Preslaughter intervention strategies to reduce food-borne pathogens in food animals[1,2]

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ABSTRACT: Food-borne pathogenic bacteria sicken more than 76 million Americans annually. Many of these illnesses are caused by consumption of foodstuffs produced from animals. Postslaughter intervention strategies effectively reduce bacterial contamination level from the abattoir to the table. However, in spite of these effective strategies, food-borne illnesses and food-related deaths still occur far too frequently. Therefore, strategies that expand the continuum of intervention from the abattoir back to the farm have the greatest potential to reduce pathogenic contamination of meats and resultant human illnesses. A broad range of preslaughter intervention strategies have been contemplated and are currently under investigation. Potential strategies to be discussed include vaccination, competitive exclusion, substrate-adapted competitive exclusion, and the use of pro- and prebiotics (e.g., fructooligosaccharides). Other strategies such as the use of bacteriophage to specifically target certain pathogenic bacteria, and the exploitation of the physiology of specific pathogens will be described. Additionally, the use of antibiotics to specifically reduce pathogens will be examined, as well as the risks incurred by antibiotic usage. The effects of management strategies (e.g., dietary changes), transportation, and stress on food-borne pathogenic bacterial populations of food animals will also be discussed. The parallel application of one or more of these preslaughter strategies has the potential to synergistically reduce the incidence of human food-borne illnesses by erecting multiple hurdles against entry of pathogens into the food chain.

Key Words: Foodborne Diseases, Intervention, Pathogens

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Introduction

Americans expect and demand a safe food supply; yet each year, more than 76 million citizens become ill from consuming foods contaminated with pathogenic bacteria (Mead et al., 1999). Many of these outbreaks have been linked to meat products or to contact with food animals or their waste. Human illnesses caused by the most common food-borne pathogens cost the U.S. economy approximately $7 billion and result in 1,600 deaths each year (ERS/USDA, 2001). The most economically important agents of food-borne illness in the United States are *Campylobacter, Salmonella*, enterohemorrhagic *Escherichia coli* (including O157:H7), and *Listeria* (ERS/USDA, 2001). All of these bacterial species can be found in the gastrointestinal microbial populations of food animals.

Traditionally, much of the research effort aimed at improving the safety of meat products has focused on postslaughter sanitation. Postslaughter antimicrobial treatments in processing plants reduce carcass contamination (Elder et al., 2000), but consumers are still sickened by food-borne pathogenic bacterial outbreaks. Until recently, little emphasis was placed on the development of intervention strategies in the live animal prior to slaughter; however, this has changed, with an increased emphasis on preslaughter intervention strategies.

Human pathogens present in the food animal gastrointestinal tract are often difficult to diagnose on the farm because they often have little or no impact on

[1]Proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product, or exclusion of others that may be suitable.

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animal health and/or production and are often shed sporadically. Because fecal shedding is correlated with carcass contamination (Elder et al., 2000), the role of the live animal in the production of a safe and wholesome food product is critical. Therefore, strategies that reduce food-borne pathogenic bacterial populations in the animal prior to slaughter could produce "the most significant reduction in human exposures to the organism and therefore reduction in related illnesses and deaths," according to Hynes and Wachsmuth (2000).

Probiotic Methods to Reduce Food-Borne Pathogens

The use of microflora to reduce pathogenic bacteria (including food-borne pathogens) in the gut has been termed a "probiotic" strategy (Fuller, 1989). The overall goal of this strategy is to promote the growth of groups of bacteria that are competitive with, or antagonistic to, pathogenic bacteria. Various probiotic techniques involve introducing a "normal" microbial population to the gastrointestinal tract or providing a limiting nutrient (sometimes termed a "prebiotic") that allows an existing commensal microbial population to expand its role in the gastrointestinal tract. The goal of these methodologies is to fill all microbial ecological niches and thereby prevent the establishment of an opportunistic pathogenic bacterial population. However, probiotics have not always been widely commercially implemented, often due to the concurrent use of noncomplimentary strategies (e.g., antibiotic use can dramatically decrease the effectiveness of competitive exclusion or prebiotics) (Steer et al., 2000). Due to increasing fears over the spread of antimicrobial resistance, it is expected in the future that antibiotics will become more closely regulated and expensive, causing probiotic strategies to become more effective and more widely accepted in the animal industry.

Competitive Exclusion

In neonates, the digestive tract is initially sterile, but it is rapidly colonized by a characteristic gastrointestinal microflora from the environment or the dam (Jayne-Williams and Fuller, 1971; Fuller, 1989). When this population becomes established, the animal is more infection-resistant, especially to bacteria that colonize the gastrointestinal tract (Fuller, 1989). This effect of the natural microbial population has been variously described as "bacterial antagonism" (Freter et al., 1983), "bacterial interference" (Dubos, 1963), the "barrier effect" (Fedorka-Cray et al., 1999), or competitive exclusion (Lloyd et al., 1977).

Competitive exclusion (**CE**), as a technology, involves the addition of nonpathogenic bacterial culture to the intestinal tract of food animals in order to reduce colonization or decrease populations of pathogenic bacteria in the gastrointestinal tract (Fuller, 1989; Nurmi et al., 1992; Steer et al., 2000). The CE culture may be composed of a single specific strain or may be composed of several strains or even several species of bacteria. Depending on the stage of production (maturity of the gut), the goal of CE can be the exclusion of pathogens from the naïve gut of a neonatal animal, or the displacement of an already established pathogenic bacterial population (Nurmi et al., 1992).

Potential Modes of Competitive Exclusion Action. Endogenous gastrointestinal bacteria compete fiercely with one another for available nutrients (Hungate, 1966). The species best adapted to each niche flourishes in the intestinal tract. Introduction of a stable, mixed microbial consortium to the naïve gut can aid in the early establishment of a normal microbial population and can create a highly competitive environment that may prevent the establishment of a pathogenic bacterial population (Nurmi et al., 1992; Crittenden, 1999; Steer et al., 2000).

As the normal (or CE) bacterial population increases throughout the gut, bacteria attach to the surface of the intestinal epithelium (Lloyd et al., 1974). This direct, physical binding can prevent opportunistic pathogens from obtaining a physical attachment site along the intestinal epithelium (Collins and Gibson, 1999). Volatile fatty acids are produced by the gastrointestinal microbial fermentation of carbohydrates or proteins and can be toxic to some species of bacteria, including the pathogenic bacteria *E. coli* O157:H7 and *Salmonella* (Wolin, 1969; Barnes et al., 1979; Prohaszka and Baron, 1983). Some bacteria produce antimicrobial compounds (traditional antibiotics, as well as bacteriocins or colicins) in order to eliminate competitive bacteria (Jack et al., 1995); these antimicrobial-producing species can be used to eliminate food-borne pathogenic bacteria. Intestinal microflora also produce vitamins that aid in the development of a healthy, vascularized intestinal epithelium with increased numbers of microvilli, and therefore, increased nutrient absorptive capacity (Collins and Gibson, 1999), potentially improving animal production efficiency as an added benefit of CE treatment.

Competitive Exclusion Applications. Providing a mixture of bacteria from healthy adult birds to newly hatched chicks (CE) provided an anti-*Salmonella* effect (Nurmi and Rantala, 1973; Nurmi et al., 1992). The beneficial effect in poultry has been widely repeated in many countries, leading to the development of several commercial CE products (Fuller, 1989; Nurmi et al., 1992). Recent studies demonstrating the effectiveness of CE in reducing *Salmonella* colonization of chicks have led to the commercial development in the United States of a mixed-culture CE product, comprised of several defined species of bacteria (Preempt, MS BioScience, Dundee, IL) (Nisbet et al., 1993a,b; 1996).

Treatment of swine with pure cultures of *Streptococcus faecium* reduced enterotoxigenic *E. coli* colonization and diarrhea (Underdahl et al., 1982; Ushe and Nagy, 1985). Other researchers have successfully demonstrated that a mucosal CE culture (mixed-CE culture) could effectively reduce *Salmonella* populations in ex-

perimentally infected piglets (Fedorka-Cray et al., 1999). Recently, a CE treatement for swine has been derived from the colonic contents of healthy pigs that reduces the incidence of *Salmonella cholerasuis* (Anderson et al., 1999) and enterotoxigenic *E. coli* (Genovese et al., 2000).

Competitive exclusion cultures have also been used to reduce *E. coli* O157:H7 in cattle (Zhao et al., 1998). Researchers isolated a defined population of multiple non-O157:H7 *E. coli* strains from naturally *E. coli* O157:H7-free cattle and found that this generic *E. coli* culture could displace established *E. coli* O157:H7 populations from cattle (Zhao et al., 1998). In more recent studies, other CE researchers have found that *Lactobacillus acidophilus* cultures (single strain addition) added to the feed of finishing cattle reduced *E. coli* O157:H7 shedding by more than 50% (Brashears and Galyean, 2002). These results indicate that CE could be a useful compliment to in-plant intervention strategies by reducing the levels of pathogenic bacteria entering the abattoir. In spite of the beneficial results of CE in several species of animals, "real-world" results have often been inconsistent and contradictory, sometimes due to interactions with other incompatible management strategies (e.g., antibiotic treatment) (Steer et al., 2000).

Prebiotics

Sugars or other organic compounds that are not digestible by the host animal, but are digestible by a segment of the microbial population are generally known as prebiotics (Walker and Duffy, 1998; Steer et al., 2000). Prebiotics have been used in humans in an effort to promote intestinal health (Crittenden, 1999). Fructooligosaccharides (**FOS**), for example, are sugars that are not degraded by intestinal enzymes that can pass down to the cecum and colon to become "colonic food" (Willard et al., 2000). Alternatively, other sugars can be used, such as galactooligosaccharides or inulin.

Prebiotics can provide energy and/or other limiting nutrients to the intestinal mucosa and substrates for the colonic/cecal bacterial fermentation to produce vitamins and antioxidants that further benefit the host animal (Collins and Gibson, 1999; Crittenden, 1999). Additionally, some prebiotics can provide specific members of the native microflora (e.g., *Bifidobacteria, Lactobacillus*) a competitive advantage (Willard et al., 2000) that can exclude pathogenic bacteria from the intestine via direct competition for nutrients or for binding sites through the production of "blocking factors" in a fashion similar to CE (Zopf and Roth, 1996). An additional benefit of prebiotic treatment is that some bacterial species that are provided a competitive advantage can produce antimicrobial substances (e.g., bacteriocins, colicins) that can directly inhibit pathogenic bacteria. An additional consideration for ruminants is that prebiotics must be able to bypass ruminal microbial degradation, requiring specific strategies tailored to allow sufficient quantities to reach the ruminant intestine. Coupling the use of CE and prebiotics (known as synbiotics) could yield a synergistic effect in the reduction of food-borne pathogenic bacterial populations in food animals prior to slaughter.

Antimicrobial Strategies to Reduce Food-Borne Pathogens

Antibiotics have often been thought of as a direct method to alter the microbial ecology of the intestinal tract. But the use of medically important antibiotics as growth promoters has become highly controversial in recent years, and is likely to become more so in the near future following recent regulatory action by the European Union. Bacteria have many complex mechanisms to resist antibiotics, and the widespread use of antibiotics in both human medicine and animal agriculture has led to the widespread dissemination of antibiotic resistance genes. Because of concern over the spread of antibiotic resistance, it is likely that the prophylactic use of antibiotics as growth promoters in food animals will become even more highly regulated, or even completely prohibited.

Antibiotics. Antibiotics have been widely used to control disease in both man and animals and to increase animal growth rate and/or efficiency. In spite of the common use of antibiotics in animals, it is sometimes difficult to target bacteria with antibiotics because they fall into diverse groups; therefore, broad-spectrum antibiotics are often included in animal rations. Antibiotic treatment to control gastrointestinal pathogens (including food-borne pathogens) can so disrupt the intestinal microbial ecosystem that opportunistic pathogens can occupy niches from which they would ordinarily be excluded. This can deleteriously impact animal health, performance, and food safety. This consideration, in addition to concerns about the role of subtherapeutic antibiotic treatment in the spread of antibiotic resistance (Witte, 2000), raises further concerns about the use of antibiotics to control food-borne pathogenic bacteria in the animal.

In spite of these potential drawbacks to antibiotic treatment, recent research has found that some antibiotics do have the potential to improve food safety at the live animal level. Neomycin sulfate is an antibiotic approved for use in cattle and has a 24-h withdrawal period. Cattle were fed neomycin for 48 h and went through a 24-h withdrawal period; they shed significantly lower generic *E. coli* and *E. coli* O157:H7 populations in their feces (Elder et al., 2002). After 5 d of neomycin withdrawal, generic *E. coli* populations had returned to near pretreatment levels, but *E. coli* O157:H7 populations remained nearly undetectable (Elder et al., 2002). The use of neomycin sulfate treatment to reduce *E. coli* O157:H7 populations has the benefit of being readily available to the industry at the present time until other strategies become market ready.

Other antimicrobial compounds are routinely incorporated into animal diets to improve animal health and/or growth performance. Ionophores are antimicrobials not related to antibiotics used in human medicine and thus do not appear to lead to an increase in antibiotic resistance. Monensin, the most widely used ionophore, has been used both as a coccidiostat in poultry and as a growth promoter in ruminants (Russell and Strobel, 1989). Because they are potent antimicrobials that are approved for use in food animals, it was hypothesized that ionophores could be used to control food-borne pathogenic bacteria populations. Unfortunately, because of the physiology of some common food-borne pathogens, it does not appear that ionophores reduce food-borne pathogenic bacteria populations (Busz et al., 2002).

Bacteriophages. Bacteria can be infected by bacterial viruses (or bacteriophages) that have very narrow target spectra, and some phages may be active against only a specific strain. This high degree of specificity allows phages to be used against targeted microorganisms in a mixed population without disturbing the microbial ecosystem, and phages have been used instead of antibiotics to treat human diseases in many parts of the world. Bacteriophages are common natural members of the gastrointestinal microbial ecosystem of food animals (Adams et al., 1966; Orpin and Munn, 1973; Klieve and Bauchop, 1988).

Phages recognize specific receptors on the outer surface of bacteria and inject their DNA into the host bacterium, which incorporates phage DNA into its chromosome. Once inserted into the chromosome, the phage "hijacks" the bacterium's biosynthetic machinery to make more phages. When intracellular nutrients are exhausted by phage replication, the host bacterium explodes (lyses), releasing thousands of new phage particles to repeat the process. An exponential increase in the number of phages continues as long as target bacteria are present and allows phages to persist in the gut rather than simply degrade over time as antibiotics do. However, phage populations are limited; if the target bacterium is removed from the environment, then phage populations diminish.

Bacteriophages have been used to control food-borne pathogenic bacteria in several species of food animals, and have been used against specific animal pathogens (Smith and Huggins, 1987; Kudva et al., 1999; Huff et al., 2002). Several research studies have examined the effect of phages on conditions or diseases that impact production efficiency or animal health (Smith and Huggins, 1982; 1983; Huff et al., 2002). The effectiveness of phage treatment in real-world conditions has been variable to date; therefore, more basic work needs to be completed before bacteriophages can be considered a viable method to control populations of food-borne pathogenic bacteria in food animals.

Specific Inhibition of Pathogens via Metabolic Pathways. Salmonella and *E. coli*, among other bacteria, can respire under anaerobic conditions by converting nitrate to nitrite via a dissimilatory nitrate reductase (Stewart, 1988). The intracellular bacterial enzyme nitrate reductase does not differentiate between nitrate and its analog, chlorate, which is reduced to chlorite in the cytoplasm; chlorite accumulation kills bacteria (Stewart, 1988). Chlorate addition to swine diets reduced experimentally inoculated *Salmonella* and *E. coli* O157:H7 fecal and intestinal populations (Anderson et al., 2001a,b). Other studies demonstrated that chlorate administered in drinking water significantly reduced *E. coli* O157:H7 populations in both cattle and sheep in the rumen, intestine, cecum, and feces (Callaway et al., 2002a). Preliminary results examining the use of chlorate in broilers and in turkeys have yielded promising results as well (J. A. Byrd, unpublished data).

Chlorate treatment does not appear to have an impact on the ruminal or the cecal/colonic fermentation in ruminants or monogastrics (Callaway et al., 2002a). It also appears that selection of chlorate-resistant mutants is not likely because chlorate resistant mutants are incapable of competing effectively against the intestinal microbial population (Callaway et al., 2001). Because of the dramatic impact chlorate has on food-borne pathogenic bacterial populations in the gut of food animals, it has been suggested that chlorate could be supplemented in the last meal prior to shipment to the slaughterhouse (Anderson et al., 2000). At the current time, however, the use of chlorate in food animals is under review by the U. S. Food and Drug Administration, but it has not been approved for use in food animals.

Immunization to Prevent Pathogen Colonization. Because food animals can be reservoirs of pathogenic bacteria, methods to exploit the animal's own immune system to reduce pathogen load have been studied. Specific immunization against pathogenic bacteria has shown great promise in reducing the levels of disease-causing pathogens in food animals. Vaccines against *Salmonella* strains responsible for disease have been developed for use in swine and dairy cattle (House et al., 2001). Vaccination has also been successfully used to combat postweaning *E. coli* edema disease in young pigs (Gyles, 1998). The introduction of "edible vaccines" has the potential to make immunization of food animals economically viable for many diseases, including food-borne pathogens.

Recently, a vaccine has been developed for cattle that reduced fecal *E. coli* O157:H7 shedding (B. Finley and A. Potter, personal communication). Preliminary studies have indicated that this vaccine is effective, and large-scale field trials are scheduled to begin in the summer of 2002. However, because *E. coli* O157:H7 and other enterohemorrhagic *E. coli* are shed sporadically by cattle, it appears that natural exposure to *E. coli* O157:H7 does not confer protection to the host (Gyles, 1998). In a similar manner, *Salmonella* can survive in an animal that has developed an antigenic response to *Salmonella* for extended periods of time (Gyles, 1998). Therefore, while some technical issues remain to be

resolved, the use of vaccination to reduce food-borne pathogens appears to hold promise, and has an added benefit in that vaccination could be used synergistically with other pathogen reducing technologies.

Dietary and Management Effects

Good animal management is crucial to the production of healthy, efficient animals. Yet it has not been conclusively demonstrated whether specific management strategies can directly impact shedding or carriage of food-borne pathogens found in animals. However, reducing the multiplication of pathogens in feed and water may reduce exposure and horizontal and vertical transmission of pathogens to and between animals (Hancock et al., 1998).

Dietary Strategies to Reduce E. coli O157:H7 Populations in Cattle. Feeding grain to cattle has a significant effect on the ruminal microbial ecosystem and overall animal health (Russell and Rychlik, 2001). Cattle in the United States are often fed high- grain rations in order to maximize growth efficiency (Huntington, 1997). Some dietary starch bypasses ruminal fermentation and passes through to the cecum and colon where it undergoes microbial fermentation (Huntington, 1997). Studies have indicated that varying the forage to grain ratio in cattle rations can have a marked effect on populations of *E. coli.* Some early studies indicated that reducing hay, over feeding grain, or switching from a better- to poorer-quality forage increased generic *E. coli* and/or O157:H7 populations (Brownlie and Grau, 1967; Allison et al., 1975; Kudva et al., 1995; 1996).

In recent research, cattle fed a feedlot-type ration had generic *E. coli* populations 1,000-fold higher than cattle fed only hay (Diez-Gonzalez et al., 1998). When cattle were abruptly switched from a finishing ration to a 100% hay diet, fecal *E. coli* populations declined 1,000-fold, and the population of *E. coli* resistant to an "extreme" acid shock (similar to that of the human stomach) declined more than 100,000-fold within 5 d (Diez-Gonzalez et al., 1998). Based on these results, the authors suggested that feedlot cattle be switched from high-grain diets to hay prior to slaughter to reduce *E. coli* populations entering the abattoir (Diez-Gonzalez et al., 1998). In a very well-controlled study, Keen et al. (1999) screened cattle on a high-grain diet for natural *E. coli* O157:H7 contamination. These cattle were divided, with one group maintained on a feedlot ration and the other abruptly switched to hay; 52% of the grain-fed cattle were positive for *E. coli* O157:H7 compared with 18% of the hay-fed cattle (Keen et al., 1999). Additional research with experimentally inoculated calves indicated that animals fed a high-concentrate diet consistently shed more *E. coli* O157:H7, and that isolates grown in ruminal fluid from grain-fed animals were more resistant to an acid shock than those grown in hay-fed ruminal fluid (Tkalcic et al., 2000). Gregory et al. (2000) stated that "the most effective way of manipulating gastro-intestinal counts of *E. coli* was to feed hay." However, other research groups have produced contradictory results indicating that forage feeding either had no effect or increased *E. coli* O157:H7 shedding (Hovde et al., 1999; Buchko et al., 2000a,b). Therefore, although it appears from most of the available literature that forage feeding does reduce *E. coli* populations (Callaway et al., 2002b), the debate is by no means complete.

In spite of the benefits potentially offered by feeding forage, the effect of hay feeding on weight gain and carcass characteristics has not been systematically examined. Recent research indicated that a switch to forage did not have a dramatic impact on carcass characteristics or final BW (Stanton and Schutz, 2000). However, other researchers have found that a switch to hay feeding resulted in a lower carcass weight (Keen et al., 1999). Thus, the economic impact of a switch from grain to forage must be carefully considered.

Water Troughs as a Source of Transmission? Cattle, as well as people, can be infected by pathogens via a water-borne route (Jackson et al., 1998; Shere et al., 2002). Researchers have demonstrated that cattle water troughs can be reservoirs of *E. coli* O157:H7 (LeJeune et al., 2001). Although the significance of this route of horizontal transmission has not been conclusively proven, interventions at the pen level offer significant promise to reduce pathogen contamination of animals (LeJeune et al., 2001). Further research into keeping pathogens from surviving in the water supply can potentially increase food safety by reducing the food-borne pathogen horizontal transmission.

Implications

Access to a safe and wholesome food supply is crucial to the American public, and the food supply in the United States is indeed the safest in the history of the world. Yet food-borne illnesses still occur, and these are often associated with products derived from animal agriculture. Although the meat industry has continuously sought improvement in the safety of its products, much of the research has focused on postslaughter strategies. Until recently preslaughter intervention points have not been given full consideration as methods to improve food safety. The use of vaccination, prebiotics, competitive exclusion, antibiotics, antimicrobials, dietary practices, and good animal management can potentially reduce the incidence of food-borne pathogenic bacteria that enter the abattoir. Further research into interventions that take advantage of this preslaughter "critical control point" is crucial to improving overall food safety.

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