Reducing Foodborne Pathogen Persistence and Transmission in Animal Production Environments: Challenges and Opportunities

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ABSTRACT Preharvest strategies to reduce zoonotic pathogens in food animals are important components of the farm-to-table food safety continuum. The problem is complex; there are multiple pathogens of concern, multiple animal species under different production and management systems, and a variety of sources of pathogens, including other livestock and domestic animals, wild animals and birds, insects, water, and feed. Preharvest food safety research has identified a number of intervention strategies, including probiotics, direct-fed microbials, competitive exclusion cultures, vaccines, and bacteriophages, in addition to factors that can impact pathogens on-farm, such as seasonality, production systems, diet, and dietary additives. Moreover, this work has revealed both challenges and opportunities for reducing pathogens in food animals. Animals that shed high levels of pathogens and predominant pathogen strains that exhibit long-term persistence appear to play significant roles in maintaining the prevalence of pathogens in animals and their production environment. Continued investigation and advancements in sequencing and other technologies are expected to reveal the mechanisms that result in super-shedding and persistence, in addition to increasing the prospects for selection of pathogen-resistant food animals and understanding of the microbial ecology of the gastrointestinal tract with regard to zoonotic pathogen colonization. It is likely that this continued research will reveal other challenges, which may further indicate potential targets or critical control points for pathogen reduction in livestock. Additional benefits of the preharvest reduction of pathogens in food animals are the reduction of produce, water, and environmental contamination, and thereby lower risk for human illnesses linked to these sources.

BACKGROUND Preharvest measures to reduce zoonotic pathogens in food animals are critical components in farm-to-table food safety approaches, which recognize that food production and safety occurs along a continuum. The encompassing goal of an integrated food safety program is to improve public health by reducing the risk of human foodborne illness, while the more specific goal of preharvest food safety strategies is to reduce the pathogen load of animals and/or animal products (such as milk or eggs) that are brought to harvest, in order to enhance the efficacy of postharvest interventions and reduce pathogens in the final product. As an example, the presence of pathogens in cattle feces and on cattle hides has been associated with beef carcass contamination (1–4). Cattle with high levels of \textit{Salmonella} on their...
hides on entry into commercial processing were often coincident with previsceration carcasses that were contaminated with the pathogen (4). Correspondingly, studies conducted in commercial beef processing plants have demonstrated that reducing *Escherichia coli* O157: H7 prevalence on cattle hides reduces its prevalence on resultant carcasses (5–7). As another example, the risk of broiler carcass contamination is greater when there is a higher degree of *Campylobacter* intestinal colonization of birds entering slaughter (8–10).

The Meat Inspection Act of 1906 (11) heralded the modern food safety era as an act of the U.S. Congress to prevent adulterated or misbranded meat and meat products (those “which are unsound, unhealthful, unwholesome, or otherwise unfit for human food”) from being sold as food and to ensure that meat animals and their products are slaughtered and processed under sanitary conditions. Provisions of the act included mandatory inspection of the live animal before slaughter, as well as mandatory postmortem inspection of each carcass. Smith et al. (12) and Oliver et al. (13) recently reviewed other historical aspects of food animal agriculture in the context of preharvest food safety, including the public health successes of the advent of widespread milk pasteurization and the reduction of human infection with *Mycobacterium bovis* as a result of the U.S. program to eradicate tuberculosis in cattle.

These examples illustrate that preharvest food safety in terms of animal health and hygiene in food production are not new concepts. However, regulatory developments in the 1990s shifted attention to the live animal and to farm-level efforts to improve the safety of food (13, 14). The enforcement of zero tolerance for *E. coli* O157:H7, the declaration of *E. coli* O157:H7 as an adulterant in raw ground beef and beef trim, and the implementation of the Pathogen Reduction/Hazard Analysis and Critical Control Point Systems regulations by the USDA Food Safety and Inspection Service brought changes to the meat industry, raising the bar on meat and poultry safety by setting pathogen performance standards on raw product (14, 15). More recently, six additional non-O157 Shiga toxin–producing *E. coli* (STEC; serogroups O26, O45, O103, O111, O121, and O145) strains have been declared to be adulterants in raw, nonintact beef products (16). The Food Safety and Inspection Service recently announced changes to poultry product inspection regulations that include the requirement for facilities to perform microbiological testing at two points in the production process to demonstrate control of *Salmonella* and *Campylobacter* (17). Current activities and discussion suggest that regulation of certain *Salmonella* serotypes as adulterants may be imminent (18).

**Advancements in Meat and Poultry Safety Research**

In response to these regulatory developments, as well as to the heightened awareness of foodborne illness outbreaks, there has been increased emphasis on food safety research initiatives and funding aimed at improving many aspects of the food animal production chain from farm to table (19). The National Food Safety Initiative (20) and the National Academy of Sciences’ report entitled “Ensuring Safe Food from Production to Consumption” (21) further reinforced the need for research and outlined goals for improving food safety and public health. A variety of tools for slaughter sanitation and intervention have been developed or improved, validated, and in many cases, widely adopted by the meat and poultry processing industries for use at animal harvest. For example, a wide variety of antimicrobial compounds have been demonstrated to reduce pathogens on carcasses of meat and poultry species, including a variety of organic acids (lactic, citric, and acetic acids) and other antimicrobials such as peracetic acid, acidified sodium chlorite, hypobromous acid, ozone, and electrolyzed oxidized water (22). Depending upon the species or process, these antimicrobial compounds may be applied as sprays or immersion dips. Hide-on carcass washing of cattle can remove bacterial contamination, thereby reducing transfer to the carcass when the hide is removed (6, 7). Heat treatments applied to animal carcasses to reduce bacteria include steam pasteurization (18, 23), hot water washing (24, 25), and scalding and singeing (26, 27). These and other postharvest interventions used for meat and poultry carcass decontamination have been discussed in more detail in recent reviews (18, 24, 28–30).

In part through these increased research investments, food safety research has also identified many preharvest interventions with the potential to reduce pathogens in the live animal. Some of these preharvest interventions currently are in use in animal production, while others are still in development and/or have not been extensively evaluated or scientifically validated as effective at reducing pathogens in animals. For others, cost and regulatory approvals are additional barriers to implementation. Probiotics, direct-fed microbials, or competitive exclusion cultures are commonly fed to livestock to increase production efficiency but may also be useful for reducing pathogen shedding by competition for attachment sites or for nutrients, by production of
antimicrobial compounds, or by promotion of immune function. Although results are inconsistent, studies have shown potential for select probiotic or competitive exclusion cultures to reduce *Salmonella* and *Campylobacter jejuni* in poultry (31, 32), *Salmonella* in pigs (33, 34), *E. coli* O157:H7 in lambs (35), and *E. coli* O157:H7 and *Salmonella* in cattle (36, 37).

In addition to their function to reduce disease in livestock, vaccines have been examined for their ability to reduce pathogens that cause foodborne illness. This approach has been explored for the reduction of *Salmonella* and *Campylobacter* in poultry (38–41), *Salmonella* in swine (42, 43), and *Salmonella* and *E. coli* O157:H7 in cattle (44–47). Vaccination to reduce *E. coli* O157:H7 in cattle has been intensively studied because of the status of this pathogen as an adulterant in ground beef and beef trim. The Epitopix *E. coli* bacterial extract vaccine with SRP technology (siderophore receptor and porin protein vaccine; Epitopix LLC, Willmar, MN) has conditional approval in the United States for use to reduce *E. coli* O157:H7 shedding in cattle (48). The Econiche vaccine (Bioniche Life Sciences, Inc., Belleville, Ontario, Canada), which targets type III secreted proteins of *E. coli* O157:H7, is licensed for use in Canada but currently is not approved for use in the United States (48). Considerable research with both vaccines demonstrates their potential to reduce *E. coli* O157:H7 in cattle, and three-dose regimens have been shown to be most effective (49, 50). However, neither vaccine has been widely used in either the United States or Canada (51, 52). A 2013 report from the USDA Animal and Plant Health Inspection Service estimated that 2.4% of large feedlots (those with a capacity of 1,000 or more head) gave cattle vaccines against *E. coli* (52). Carriage of *E. coli* O157:H7 does not affect the beef production efficiency, so the cost of the vaccinations in combination with the lack of economic incentives to cattle producers has limited their adoption to date (13, 18, 48, 53).

The USDA Food Safety and Inspection Service has approved the use of bacteriophages to reduce bacterial pathogens for limited applications, including application as a spray or wash to reduce *Salmonella*, *E. coli* O157:H7, and non-O157 STEC on the hides of live animals before slaughter and *Salmonella* on the feathers of live poultry before slaughter (22, 48). Oral dosing of bacteriophages to reduce pathogens in the gastrointestinal tract of live animals also has been explored to control *E. coli* O157:H7 in cattle and sheep (54–56), *Salmonella* in swine (57, 58), and *Salmonella* and *Campylobacter* in poultry (59–61). The addition of sodium chlorate to the water or feed of food animals before shipping and harvest has been proposed for reduction of *E. coli* O157:H7 and *Salmonella* in the intestinal tract. These pathogens contain the intracellular enzyme nitrate reductase, which reduces chlorate to chloride, which then accumulates to lethal levels in the bacterial cells (62). Chlorate treatment has been demonstrated to reduce *Salmonella* in swine (63) and poultry (64, 65) and *E. coli* O157:H7 in swine (66), sheep (67), and cattle (68, 69). The use of chlorate to reduce pathogens in livestock currently is not approved, but the application is under review by the U.S. Food and Drug Administration. These preharvest interventions and other potential approaches for pathogen reduction in food animals (such as dietary supplementation with organic acids/fatty acids, essential oils and other phenolic compounds, prebiotic sugars) are further discussed in other chapters in this book and have also been topics of many recent reviews (13, 18, 62, 70–78).

**Good Animal Management Practices Are the Foundation**

Hygiene, disinfection, and biosecurity measures are at the heart of an effective preharvest food safety program and protect animal health in addition to limiting animal exposure to those zoonotic pathogens that can cause human foodborne disease. The core elements of a preharvest animal management program include (i) preventing the introduction of infection, (ii) preventing the survival and spread of infection within the herd or flock, and (iii) reducing or eliminating an established infection (79). Best management practices include provision of clean feed and water, maintenance of a clean, well-drained environment, and biosecurity procedures to isolate sick or infected animals and, to the extent possible, to exclude wildlife and such pests as rodents and insects (48, 74, 75, 80). Wild animals, birds, and insect pests as potential pathogen vehicles may be difficult or impossible to control for animals raised outside in pastures or lots, compared to animals in enclosed confinement buildings. Various treatments and additives to reduce pathogen contamination in animal feed and drinking water have been examined and are discussed in recent reviews (74, 75, 81, 82). Direct or indirect fecal–oral exposure is a significant route of pathogen transmission among animals in the production environment, so regular removal and treatment of manure will reduce an important source of pathogens. Reducing pathogens in manure will also reduce the risk for transmission of these pathogens to human food crops and water, and this is discussed further below.
Preharvest food safety research to date suggests that these good production and management practices alone will not eliminate foodborne pathogens (18, 62, 80). However, there are success stories, and notable examples are Denmark’s Salmonella control programs for various food animal species (83–85). These programs are integrated “feed-to-food” systems that employ intensive surveillance and rigorous biosecurity measures. Preharvest controls differ depending upon species (chickens, pigs, cattle) or segment (e.g., broilers versus laying hens), but elements include elimination of infected breeding poultry flocks (83), classification of cattle and swine herds according to Salmonella risk (83, 86), animal movement and trade restrictions (83, 85, 86), feeding strategies (83, 87), cleaning and disinfection of houses between flocks (83), and various management recommendations (e.g., all in–all out pig flow scheme, calving management; 83, 85, 87, 88). Animals that are suspected or confirmed to be Salmonella-positive may be slaughtered separately using special hygienic precautions (83, 86). Using the Salmonella control program for swine as an example, identified risk factors for high Salmonella prevalence in pig herds include feed, management, and hygiene (87). The success of these programs in reducing Salmonella in meat and poultry and reducing the incidence of human salmonellosis illustrate the potential for preharvest management approaches to reduce foodborne pathogens (83).

**PERSISTENCE AND TRANSMISSION IN PREHARVEST ENVIRONMENTS: CHALLENGES AND OPPORTUNITIES**

Oliver et al. (13) discuss the on-farm contamination cycle of zoonotic foodborne pathogens, which begins with the infection of animals by ingestion of contaminated feeds and water or other oral exposure (e.g., by grooming [89]) and proceeds by shedding of pathogens in feces, which in turn contaminates additional feed, drinking water, and/or the environment, thereby causing new infections or re-infection of animals. They further illustrate a scenario in which pathogen amplification in the animal, shedding, and distribution of pathogens in the farm environment leads to the persistence of pathogens on the farm, for which the outcome is a maintained reservoir of foodborne pathogens. Current experience suggests that this scenario may be the case for many pathogens and that interrupting the infection-reinfection cycle will be key for progress in preharvest food safety. Even though complete elimination of pathogens in a preharvest animal production environment is unlikely, measures aimed at reducing infection levels, numbers, and persistence of pathogens should have a positive impact on food safety.

While recent preharvest food safety research efforts have advanced the science, they have also revealed the complexity of the problem; there are multiple pathogens of concern, multiple animal species under different production and management systems, and a variety of sources of pathogens in a farm environment, such as other livestock and domestic animals, wild animals and birds, insects, water, and feed (as reviewed in 70, 74, 75, 90). The broad problem is further complicated by the fact that some of the pathogens are not pathogenic to the animal or may result in asymptomatic infection. These research efforts have led to greater understanding of the transmission of pathogens and their ecology on the farm and have identified numerous factors that can impact pathogens on the farm, including seasonality, production systems, diet, and dietary additives. Moreover, this work has identified both challenges and potential opportunities for reducing pathogen persistence and transmission in food animals and their production environments.

**Can Targeting Super-Shedders Reduce Food Safety Risk?**

Enumeration of select pathogens in complex samples with high background microflora is technically difficult, so most studies determine the prevalence of the target pathogen (presence/absence). However, use of quantitative techniques to determine pathogen levels can provide information for risk assessments or for determining the relative impact of various factors on transmission and persistence of pathogens. Furthermore, the use of these techniques has led to important discoveries regarding animals that shed high concentrations of pathogens, often referred to as super-shedders. Cattle that are super-shedders of E. coli O157:H7 (those animals that excrete ≥10⁴ CFU/g in their feces) typically are a small proportion of the total animals in a herd but have a large impact on the prevalence of this pathogen in the remaining cattle and their production environment (91–97). As an example, Chase-Topping et al. (92) found that high prevalence of E. coli O157 among cattle on a farm was associated with the presence of a cohort animal shedding high levels of the pathogen (>10⁸ or >10⁴ CFU/g of feces) on that farm. Several studies have shown that the presence of super-shedders in feedlot pens is associated with higher E. coli O157:H7 prevalence for those pens (91, 94, 97). Super-shedding of E. coli O157:H7 by cattle has also been associated with...
higher levels of hide contamination, which can increase the risk of carcass contamination at harvest (97–100). Correspondingly, the probability of preintervention beef carcass contamination with E. coli O157:H7 was strongly correlated with the presence of a high-shedding animal in the same truckload of cattle brought to harvest (101). Given that preharvest interventions may not completely eliminate foodborne pathogens in animal production, these data do illustrate that reducing the load of these pathogens can have an impact on food safety. Accordingly, identifying super-shedding animals for removal or targeted interventions has been suggested for reducing E. coli O157:H7 prevalence in cattle (96, 102).

The host, pathogen, and/or other environmental factors or mechanisms that result in super-shedding of E. coli O157:H7 by cattle have remained elusive, but their discovery may suggest strategies to reduce this occurrence. Colonization at the recto-anal junction is linked both to high levels of shedding and to longer duration of shedding (93–95, 103–105), although some cattle may shed E. coli O157:H7 for only a short time (98, 106). In a Scottish study to identify risk factors for the presence of cattle shedding high levels of the pathogen, E. coli O157 from high-level shedders was more likely to be phage type 21/28, leading these researchers to hypothesize that this phage type may be a marker for a genotype or altered gene expression that may result in the tendency for high-level shedding or the ability to persist outside of the host (92, 93). Arthur et al. (107) characterized a diverse set of E. coli O157:H7 from super-shedding cattle using pulse-field gel electrophoresis (PFGE), phage typing, Stx-associated bacteriophage insertion site determination, lineage-specific polymorphism assay, and variant analysis of Shiga toxin, tir, and antiterminator Q genes. They found no genotype that was common to all super-shedder isolates, but the super-shedder isolates tended to have higher frequencies of traits associated with human disease isolates with regard to lineage and tir allele. Interestingly, this U.S. study found 19 phage types among the 102 super-shedder isolates, of which 30% were phage type 4 and none were phage type 21/28, indicating regional or global differences (92, 93, 107). Xu et al. (108) used 16S rRNA gene pyrosequencing to examine differences in bacterial communities in the feces of E. coli O157:H7 super-shedding and nonshedding cattle. These researchers found distinct differences in fecal microbial communities between these two groups of cattle, with a more diverse microflora associated with super-shedding animals, although the mechanism(s) for these differences is as yet uncertain. Hallewell et al. (106) found a higher prevalence of endemic bacteriophages, including T4-like phages of Myoviridae, in feces of cattle that were low shedders of E. coli O157:H7, compared to super-shedders (<10⁵ versus ≥10⁶ CFU/g of feces). The T4-like phages exhibited broader host range and stronger lytic capability for E. coli O157:H7, further suggesting that bacteriophages may be involved in differences in shedding levels of this pathogen by cattle.

The super-shedding of E. coli O157:H7 by cattle is receiving intensive research attention, given the status of this pathogen as an adulterant in beef products; however, super-shedding and its impact on pathogen transmission and environmental contamination has been reported for other zoonotic pathogens and animal species, including Mycobacterium avium species paratuberculosis (MAP) in dairy cattle (109, 110), Salmonella enterica serovar Typhimurium in mice (111), and Clostridium difficile in mice (112).

What Drives Pathogen Persistence in Animal Production?

Studies that examined horizontal transmission of zoonotic pathogens among food animals, such as E. coli O157:H7 and MAP in cattle (89, 113, 114), Salmonella in cattle, pigs, chickens, and turkeys (115–118), and Campylobacter in chickens (116), point to the importance of environmental contamination in the pathogen transmission process. This is further suggested by research on the impact of super-shedding animals on the transmission of zoonotic pathogens to other animals in a feedlot or on a farm (91, 94, 97, 98, 109; as reviewed by Chase-Topping et al. [93]). While colonization of the gastrointestinal tract and subsequent amplification and shedding are key steps in the infection cycle, environmental persistence is also involved in the maintenance of zoonotic pathogens in animal production. As an example, E. coli O157:H7 and some non-O157 STEC have been demonstrated to persist for long periods in soils, manure, or feedlot surface soils (119–123; as reviewed by Berry and Wells [70]), and the presence of the pathogen in these materials has been observed to be important to the spread of E. coli O157:H7 to other animals in the pen or herd (124, 125). The greater persistence of E. coli O157:H7 in manure from cattle fed 20 and 40% corn wet distillers grains with solubles compared to corn may contribute to the higher prevalence and levels of this pathogen in feces and on hides of cattle fed wet distillers grains with solubles (126, 127). The abilities of other foodborne pathogens, including Salmonella, Campylobacter species, and Listeria monocytogenes, to survive...
in animal manures and/or soils have been described (128–131). As recently reviewed by Vivant et al. (132), L. monocytogenes is a natural inhabitant of soils, and this pathogen is commonly isolated from animal manures and the environment (133).

The significance of persistence to maintain zoonotic pathogens in preharvest production environments is further suggested by reports that have found that most isolates on a farm or feedlot are of one or a few predominant genetic subtypes of the target organism, which may persist for months or even years. Baloda et al. (128) repeatedly isolated a single PFGE subtype of S. Typhimurium for over a 3-year period in the animals and environment of a Danish pig farm. The persistence of predominant Salmonella subtypes during broiler production (134) and in beef cattle at the feedlot (135) has also been described. Petersen and Wedderkopp (136) typed fecal C. jejuni isolated from chickens from 12 broiler houses on 10 different farms, and found farm-specific PFGE subtypes of C. jejuni that persisted in houses through multiple rotations of broiler flocks. In addition, the long-term persistence of specific clones of Campylobacter species (137, 138) and MAP (139) in cattle has been reported. Numerous studies describe the occurrence of persistent, predominant genotypes of E. coli O157:H7 in cattle in feedlots and on farms (98, 124, 139–143). A recent study provided evidence of the persistence of E. coli O157:H7 of indistinguishable PFGE types on cattle farms for 3 to 4 years (143). The cattle production environment may be more important as a source of E. coli O157:H7 than are incoming cattle (139, 141).

Pathogen persistence in animals and the production environment may also be the result of persistent latent infection in animal hosts. Infection can often be asymptomatic, but in some cases the asymptomatic host can become chronically infected and the pathogen can persist in the host for long periods of time. A classic example of this is Mary Mallon (Typhoid Mary), who was a cook that inadvertently transmitted S. enterica serovar Typhi to numerous households in New York City a century ago. Many foodborne pathogens are asymptomatic to the food animal host, and some animals can become chronic carriers of the pathogen. In cattle, Johne’s disease is a slowly progressing gastrointestinal disease caused by MAP. At slaughter, nearly all culled cattle carried MAP on their hides and more than a third of the ileocecal lymph nodes of culled cattle tested positive for MAP, whereas less than 1% of fed cattle were positive (144).

When infected with different Salmonella species, the bovine calf has enumerable levels for all species in the intestinal tissue, intestinal lymph nodes, spleen, liver, and peripheral lymph nodes by seven days (145). At slaughter nearly 72% of cattle tested positive for Salmonella in the mesenteric lymph nodes (146). Peripheral nodes can end up in ground beef, and recent research has indicated that bovine peripheral nodes were Salmonella-positive by culture (147). In cattle, the subiliac lymph node may be most contaminated with Salmonella species, with nearly 12% of these lymph nodes from fed cattle being culture positive (148). In swine the cecum and the ileocecal lymph nodes have the highest prevalence for Salmonella (149), and infected piglets can shed Salmonella for months (150). However, the subiliac (151) and the prescapular (152) lymph nodes of pigs were typically culture negative for Salmonella at slaughter. Poultry can harbor Salmonella in the ceca, liver, spleen, and reproductive organs for long durations (153, 154).

Piglets experimentally infected with Campylobacter coli had detectable levels in multiple tissues for a few animals, but all piglets had detectable levels in the intestines, with the highest levels in cecum and ascending colon tissues (155). Poultry are reservoirs for C. jejuni, which can persist at high levels in the intestinal tract (156). L. monocytogenes can be an intracellular pathogen (157), and infected sheep shed this pathogen from their rumen for several weeks (158). E. coli O157:H7 has not been shown to be a chronic colonizer of cattle.

The above examples demonstrate that greater understanding of the mechanisms that contribute to pathogen persistence is needed to develop improved strategies for breaking the infection–reinfection cycle. The ability to genotype and identify unique persistent strains by molecular or other means (e.g., by PFGE, lineage-specific polymorphism assay, multilocus sequence typing, or genome sequencing) presents opportunities to learn what these strains can do that other less persistent strains cannot, which allows for greater capacity to survive in the external environment or superior ability to colonize animals.

E. coli O157:H7 isolates of a persistent PFGE type that was shed predominantly during the finishing of feedlot cattle also had greater ability to adhere to Caco-2 human intestinal epithelial cells compared to the less persistent types, which indicated that the more prevalent strains may be better adapted to colonize and persist in the gastrointestinal tract (140). Jeong et al. (159) characterized and compared bovine E. coli O157:H7 strains from a dairy farm that were either persistent and predominant or less commonly isolated over a 2-year sampling period. Compared to the less persistent E. coli
O157:H7 strains, the dominant strain utilized the most carbon sources and was the only strain to oxidize five of the carbon sources, which indicated that more flexibility to use different carbon sources may be advantageous for colonization of cattle or survival in the environment. E. coli O157:H7 persistence in soils and resistance to predation by protozoa was associated with the curli-negative phenotype (160). A proteomics approach was used to compare E. coli strains associated with persistent or transient bovine mastitis; proteins associated with swimming and swarming motility were more highly expressed in E. coli from persistent mastitis cases (161). While mastitis-causing E. coli strains are not associated with human foodborne disease, a similar approach may be useful for determining protein expression differences in other persistent versus nonpersistent pathogens from animal production environments. The persistence of L. monocytogenes in food processing environments is a critical food safety issue because of the increased risk of cross-contamination of finished products. Fox et al. (162) used a phenotype microarray and transcriptome sequencing to characterize persistent and nonpersistent L. monocytogenes and identified gene clusters they hypothesized to be important to persistence of this pathogen in environments outside the human host. With the advances in sequencing technology, and the increased application of functional genomic and comparative genomics/phenomics approaches, progress can be anticipated for determining variations among pathogen species that lead to their persistence in animals and the production environment (163–165; as reviewed by Bronowski et al. [166]). Recent reviews further discuss aspects of survival and/or persistence of E. coli, Salmonella, and/or Campylobacter species in preharvest animal production and other environments (78, 167).

Can We Select for Resistant Animals?
Animal selection by mankind has occurred for centuries. Humans have intentionally selected agricultural animals for meat, milk, and fiber, and in the process have selected for traits such as coat color, size, muscling, milk production, and a variety of other economically important traits. Selection for these traits has been based on obvious and easily measured phenotypes. However, most zoonotic pathogens provide no obvious phenotype for trait evaluation, and as a consequence very little research has been directed into these important avenues.

Heritability (h²) is an estimate of a trait’s variation that can be due to genetic differences in a population. Heritability is important for selective breeding, and measures of heritability have been estimated for a few pathogen-host phenotypes where the phenotype measure can be easily collected. However, heritability of a phenotype does not attribute a specific genetic cause, and the genetic contribution for a phenotype can be polygenic. With recent advances in genotyping, associations between pathogens and specific host genotypes can be better determined. Single nucleotide polymorphisms (SNPs) represent biallelic differences in the genomic sequence and are widely distributed in mammalian genomes (168). These genotypes, or markers, can be used to associate a phenotype with functional genomics and to assist with selection for resistant populations or against susceptible animals.

The poultry industry produces eggs and meat for human consumption, and poultry products have been a major source of foodborne outbreaks of Salmonella (169; as reviewed by Doyle and Erickson [74]). Breeds of poultry have been selected for either eggs (layers) or meat products (broilers), and both types of poultry can be chronically infected with Salmonella. Salmonella infection often occurs early in a bird’s life, typically from contaminated eggs (170), and can persist into adulthood (154). Salmonella infection is a polygenic disease in poultry, and a variety of heritability estimates for disease susceptibility/resistance have been determined in poultry using pathogen challenge models with different breeding lines and different sites of pathogen localization (171). The resistance to becoming a Salmonella carrier (i.e., the carrier state) has been evaluated, and depending on the specific pathogen infection site sampled, age of the bird at infection, and breed type and line, the h² for S. enterica serovar Enteritidis infection can be as high as 0.29 (172). Similarly, candidate genes have been evaluated and localized single nucleotide polymorphisms have been associated with Salmonella infection. The Slc11a1 (Nramp1) gene encodes for a solute carrier protein, and polymorphisms in this region have been associated with S. Enteritidis carriage in layers (172, 173). The Cd28 gene encodes a transmembrane protein found on T-cells, and the Md2 gene encodes a protein required for toll-like receptor signal recognition; polymorphisms in both genes have been associated with S. Enteritidis response and carriage in broilers (174). In contrast, other researchers have evaluated S. Typhimurium-resistant and –susceptible lines and located a large multigene region associated with infection resistance (175). Swaggerty et al. (176) have evaluated poultry for cytokine/chemokine responses, and based on selection of extreme phenotypes, they were able to generate broiler lines that differed in pathogen colonization when challenged with S. Enteritidis, Enterococcus gallinarum, or C. jejuni.
In swine, markers associated with *S. enterica* serovar Choleraesuis susceptibility were located throughout the genome (177), but none of these regions were mapped to candidate genes. Using inoculated piglets, Uthe et al. (178) identified a single nucleotide polymorphism in the CCT7 gene that encodes a chaperonin protein subunit (T-complex protein 1 subunit eta) that was associated with colonization and shedding of *S. Typhimurium*. As in poultry, much research in swine has been directed at the immune response and its control (179). Heritability estimates for immune response can range from 0.2 to 0.8 (180), but relationships with specific pathogens have yet to be reported. However, selection for stronger general immune function could benefit the host against multiple pathogens.

In large animals, the rationale for animal selection for pathogen traits is supported in part by studies of disease-causing organisms in production animals, such as with mastitis. Mastitis in dairy cattle is the inflammation of the udder, often a result of bacterial invasion through the teat. This is a costly disease to the dairy industry and is routinely monitored to prevent the sale of milk from diseased animals. The heritability for mastitis is low (0.01 to 0.15), but clinical records are not uniformly collected (see Pighetti and Elliott [181] for a review). The somatic cell count and its normalized transformant, the somatic cell score, are attributes correlated with mastitis, and these parameters offer greater heritability estimates. Host genetic loci associated with mastitis traits appear to be widely distributed across the bovine genome. Mastitis is a complex disease in cattle, and both Gram-positive and Gram-negative bacteria can cause the disease. Heritability estimates for any specific pathogen was low, but collectively, infections caused by Gram-positive organisms exhibited greater heritability than infections attributed to Gram-negative organisms (182). This latter observation is likely a reflection of differing immune responses for the different types of bacteria. Genetic correlations between type of bacterial infection and mastitis are high (0.7 or better), and polymorphisms in a chemokine receptor gene (CXCR1) have been associated with the bacterial type infecting mammary glands of dairy heifers (183).

### Can the Bacteria in the Gastrointestinal Ecosystem Affect Pathogen Persistence?

Digestive systems are nutrient-rich, and animals have evolved a number of different digestive systems to digest food and absorb nutrients (184). Microbes have evolved in symbiosis with the digestive tracts, and the bacterial levels in the lumen of the gastrointestinal tract can be variable. Bacterial concentrations typically are highest in the colonic digesta and feces.

Swine have a simple digestive system and are considered omnivores. Young swine are typically susceptible to pathogens, and diet can modulate the gastrointestinal environment (185). Diets low in host nondigestible but microbial-fermentable protein can result in production of ammonia and toxic amines, whereas diets high in fermentable carbohydrates can result in beneficial short-chain fatty acids. In young piglets inoculated with *S. Typhimurium*, the shedding of the pathogen is variable (186). On day 0, animals that were later characterized as low shedders had higher levels of *Ruminococcaceae* family, whereas feces of animals that were to become high shedders had higher levels of two bacterial genera: *Phascolarctobacterium* and *Coprobacillus*. The microbial ecology was significantly altered in the high-shedders and was likely associated with immune response, similar to a study reported for *S. Typhimurium*-challenged mice (187, 188). Interestingly, when swine were challenged with *Salmonella*, the fecal microbiome of the challenged pigs was different 21 days postinfection compared to nonchallenged controls (186). Lysozyme has been demonstrated to improve performance and reduce pathogen shedding in piglets (189, 190), and this compound can reduce enterotoxigenic *E. coli* in challenged piglets (191). Maga et al. (192) noted a change in fecal microflora for piglets supplemented with lysozyme, particularly increases in *Prevotella* species, as well as increases in beneficial *Firmicutes*, such as bifidobacteria and lactobacilli.

Cattle are ruminant animals with a complex gastrointestinal tract dominated by pregastric fermentation in the reticulo-rumen. Nonetheless, like simple stomach animals, the fecal microbiota of the bovine is mainly composed of the *Firmicutes* and *Bacteroidetes* (193). In recent years, diet has been shown to influence *E. coli* O157:H7 shedding, and diets with high levels of distillers grains can increase *E. coli* O157:H7 in feces (127, 194). In addition, high levels of distillers grains in the diet increased generic *E. coli* concentrations and altered the habitat (127). The composition and diversity of the fecal microbiome can be greatly influenced by diet (195). The fecal microbiome composition of cattle fed high levels of distillers grains differed from that of cattle fed a corn diet, but no association with *E. coli* O157:H7 was reported (196). Recent research with cattle characterized as super-shedders of *E. coli* O157:H7 revealed more than 70 bacterial groups that differed in abundance compared to cattle negative for *E. coli* O157:H7 shedding (108). It appears possible that the microbiome...
composition may increase or decrease the shedding of *E. coli* O157:H7; however, most of these bacterial groups were classified as *Ruminococaceae* family members, and there may be interactions among the *Ruminococaceae* that associate with *E. coli* O157:H7 colonization. In contrast, *Salmonella* shedding in cattle feces may not be greatly influenced by gastrointestinal microbiota (197), but animal-to-animal variation in the microbiota may have been too great to observe significant differences for individual bacterial groups in this small study.

Poultry also have a complex digestive system with pregastric and postgastric compartmentalization. The ceca (dual cecum) in poultry are primary microbial habitats and sites for pathogen colonization, and healthy chickens can harbor high levels of pathogens asymptomatically (198). *Firmicutes* is the predominant bacterial phylum present in the broiler ceca, and there is much bird-to-bird variation (199). The microbiome between ceca has some variation within each bird but much less than variation between birds (200). Based on these previous reports, the microbiota composition of the ceca can be highly variable and, unlike cattle and swine, predominated by a few genera. Early research by Rantala and Nurmi (201) indicated that when 1-day-old chicks were pretreated with enriched gastrointestinal digesta from a *Salmonella*-free adult chicken, the chicks were resistant to *Salmonella* Infantis infection when challenged. As a consequence, a number of competitive exclusion products have been developed for poultry (202). Recently, *Bdellovibrio bacteriovorus*, a predator of Gram-negative bacteria, was effective at reducing *S. Enteritidis* infection in chicks (203).

**ENVIRONMENTAL BENEFITS OF PREHARVEST PATHOGEN REDUCTION: REDUCING WATER AND PRODUCE CONTAMINATION**

Animal feces and manures are significant vehicles of zoonotic pathogens for the direct or indirect contamination of produce, water, and the environment, so the preharvest reduction of pathogens in food animals may further reduce the risk for pathogen dissemination from production and the risk of human illness linked to these sources. Fresh produce has increased in prominence as a source of pathogens contributing to human foodborne illnesses and outbreaks (204, 205). In addition, water contamination and waterborne disease outbreaks have been linked to livestock production as a result of runoff from farms or manure-amended fields (206–209).

The risks associated with the use of raw manures for soil amendments are well understood, and procedures for treating manures to reduce pathogens have been reviewed (70, 75, 210, 211). The recent Food Safety Modernization Act Final Rule on Produce Safety is anticipated to improve produce safety by providing guidelines for the safe use of animal manures as soil amendments for fields used to grow produce crops (212).

Recent research has examined pathogen carriage and shedding by insects and wildlife and the risks for these creatures to disseminate pathogens from livestock to contaminate produce or irrigation water (213–215). *E. coli* O157:H7 and *Campylobacter* species were isolated from feral swine living near beef cattle in a major leafy green-growing region on the central California coast; this same swine population was implicated as a potential source of *E. coli* O157:H7 for the contamination of baby spinach linked to the large 2006 foodborne illness outbreak (213, 214). Talley et al. (215) detected STEC genes in pest flies captured in a leafy green crop that was located near a cattle production area and further demonstrated in laboratory studies that house flies can transfer *E. coli* O157:H7 to spinach leaves. Considerable work has examined zoonotic pathogen carriage by bird species that are often closely associated with cattle, including starlings (216–218), pigeons (219), brown-headed cowbirds (220), common grackles (220), and cattle egrets (220). Pathogen species found in these birds include *E. coli* O157:H7 (216, 220), *Salmonella* (217, 219, 220), *Campylobacter* (218), and MAP (217). In addition to disseminating pathogens to animals on different farms, these birds may play a role in transporting pathogens to produce crops (221–223). Additional research has been concerned with the bioaerosol dissemination of pathogens and the risks for contamination of produce grown near livestock production facilities (224, 225). Riparian buffers, windbreaks, or hedgerows can protect produce crops from airborne pathogens from animal production but may also provide habitat or harborage for wildlife that may carry pathogens and potentially contaminate crops. Comanagement of food safety objectives and conservation of water, soil, and other natural resources is a high-priority area (226).

**ADDITIONAL CONSIDERATIONS FOR PREHARVEST RESEARCH AND DEVELOPMENT**

The bacterial pathogen species that are discussed in this review are important in terms of their public health impact and regulatory significance but are by no means...
the only pathogens of concern. Preharvest control of a number of other zoonotic bacteria, as well as many species of parasitic protozoa and viruses, will be vital for improving food safety. Indeed, the long list of zoonotic pathogenic microorganisms reveals the complexity of the problem. Intensive efforts in preharvest food safety research have identified a number of interventions and management strategies that are in use and/or under investigation, with the potential to reduce zoonotic foodborne pathogens in live animals. However, preharvest reduction of foodborne pathogens in food animals and their production environments remains a significant challenge. Another important product of these on-farm food safety research efforts has been the development of greater knowledge regarding the sources, transmission, and ecology of zoonotic pathogens in animal production (Fig. 1). This work has identified both particular challenges and potential opportunities for preharvest food safety. Animals that super-shed high numbers of pathogens and predominant pathogen strains that exhibit long-term persistence appear to play large roles in maintaining the prevalence of pathogens in animals and their production environment. Continued study and advances in sequencing and other technologies should divulge the mechanisms that result in super-shedding and persistence, in addition to enhancing prospects for the selection of pathogen-resistant food animals and the understanding of the microbial ecology of the gastrointestinal tract as it relates to colonization by zoonotic pathogens. It is likely that additional research will reveal other as-yet-unrecognized challenges, which may also point to potential targets or critical control points to exploit to reduce pathogens in animal production.

Research efforts to date indicate that longitudinal studies are valuable for clarifying the nature of pathogen sources, occurrence, and transmission, as well as for identifying the factors that affect pathogens in preharvest environments and determining the efficacy of preharvest intervention treatments. For example, the fecal shedding of E. coli O157:H7 by cattle can fluctuate widely in terms of both prevalence (positive/negative) and the concentrations shed (98, 127, 194). Multiple
samplings over time and sufficient replication (versus sampling at a single point in time) clarified the role of *E. coli* O157:H7 super-shedder cattle for hide and environmental contamination and further suggested *E. coli* O157:H7 shedding reduction targets to reduce hide contamination (98). Likewise, longitudinal studies were needed to demonstrate the impact of feeding corn wet distillers grains on *E. coli* O157:H7 prevalence in cattle (127, 194).

Demonstration of the ability of preharvest procedures to contribute to the improved microbial safety of foods at the endpoint of the farm-to-table continuum will be essential for broad animal producer adoption of new technologies. Key to the success of these preharvest procedures will be the development and use of systems-based approaches or integrated food safety systems that link preharvest and postharvest food safety along the animal production and processing chain and avoid breaks in the chain that negate or neutralize the previous food safety efforts. Perhaps the best examples of the need for these systems approaches are demonstrated by studies showing that carriage of pathogens such as *E. coli* O157:H7 and *Salmonella* can increase in cattle or swine during transportation to and lairage at processing plants as a result of contact with one another or with contaminated feces, transport trailers, or holding pens at lairage (101, 227, 228). These exposures may nullify any benefits of previous control efforts and indicate that preserving any pathogen reduction beyond these stages would require wide adoption of preharvest practices by animal producers, and/or the application of additional antipathogen interventions during transportation and lairage. Adoption of preharvest control practices will also be favored if the procedures are economical and easy to implement. Furthermore, preharvest food safety approaches should contribute to the sustainability of farming, ranching, and feeding enterprises. Finally, successful preharvest food safety strategies directed at food animals can provide additional benefits to public health beyond the production of safer animal-based products, by reducing the risk for dissemination of zoonotic pathogens to produce, water, and the environment.

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