells and bovine intestinal tissue explants, the H7 flagellum acted as an adhesin to bovine intestinal epithelium and contributed to initiation of intestinal colonization (46, 47). A following study showed that immunization of cattle with H7 flagellin reduced colonization rates and delayed peak bacterial shedding following subsequent oral challenge with *E. coli* O157 (48). Based on these data, incorporation of one or more of these antigens, perhaps in combination with antigens used in the currently available vaccines, may further enhance vaccine efficacy.

CATTLE INFECTION WITH *E. coli* O157 AS AN ECOLOGICAL PROBLEM

As microbiological methods were developed to efficiently detect *E. coli* O157 and other pathogenic STEC strains in cattle feces and environmental samples, and as more epidemiological studies in cattle herds were completed, several observations with profound implications for preharvest food safety were made. These included (i) the ubiquitous presence of *E. coli* O157 and other pathogenic STEC strains on cattle farms during the summer (49) but its relative absence during the winter, (ii) the similarity in prevalence of infection among cattle raised under drastically different management conditions ranging from dispersed distribution of animals on pastures to housing in a highly concentrated fashion in feedlots, (iii) the transient nature of STEC colonization of individual animals, typically lasting one to a few weeks, (iv) the sporadic occurrence of herd outbreaks of high prevalence *E. coli* O157 fecal shedding that present all the hallmarks of food- or waterborne transmission, and (v) the detection of *E. coli* O157 fecal shedding in a very wide range of other mammalian and avian species. Basically, these observations are inconsistent with the widely held idea that cattle are the central sustaining reservoir for *E. coli* O157 and instead support the idea that cattle are just one more mammalian host periodically infected with this agent following oral exposures, albeit a host with particular significance for human exposure due to its use for producing human foods. The following sections of this article explore what is known of the ecology of this agent.

"Reservoirs" of *E. coli* O157 on Cattle Farms

The study of reservoirs is complex, and a variety of reservoir models exist for different pathogens. Much work has gone into identifying the reservoir for *E. coli* O157 to formulate strategies for controlling this pathogen on farms in pursuit of preharvest food safety. The clearest type of reservoir is a *biological reservoir*, a site or host where the agent can always be found and serves as a source of the infection for target populations. Complex reservoirs may include maintenance host populations that persistently harbor the infectious agent, as well as nonmaintenance (incidental or amplifying) host populations that do not harbor the microorganism indefinitely, but aid in the dissemination and amplification of the pathogen. Haydon et al. explain that the number of maintenance host populations is generally limited, whereas the number of nonmaintenance host populations may be unlimited (50). These definitions may be useful in considering the role(s) such populations may play in the seasonal occurrence of *E. coli* O157 on farms and in understanding how these populations may serve as targets for preharvest control of these bacteria.

Cattle as Reservoirs

Many human outbreaks with *E. coli* O157 have been associated with the consumption of contaminated foods of bovine origin or with direct contact with cattle or farms where infected cattle are raised (51, 52). Cattle are the sole animal host known to demonstrate site-specific intestinal colonization with this agent, at the recto-anal junction (RAJ). RAJ colonization among cattle has been observed on several dairy and beef farms without resulting in a detectable illness in these animals (53). Nearly all cattle herds, including both beef and dairy types, may be colonized. As discussed previously in this article, fecal shedding is associated with hide contamination, which has been demonstrated as a main source of meat contamination at slaughter (1, 54); thus research has been directed to the identification of preslaughter interventions that can decrease RAJ colonization and fecal shedding of these bacteria. Vaccination (discussed above) of cattle may be promising to accomplish this goal; however, identifying ways to reduce or eliminate the source of cattle infection is equally important.

While cattle are likely an important part of the reservoir for *E. coli* O157 on farms, several pieces of evidence have raised questions on whether cattle are truly a maintenance population for this pathogen. First, cattle typically shed *E. coli* O157 only transiently during summer months, and levels and prevalence of cattle shedding cease or decrease drastically during winter months (55, 56). Second, a single strain of *E. coli* O157 frequently predominates on individual farms over periods of multiple years, despite essentially disappearing from cattle populations each winter. This tendency is particularly interesting on large feedlots that go through multiple animal population turnovers annually, with incoming cattle originating from many diverse sources (57). These data suggest that farms may contain other noncattle maintenance hosts (reservoirs) of *E. coli* O157 and also cast doubt on whether the cattle themselves make up the true maintenance host population. Recently, it has been experimentally demonstrated that the seasonal differences in *E. coli* O157 shedding by cattle are not due to intrinsic factors within the animals. Cattle given identical challenge doses of *E. coli* O157 shed the agent in similar amounts and for similar durations, regardless of the season of exposure (58); this

is the outcome predicted of an amplifying host population, where the source is the key factor in duration and level of bacterial colonization in cattle. If cattle are simply an amplifying host population, it seems clear that identification of the true maintenance reservoir(s) of *E. coli* O157 is critical to the development of truly preventive systems for management of *E. coli* O157 on cattle farms.

Survivability of *E. coli* O157 in the Environment

E. coli O157 is surprisingly persistent in environmental sites, documented to survive in ovine manure for 21 months (19). The environments (bedding materials and water) of experimentally infected steers maintain detectable viable *E. coli* O157 for at least 14 weeks after inoculation of the cattle (59). Interestingly, in this study, *E. coli* O157 was cultured from the bedding and water even during weeks when it was not possible to recover *E. coli* O157 from cattle fecal samples (59). In a longitudinal, year-long study of naturally occurring *E. coli* O157 infection of cattle on two feedlots in southern Alberta, *E. coli* O157 was cultured from only 0.8% of the fecal pats, but 12% of the water troughs sampled were found positive. *E. coli* O157 was also cultured from 1.7% of feed bunk feed samples but not from fresh total mixed rations (60). Culture-positive water troughs occurred seasonally: 35% of water troughs sampled during the summer on one feedlot were culture-positive for *E. coli* O157, compared to 0% sampled during the winter (60). This seasonal variation clearly parallels the seasonality of cattle infection on farms and also raises the question of whether the water contamination is the source of, or results from, the cattle infection.

Water as a Reservoir

As described above, water is one of the most commonly contaminated materials on cattle farms. In culture-positive water troughs *E. coli* O157 is consistently detected more frequently in sediments than in the water column (59, 61). Water trough sediment consists of feed and fecal material admixed with numerous bacteria and protozoa, with rare metazoan species (nematodes and rotifers). Viable *E. coli* O157 in the sediment layers of water troughs can persist for greater than 245 days (61). One hypothesis is that ambient temperature during the summer is more permissible for growth of bacteria and, therefore, may result in increased bacterial populations in water troughs during the summer compared to the winter season (62). While increased ambient temperature likely plays a role in proliferation of bacteria, there may be other factors that influence seasonal variation and overall survival of these bacteria in water troughs. For example, mean coliform counts were significantly higher in water troughs that were cleaned at least every 2 months compared to those that were cleaned less frequently (63). Additionally, use of chlorinated or hyperchlorinated water in trough microcosms failed to eliminate *E. coli* O157 (61). These data suggest that there are likely additional factors other than ambient temperature that contribute to the survival and proliferation of *E. coli* O157 in water troughs.

Role of Protozoa

While the relationship between *E. coli* O157 and protozoa has not yet been clarified, LeJeune et al. demonstrated a significant increase in the quantity of free-living protozoa within water trough sediment in the winter compared to the summer (63). Other studies have demonstrated grazing of bacteria by protozoa collected from soil, lakes, and streams. While many bactivorous protozoa will feed on any available food, preferential grazing for bacteria also occurs. For example, *E. coli* O157 containing Stx2a-encoding bacteriophage are relatively resistant to grazing by *Tetrahymena* sp. (64, 65). Survival of *E. coli* O157 within the food vacuoles and excretory vacuoles of protozoa isolated from dairy lagoon wastewater suggests that protozoa may be vehicles for dissemination of the bacterium to crops (66). Many free-living protozoa form cysts under stressful conditions such as temperature or salinity changes and food deprivation, and these cysts can persist in the environment for decades. While several bacterial genera including *Legionella, Mycobacterium, and Listeria* spp. have been shown to survive within such protozoan cysts (67–70), research is needed to determine whether this may also be true for *E. coli* O157.

Role of Environmental Invertebrates

Apart from protozoa, invertebrate organisms such as nematodes and rotifers that have the potential for harboring *E. coli* O157 also inhabit water troughs and soils on cattle farms. Research has shown that *E. coli* O157 can amplify and persist for 5 days or more within one such free-living nematode, *Caenorhabditis elegans* (71). The association of *C. elegans* with *Salmonella* spp. has been more thoroughly investigated; when *C. elegans* is exposed to *Salmonella* serovar Newport, these bacteria can be detected in nematode progeny for at least the subsequent two generations (71). Another bactivorous, free-living nematode, *Diploscapter* spp., has been demonstrated to migrate rapidly toward colonies of *E. coli* O157 and to shed viable bacterial cells for at least a

day after exposure (72). Free-living nematodes protect themselves in scarcity of food or harsh environmental conditions by forming arrested-development larvae (dauer) stages, however, it has not yet been determined whether dauer stages can harbor food-borne pathogens and subsequently act as a source of contamination or as a reservoir for these pathogens.

Role of Flies

Many different families of flies are present on cattle farms. Flies mostly multiply during the spring and are in constant contact with cattle and feed during summer and early autumn months. Many flies lay their eggs in cattle feces, which hatch into larvae (maggots) that feed on manure before maturing into pupae within a week (73). Pupae contain a hard durable shell that allows them to survive under harsh conditions; most flies survive in this stage over the winter (74, 75). Adult flies that emerge from pupae typically survive for only a few weeks. Because of flies' close interactions with cattle on farms, some investigators have studied flies as a component of the reservoir of *E. coli* O157. These bacteria can be cultured from adult houseflies found in feed bunks and cattle feed storage sheds during summer months (76). In an *E. coli* O157 outbreak at a nursery school in Japan, the strains of *E. coli* O157 isolated from patients matched those detected in houseflies collected from within the school (77), and the possibility that the flies were acting as mechanical vectors able to disseminate bacteria to food and eating utensils was considered. Subsequent research suggested that flies may be more than just mechanical vectors. After oral infection of adult houseflies with *E. coli* O157, bacteria were identified in the alimentary canals of 30% of these flies up to 3 days postinfection. Orally infected flies with actively proliferating *E. coli* O157 on their mouthparts demonstrate cellular lesions similar to the attaching and effacing lesions seen in the colonized RAJ of cattle (78).

Role of Birds

E. coli O157 has been cultured from wild birds on cattle farms in many investigations. Birds, much like flies, may be seen as a general nuisance on farms and may act to contaminate cattle feeds and water sources, as well as disseminate bacteria within and between farms. A surveillance study determined that 3% of European starlings and 4% of the cattle study population were culture-positive for *E. coli* O157. In addition, these birds frequently visited the same farms on daily feeding forays but returned nightly to share a communal roost with birds that visited other farms, providing a potential method for pathogen dissemination (79). Poultry are readily experimentally colonized with *E. coli* O157 (80), but contamination of poultry products is very rare and human infection with *E. coli* O157 resulting from contaminated poultry has rarely been documented.

Role of Mammals

E. coli O157 fecal shedding has been detected in many different domestic animal species, including dogs, cats, horses, and sheep. Colonization of the ovine RAJ has been demonstrated but seems to occur less efficiently (81). Colonization in wildlife including feral swine, deer, raccoons, opossums, and rats has also been reported. Deer have been frequently documented to shed *E. coli* O157, and human infections have been traced to contaminated venison (82, 83). Swine are readily experimentally colonized with *E. coli* O157, but the prevalence of natural infection is very low (84, 85). In contrast, feral swine have been demonstrated to shed *E. coli* O157 and were suggested to play a role in dissemination of this agent to fresh produce that resulted in a large human outbreak of disease (86).

The Need for a Better Understanding of the Ecology and Reservoir Structure of *E. coli* O157

As mentioned previously, the reservoir for *E. coli* O157 is very complex. Based on Haydon et al.'s descriptions of complex reservoirs (50), there is likely one or more maintenance host populations that could include role(s) for organisms such as protozoa, invertebrates, or flies on cattle farms. Presence of a maintenance host population outside cattle is suggested by the fact that although swine and poultry, like cattle, are readily colonized with E. coli O157 in experimental settings, contamination of pork or poultry meats with this agent is relatively rare (87, 88). One possible explanation for this low prevalence may be that swine and poultry are typically reared in confinement in the United States, which may shield them from exposure to environmental sources of *E. coli* O157 infection. If so, this suggests that management systems to reduce cattle exposure to environmental sources of *E. coli* O157 may be required to reduce their prevalence of infection.

It is also possible that the bacteria can survive without hosts in soil or water environments during the winter, amplifying each spring (as ambient temperatures increase) to levels that are infectious to cattle. Several vertebrates, including birds, cattle, and other mammals, likely act at least as nonmaintenance host populations that aid in dissemination and amplification of these bacteria, especially during the summer months. More

research leading to a better understanding of the complex reservoirs of *E. coli* O157 may lead to improved targeting of these bacteria and improved preharvest control on cattle farms along with better strategies to reduce environmental and non-beef-product-related exposures contributing to human infection.

E. coli O157 GENOTYPES, HUMAN DISEASE, AND PREHARVEST FOOD SAFETY

Various genotyping methods including multilocus enzyme electrophoresis (89, 90), octamer-based genome scanning (91, 92), whole-genome PCR scanning (93), pulsed-field gel electrophoresis(94), Shiga toxin-associated bacteriophage insertion, typing (95), lineage-specific polymorphism assay, (96), comparative genomic hybridization, (97, 98), optical mapping (99), and single nucleotide polymorphism typing (100, 101) have been used to decipher the population structure of *E. coli* O157 (102). These studies revealed that bacteriophages play an important role in establishing the genetic diversity among *E. coli* O157 isolates and that certain specific genetic lineages of *E. coli* O157 are associated with most human disease. These strongly disease-associated genotypes have been termed clinical genotypes whereas other lineages, less frequently isolated from humans with illness compatible with *E. coli* O157 infection, have been termed bovine-biased genotypes (91, 92, 96, 103–107). In general, the various genotyping methods are concordant in their identification of clinical genotypes of *E. coli* O157 (108, 109). Populations of *E. coli* O157 in different geographical regions differ significantly in the relative frequency of particular genotypes in different countries, and generally clinical genotypes are more frequent in cattle populations in countries with higher incidences of hemolytic-uremic syndrome, a severe form of illness associated with *E. coli* O157 infection (110–115). On the other hand, at least some genotypes isolated from clinical illness in humans are not represented in cattle, indicating the presence of non-cattle-associated reservoirs or sources of human infection (101).

Given the similar prevalence of cattle infection with clinical and bovine-biased lineages in the United States, it seems likely that people in this country are similarly exposed to both clinical and bovine-biased genotypes of *E. coli* O157 via ground beef, other cattle-origin meats, and cattle environments. Therefore, the preponderance of human disease associated with clinical genotypes in the United States may simply be the result of relatively higher virulence of clinical genotype strains. This possibility has two important implications for preharvest food safety: First, the virulence differences among *E. coli* O157 genotypes suggest the possibility or likelihood that these genotypes may respond differently to preharvest food safety interventions due to other intrinsic biological differences associated with their genotypes, and second, that in evaluating the efficacy of preharvest food safety interventions it is important to demonstrate specific reductions of clinical genotypes, rather than assuming that any prevalence or shedding reductions include clinical genotypes. Recent studies have shown that different lineages of *E. coli* O157 may differ in their ability to persist on cattle farms through various seasons, cattle diets, and animal husbandry practices. Vanaja et al. (116) demonstrated that certain cattle-associated genotypes expressed gene repertoires expected to improve their resistance to adverse environmental conditions in comparison to genotypes more commonly associated with clinical disease. Some genotypes of *E. coli* O157 are more resistant to stress factors such as heat and starvation compared to other genotypes (117). It is similarly possible that different lineages of *E. coli* O157 may respond differently to preharvest control measures such as vaccines, probiotics, bacteriophage treatments, or animal husbandry interventions. Therefore, further studies are required to (i) specifically target bacterial genetic factors that are responsible for the differential response of different lineages of *E. coli* O157 to various preharvest control measures, and (ii) to confirm that any preharvest control measures put into practice are effective against clinical genotypes. These studies will aid in identifying tools to improve the current preharvest food safety measures or formulate new better ways to reduce prevalence and shedding of *E. coli* O157 on cattle farms with consistent and reliable results.

CONCLUSION

Preharvest food safety for *E. coli* O157 is the term used for management systems that reduce the prevalence and/or magnitude of shedding of this agent by cattle populations to reduce the risk of contamination of cattle-derived food products and subsequent human exposures. Decades of research have provided a better understanding of the epidemiology and ecology of *E. coli* O157 on cattle farms, but only limited progress on preharvest food safety goals has been made. Although several interventions (certain feed ingredients, probiotics, and vaccines) have been identified with statistically significant impacts on cattle shedding of *E. coli* O157, the impact of these potential interventions remains insufficient due to their limited efficacy, practical

difficulties with their implementation, or inconsistency in their results, leading to limited uptakes by producers. To date, the promise of the preharvest food safety approach to reducing human infection with *E. coli* O157 has not been fulfilled. A more holistic approach, with complex ecology and genetics of this bacterium in mind, is needed toward identifying true maintenance host populations and developing strategies to control *E. coli* O157 and other pathogenic STEC strains in these maintenance host populations.

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