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Practical Mechanisms for Interrupting the Oral-fecal Lifecycle of *Escherichia coli*

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Abstract

***Escherichia coli* is a common gut inhabitant, but it is usually out numbered by strictly anaerobic bacteria. When fecal material is exposed to oxygen, fermentation acids can be respired, and *E. coli* numbers increase. *E. coli* can survive for long periods of time in feces, but subsequent proliferation is dependent on its ability to re-enter the gastrointestinal tract via contaminated water and food. The oral-fecal lifestyle of *E. coli* is facilitated by its ability to survive the low pH of the human gastric stomach. Most strains of *E. coli* do not cause human disease, but some strains produce toxins and other virulence factors. Mature cattle carry *E. coli* O157:H7 without showing signs of infection, and beef can be contaminated with cattle feces at slaughter. Cattle manure is often used as a fertilizer by the vegetable industry, and *E. coli* from manure can migrate through the soil into water supplies. Sanitation, cooking and chlorination have been used to combat fecal *E. coli*, but these methods are not always effective. Recent work indicates that cattle diets can be modified overcome the extreme acid resistance of *E. coli.* When cattle were fed have for only a few days, colonic volatile fatty acid concentrations declined, pH increased, and the *E. coli* were no longer able to survive a pH shock that mimicked the human gastric stomach. *E. coli* in stored cattle manure eventually become highly acid resistant even if the cattle were fed hay, but these bacteria could be killed by sodium carbonate (150 mM, pH 8.5). Because the diet manipulations and carbonate treatments affected *E. coli* in general rather than specific serotypes, there is an increased likelihood of successful field application.**

Introduction

Escherichia coli was one of the first bacteria to be characterized, and it is the "best known prokaryotic organism" (Brock *et al.,* 1994). *E. coli* has a physiology and metabolism that is both varied and adaptive, and the discovery of conjugation and transduction sealed its use as a model for genetic and biochemical experiments (Schaechter and Neidhardt, 1996). Some strains of *E. coli* are pathogenic, but most types are nonpathogenic and can be easily cultivated in the laboratory.

E. coli is found in the GI tract of virtually all mammals, but it is outnumbered 100-fold or more by strictly anaerobic gut bacteria (Drasar and Barrow, 1985). When oxygen is lacking, *E. coli* has a fermentative metabolism, but it can use either oxygen or nitrate as terminal electron acceptors for respiration (Gottschalk, 1986). *E. coli* counts have long been used as an index of fecal contamination (Jay, 1989), and contaminated food and water can serve as a means of GI tract re-colonization (Figure 1).

Foods can be contaminated with human fecal material if sanitation procedures are inadequate, and meat supplies can be contaminated with livestock feces at slaughter. Livestock manure is often used as a fertilizer for fruits and vegetables, and *E. coli* from manure can migrate through the soil into water supplies (USEPA, 1998; Craun *et al.,* 1997).

GI Tract and Fecal Habitats

The GI tract is an anaerobic habitat, and *E. coli* has a fermentation scheme that produces acetate, formate and ethanol (Gottschalk, 1986). If the pH is acidic, the pyruvate formate lyase is inhibited and lactate and succinate are produced (Stokes, 1949). The fumarate reductase of *E. coli* is a cytochrome-linked enzyme (Gottschalk, 1986), and iron availability can affect the growth of *E. coli* in the GI tract (Drasar and Barrow, 1985)

Most simple sugars are readily used by mammals, and free sugars are only found in the proximal regions of the small intestine. However, adult mammals often become lactose intolerant, and most strains of *E. coli* can ferment lactose (Brenner, 1984). *E. coli* does not digest starch, but it can utilize maltodextrins and maltose that would arise from the extracellular amylases of other bacteria (Schwartz, 1987). *E. coli* is a 'proteobacterium' that utilizes amino acids as an energy source even if oxygen is not available (Brenner, 1984).

Once mammalian fecal material is excreted, oxygen drives respiration, and *E. coli* then utilizes acetate as an energy source for growth (Gottschalk, 1986). The *E. coli* counts of fresh cattle feces stored aerobically increased 2 to 4 logs in the first 3 days of incubation, and declined slowly thereafter (Figure 2a). *E. coli* can survive for long periods of time in fecal material (Himathongkham *et al.,* 1999), but its lifecycle is terminated if it does not re-enter the GI tract via contaminated food (Figure 1).

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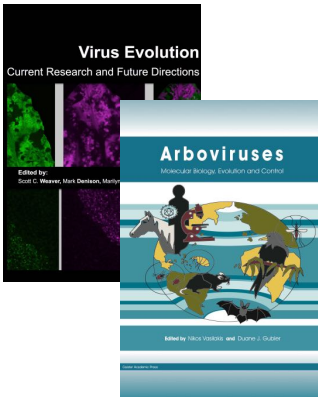

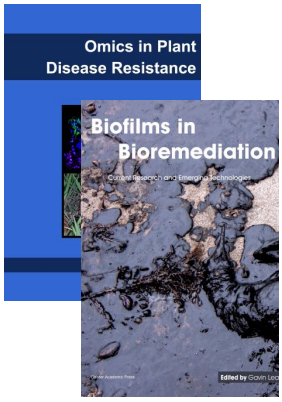

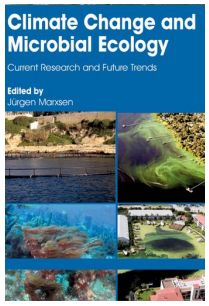

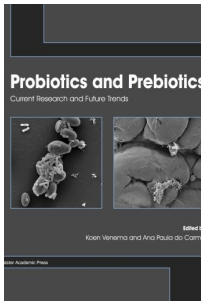

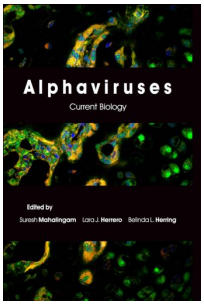

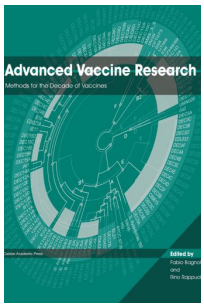

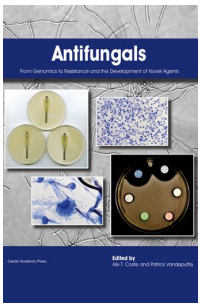

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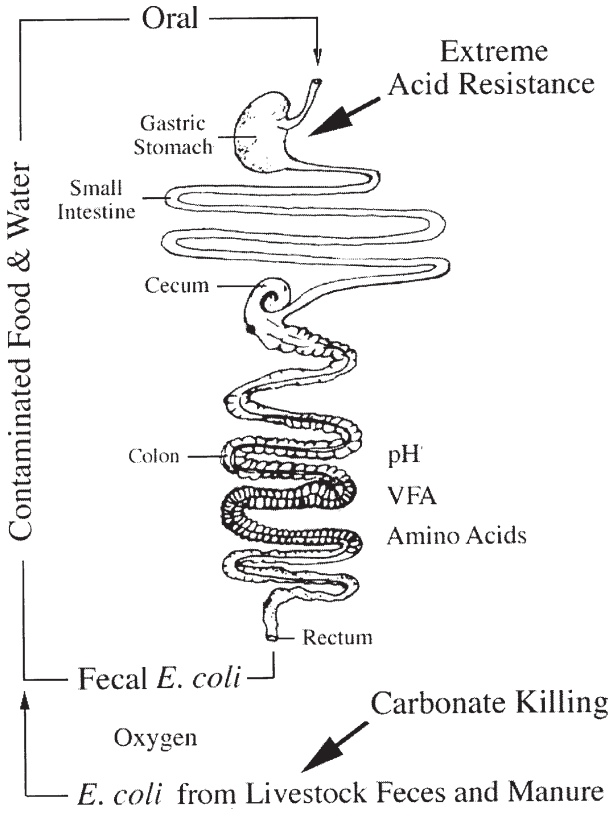

Figure 1. A schematic showing the oral-fecal lifestyle of *E. coli* and its critical control points.

Pathogenic and Non-pathogenic *E. coli*

In the 1950's, animal feeding trials demonstrated that feed efficiency and weight gain could be enhanced by sub-therapeutic doses of antibiotics, and the response was greatest if animals were housed in unsanitary conditions (Solomons, 1978). Because germ-free animals did not show an antibiotic response, it appeared that even the 'normal gut microflora' could have a negative impact on animal performance. Germ-free animals had a thinner gut mucosa, and it appeared that the attachment of gut bacteria to the intestinal mucosa was causing an inflammation (Bruckner and Szabo. 1984).

Most strains of *E. coli* are thought to be harmless, but it has long been recognized that any *E. coli* can cause disease if it penetrates the gut mucosa and enters the blood stream (Jawetz *et al.,* 1974). When *E. coli* reaches the blood and lyses, it releases an endotoxin that causes fever and even death. Endotoxin is a normal part of the *E. coli* cell wall (chiefly lipid A of the lipopolysaccharide). When even small amounts of *E. coli* lipopolysaccharide were injected into healthy animals, macrophages released cytokines, food intake decreased, and the animals stop gaining weight (Webel *et al.,* 1998).

Some strains of *E. coli* produce an enterotoxin that resembles cholera toxin, and this protein causes acute diarrhea even if bacteria never cross the intestinal epithelium (Jawetz *et al.,* 1974). Enterotoxin-producing *E. coli* can attach tightly to the gut mucosa, and this attachment process is mediated by specific proteins or adhesions. The type I fimbriae of *E. coli* binds to a galactosylated receptor protein complex of the susceptible animals, and it also causes hemagglutination of erythrocytes (Jeyasingham *et al.,* 1999).

In the early 1980's, a strain of *E. coli* designated as O157:H7 was isolated from the bloody feces of patients that had consumed contaminated hamburgers (Riley *et al.,* 1983), and subsequent work demonstrated that *E. coli* O157:H7 attached tightly to the gut mucosa via a protein known as intimin (Kaper *et al.,* 1998). The virulence of *E. coli* O157:H7 was enhanced by Shiga toxins that cause kidney malfunction and a hemolysin (Su and Brandt, 1995). *E. coli* O157:H7 causes approximately 60,000 infections and 50 deaths each year (Hoyle, 2000). The recovery

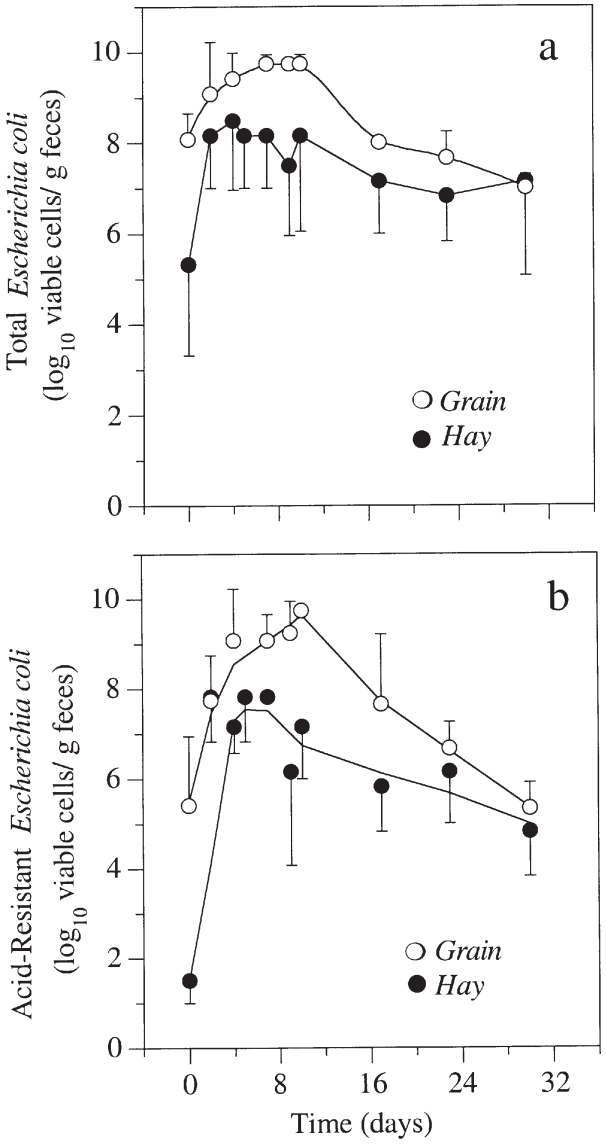

Figure 2. The effect of cereal 90% grain (open symbols) or hay diets (closed symbols) on the survival of *E. coli* in stored cattle feces. Total counts are shown in part (a) and acid-resistant counts (pH 2.0, 1.0 h) are shown in part (b). Unpublished data.

period from *E. coli* O157:H7 infections is relatively long, and the kidney damage can be permanent (Su and Brandt, 1995).

Shigella, a bacterium closely related to *E. coli*, produces intestinal inflammation and bloody diarrhea, but *Shigella* is not normally found in the gastrointestinal tract at high numbers (Jawetz *et al.,* 1974). Because *E. coli* O157:H7 produces toxins that are homologous to the ones carried by *Shigella* (Nataro and Kaper, 1998), and temperate phages with the Shiga-toxin genes were isolated from *E. coli* O157:H7, phage is suspected as the route of transmission from *Shigella* to *E. coli* (Su and Brandt, 1995). *E. coli* O157:H7 has a hemolysin that enhances its virulence, and the gene encoding this protein is located on a large plasmid (Nataro and Kaper, 1998). The intestinal adherence factor of *E. coli* O157:H7, intimin, is encoded by a chromosomal gene (Nataro and Kaper, 1998). The hemolysin and intimin genes have some homology to previously identified proteins from enteric bacteria, but the exact origins are not known (Kaper *et al.,* 1998).

Outbreaks of *E. coli* O157:H7 have been linked to a variety of foods and drinking water, but hamburger is still a common source of this bacterium (Armstrong *et al.,* 1996). Recent work indicated that 28% of the beef cattle entering Midwest slaughter houses were O157 positive, and *E. coli* carrying the O157:H7 antigen were found in approximately 2% of the processed meat samples (Elder *et al.,* 2000). Adult cattle are asymptomatic carriers of *E. coli* O157:H7 and do not show any outward signs of infection (Armstrong *et al.,* 1996).

Researchers have pursued a variety of approaches to combat *E. coli* O157:H7 in cattle, but these efforts have provided few successes. Because *E. coli* O157:H7 is antigenically unique, Johnson *et al.* (1999) tried to develop a vaccine. However, even calves that had strong and sustained antibody responses did not shed significantly fewer organisms in their manure than untreated controls. Doyle and his colleagues (Zhao *et al.,* 1998) noted that some nonpathogenic *E. coli* produced colicins that killed *E. coli* O157:H7, and they hypothesized that these bacteria would competitively exclude *E. coli* O157:H7 from the GI tract. However, this approach was only tested with experimentally inoculated animals.

The ability of researchers to assess *E. coli* O157:H7 prevalence in cattle has in many cases been stymied by sometimes ineffective detection methods. Most strains of *E. coli* O157:H7 are sorbitol negative and tellurite-resistant (Hancock *et al.,* 1994), but these enrichment procedures do not always detect *E. coli* O157:H7 in cattle (Elder *et al.,* 2000). Sorbitol negative, tellurite resistant-*E. coli* often have Shiga-toxin genes, but isolates lacking one or both of the Shiga-toxin genes have been detected (Park *et al.*, 1999). Because *E. coli* O157:H7 is phage susceptible, it has a relatively plastic genome, and the pulsed gel electrophoresis pattern is not always constant genome (Plunkett *et al.,* 1999). Immunomagnetic beads coated with an antibody to the O157 antigen are commercially available, and their use had improved *E. coli* O157:H7 detection (Chapman *et al.,* 1997; Keen *et al.,* 1999). When *E. coli* O157:H7 in cattle feces were captured with immunomagnetic beads, 28% of the cattle entering slaughter houses in the Midwest were shown to be *E. coli* O157 positive (Elder *et al.,* 2000), and this value was 10-fold greater than previous estimates (Hancock *et al.,* 1994).

In the 1980's, virtually all cases of human bloody diarrhea could be linked to *Shigella* or *E. coli* O157:H7, but recent studies from other countries indicate that nearly half of the reported cases can be caused by other strains of *E. coli* (e.g. O111, O23, O26, O157:H-, and O91) (Goldwater and Bettelheim, 1996; Park *et al.*, 1999). If methods of combating *E. coli* O157:H7 are highly specific, another serotype may simply take its place. Recent work, however, indicates that virtually all *E. coli* have common properties than can be exploited to decrease either total number or infectivity.

Gastric Barrier

Many bacteria are killed by the low pH of the gastric stomach (Cash *et al.,* 1974; Peterson *et al.,* 1989), and the oral-fecal lifestyle of *E. coli* is facilitated by its ability to survive low pH (Figure 1). However, the extreme acid resistance of *E. coli* is an inducible characteristic that is influenced by a variety of environmental factors. Until recently the physiology and genetics of extreme acid resistance were poorly understood, but recent work has identified key features of this adaptation (Waterman and Small, 1996; Hall *et al.,* 1995; Diez and Russell, 1999).

Food ingested by simple stomached warm blooded animals is first deposited in the gastric stomach. The gastric glands secrete HCl was well as pepsin, and this enzyme that has a pH optimum of 2.0 (Guyton, 1971). Humans secrete approximately 3 liters of gastric juice per day, and this juice has an HCl content of approximately 0.17 N and a pH of approximately 0.9. Stomach pH can be as high as 6.0, if a large amount of food has just been ingested, but the pylorus does not open until "the acidity of the gastric contents reaches a relatively high value" (Williams, 1931). The mean stomach pH of humans is 2.0. Residence time of food in the stomach varies, but the typical half residence time is 1.5 hours (Texter *et al.,* 1968). "Because acidity develops more quickly in the presence of carbohydrate, the stomach empties much more rapidly when carbohydrate food is eaten than when proteins are being digested" (Williams, 1931).

Mild versus Extreme Acid Resistance

Many bacteria are inhibited by weak acids, and fermentation acids have long been used as preservatives (Russell and Diez-Gonzalez, 1998). Lactic acid bacteria have circumvented fermentation acid toxicity, by evolving an intracellular metabolism that tolerates a low intracellular pH. When intracellular pH declines, the pH gradient across the cell membrane is low, and fermentation acid anions do not accumulate intracellularly. Lactic acid bacteria can grow at pH values as low as 4.5, but they are killed by the low pH of the gastric stomach (O'Sullivan and Condon, 1997). Conversely, *E. coli* does not grow well in the presence of mild fermentation acids, but it can survive pHs that mimic the human gastric stomach (Russell and Diez Gonzalez, 1998). The term "acid resistance" has been used interchangeably to describe growth at mildly acidic conditions as well as survival at very low pH, and this lack

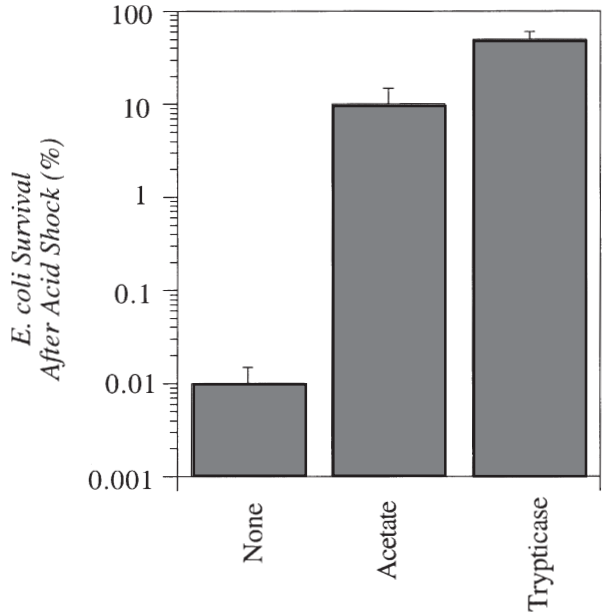

Figure 3. The effect of sodium acetate addition (50 mM) and Trypticase (1.5 mg/ml) on the ability of *E. coli* to survive an acid shock that mimicked the human gastric stomach (pH 2.0, 1 h). *E. coli* that were grown in minimal medium with little amino acid (< 0.25 mg/ml) and maltose as an energy source are also shown (none). The figure was re-drawn from the data of Jarvis and Russell (2001).

of specificity has created confusion. Lin *et al*. (1995) used the term "extreme acid-resistance" to describe viability after low mineral acid shock, and we will use this same terminology.

Extreme Acid Resistance of *E. coli*

Poynter *et al*. (1986) noted that significant numbers of *E. coli* survived an HCl acid shock (pH 2.5, 30 min), and subsequent work indicated that this survival was even greater if the cells were first grown at pH 5.0 (Goodson and Rowbury, 1989). Gale and Epps (1942) reported that the amino acid decarboxylases of *E. coli* were induced by growth at low pH, and Guilfoyle and Hirshfield (1996) noted that arginine and lysine decarboxylase genes could be induced by short chain organic acids. Because mutants defective in arginine (Lin *et al.,* 1996) and glutamate (Hersh *et al.,* 1996) decarboxylases were more sensitive to acid shock than wild-types, it appeared that amino acid availability might be a regulator of extreme acid resistance in *E. coli*. When a freshly isolated *E. coli* strain was grown anaerobically on maltose with a low concentration of yeast extract and amino acids, less than 0.1% of the bacteria survived a 1 h, pH 2.0 acid shock (Jarvis and Russell, 2000). However, if Trypticase was added, the survival increased approximately 4 logs (Figure 3). When the same strain was grown aerobically in Luria broth, a medium rich in amino acids, the cell survival was nearly 100% (pH 2.0, 1 h). The mechanism of this amino acid protection is not entirely clear. Are the amines preventing a decline in intracellular pH or are they acting as chaperones to protect pH sensitive cell components?

When a freshly isolated *E. coli* strain was grown anaerobically on maltose in a minimal medium at near neutral pH, acetate addition caused a large increase in acid shock survival (Figure 3), and this result indicated that fermentation acids could induce extreme acid resistance even if amino acids were lacking (Jarvis and Russell, 2001). Some undissociated weak acids can traverse the cell membrane and cause a decrease in intracellular pH, and Slonczewski *et al*. (1987) noted that these acids induced a variety of genes in *E. coli*. However, recent work with *E. coli* O157:H7 indicated that the synthetic uncoupler, carbonylcyanide-*m*-chlorophenylhydrazone, alone could not trigger an increase in acid shock survival (Diez-Gonzalez and Russell, 1999).

When *E. coli* O157:H7 was grown in a medium with increasing amounts of sodium acetate at pH values ranging from 5.5 to 7.0, cell survival was highly correlated with the concentration of undissociated acetate in the growth medium (Diez-Gonzalez and Russell, 1999). Propionate and butryate also caused a marked increase in *E. coli* survival, but formate, lactate and benzoate were 100 to 1000-fold less effective. These results indicated that the acid-dependent induction of extreme acid resistance has at least some specificity.

E. coli can be grown either aerobically or anaerobically, and redox also appears to have significant impact on extreme acid resistance. When *E. coli* O157:H7 was grown anaerobically in a reduced medium at pH 7.0, acid shock survival was not maximal until the VFA concentration was greater than 50 mM (Diez-Gonzalez and Russell, 1999). If the same cultures were grown aerobically without a reducing agent, 10-fold less VFA was needed. The genetics of extreme acid resistance are poorly understood, but it should be noted that the global regulatory protein, Fnr, is a redox sensitive protein that is induced under anaerobic conditions (Unden and Schirawski, 1997).

Exponential versus Stationary Phase Cells

In *E. coli*, a variety of stress responses are regulated by the stationary phase sigma factor, RpoS, and *rpo*S-defective mutants are more sensitive to acid shock than wild-types (Cheville *et al*., 1996). The idea that extreme acid resistance is a stationary phase induction was supported by the observation that exponentially growing cells were 100-fold more sensitive to acid shock (Diez-Gonzalez and Russell, 1999). However, growth experiments indicated that acetate was only able to induce extreme acid resistance if it was added to cultures that were still growing (Diez-Gonzalez and Russell, 1999), and at least some RpoS regulated proteins are synthesized in late exponential phase, just before growth has ceases.

Fresh Fecal Contaminations at Slaughter

When cattle are slaughtered, the digestive system is carefully removed from the body cavity, but fecal contamination is still a problem (Elder *et al.,* 2000). Many slaughter facilities have implemented washing procedures (water or vinegar water) to minimize fecal contamination, but these remedies do not always remove all of the fecal bacteria (Berry and Cutter, 2000). Meats are routinely monitored for *E. coli*, but these procedures do not indicate

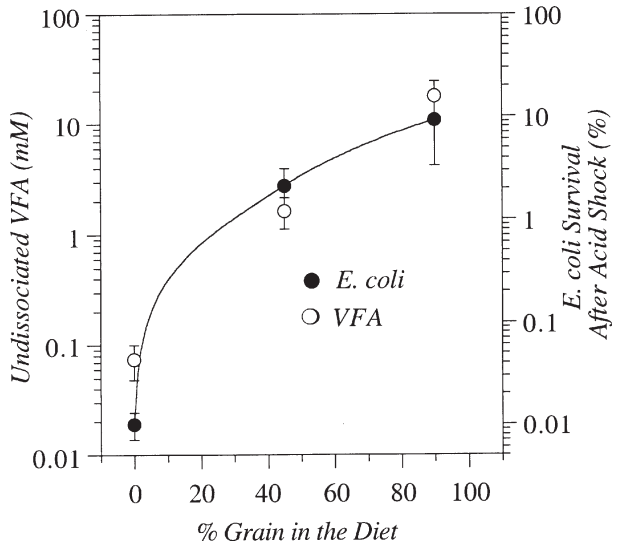

Figure 4. The effect of cereal grain on colonic undissociated volatile fatty acid concentrations and the percentage of acid-resistant *E. coli*. Open symbols show undissociated VFA (volatile fatty acids) and the closed symbols show *E. coli* survival after acid shock. The bars indicate standard deviations of the mean (3 animals, 4 sampling days). Figure re-drawn from the data of Diez Gonzalez *et al.,* 1998.

if the *E. coli* are acid-sensitive or acid-resistant.

Cattle evolved as grazing animals, but they grow more rapidly if they are fed diets rich in cereal grain (Waldo, 1973). Since World War II, cattle have been fed increasing amounts of grain, and fattening beef cattle in the United States are routinely fed diets that are 90% grain. Cattle have a complicated digestive system, and feeds are subjected to fermentation in the rumen prior to gastric and intestinal digestion. Much of the grain is fermented in the rumen, but some grain passes by the rumen to the colon where it is subjected to another fermentation (Waldo, 1973).

Recent work (Diez-Gonzlez *et al.,* 1998; Scott *et al.,* 1999) indicates that cattle diets can have a profound effect on the number and acid resistance of *E. coli*. When the proportion of grain in the diet was increased, colonic volatile fatty acid concentrations increased, colonic pH decreased, *E. coli* numbers were greater, and the *E. coli* cells were more acid-resistant (Figure 4). Based on these observations, it appeared that grain feeding might be increasing the risk of food borne illness, but a brief period of hay feeding was able to counteract this effect (Figure 5) (Diez-Gonzlez *et al.,* 1998; Scott *et al.,* 1999).

Hancock *et al.* (1999) challenged the idea that diet shifts would improve food safety on the grounds that the *E. coli* originate "from the hide, hooves or the equipment used in slaughter and processing rather than directly from the colon," but the experiments of Elder *et al.* (2000) showed that 90% of the carcasses positive for O157 antigen had *E. coli* O157:H7 in their fresh feces. Because cattle carcasses are immediately chilled to prevent bacterial growth (Gill and McGinnis, 1993), the argument that the contaminating *E. coli* would "have replicated extensively outside the colon" is also doubtful. When refrigerated hamburger was inoculated with either non-induced *E. coli* cultures or feces from cattle fed hay, acid resistant *E. coli* were never detected (Russell *et al.,* 2000).

The strategy of feeding hay immediately before slaughter appears to have another added benefit. Keen *et al.* (1999) noted that beef cattle that were fed a 90% grain diet typical of feedlots in the Midwest had an *E. coli* O157:H7 prevalence of 53% (n = 200). Cattle that remained on grain (n =100) continued to have a prevalence of 53%, but only 18% of the cattle that were switched to hay for 7 days were O157 positive.

Contaminated Water

In the past, farmers spread their manure on a daily basis, and field application rates were generally low. When the scale and intensity of livestock production increased, disposal became a problem (Strauch and Ballarini, 1994). Livestock manure is now frequently stored in large tanks or ponds prior to spreading, and the soil application rates can be very high. A recent EPA survey (USEPA, 1998) indicated that as many as 40% of the wells in rural America were contaminated with *E. coli*. The sources of these bacteria could not be precisely defined, but livestock manure was cited a likely source of contamination.

When cattle feces were stored aerobically for only three days, volatile fatty acids supported the growth of *E. coli*, and the *E. coli* became highly acid resistant, even if the cattle had been fed hay (Figure 2b). The increase in acid-resistant *E. coli* is consistent with the observation that little VFA is needed to induce acid resistance when oxygen is available and the redox state is high (Diez Gonzalez and Russell, 1999).

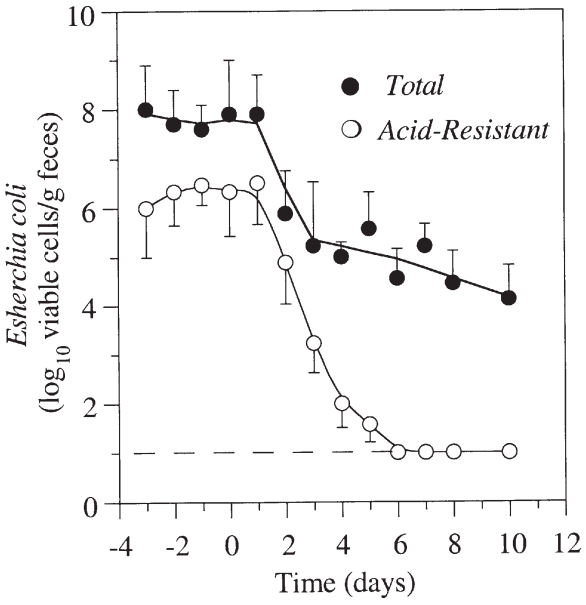

Figure 5. The effect of a diet switch (90% grain to day on day zero) on the colonic *E. coli* of cattle. Open symbols show acid resistant counts (pH 2.0, 1.0 h) and the closed symbols show total counts. The bars indicate standard deviations of the mean (3 animals, 4 sampling days). Figure re-drawn from the data of Diez Gonzalez *et al.,* 1998.

Livestock manure can be treated with harsh chemicals to eliminate coliforms and other bacteria, but these methods are expensive or environmentally unsound (Strauch and Ballarini, 1994). Anaerobic sewage digesters and composts can also kill bacteria in manure, but temperatures generated are often too low to destroy all the pathogens. Strauch and Ballarini (1994) noted that anaerobic digester technology was only suited to large scale animal enterprises, and few such systems have ever been installed.

Carbonated Cow Manure

It has long been recognized that urine has antimicrobial activity, but the nature of this effect was not defined (Mulholland *et al.,* 1969; Lees and Osborne, 1979). When cattle manure was mixed in equal parts with cattle feces, fecal urease caused an increase in ammonia and pH, but neither of these effects alone could explain the decrease in *E. coli* number (Diez Gonzalez *et al.,* 2000). Because human urine did not kill *E. coli* until urease was added, acidified cattle urine lost all activity, and little killing was observed until the pH was greater than 8.5, it appeared that carbonate might be the antibacterial agent.

Cattle typically produce 2.2 times as much feces as urine (Agricultural Engineers Handbook, 1995), and little decrease in *E. coli* was observed if the urine to feces ratio was 2.2 to 1.0 (Diez Gonzalez *et al.* 2000). However, if cattle manure was supplemented with 4 and 2 g of sodium carbonate and sodium hydroxide, respectively, *E. coli* was eliminated (Figure 6), and the estimated cost was less than 3 cents per cow per day. Because carbonate killed a variety of bacteria as well as *E. coli*, the carbonate and alkali treatment could prevent the spread of other food- or water-borne pathogens (Table 1).

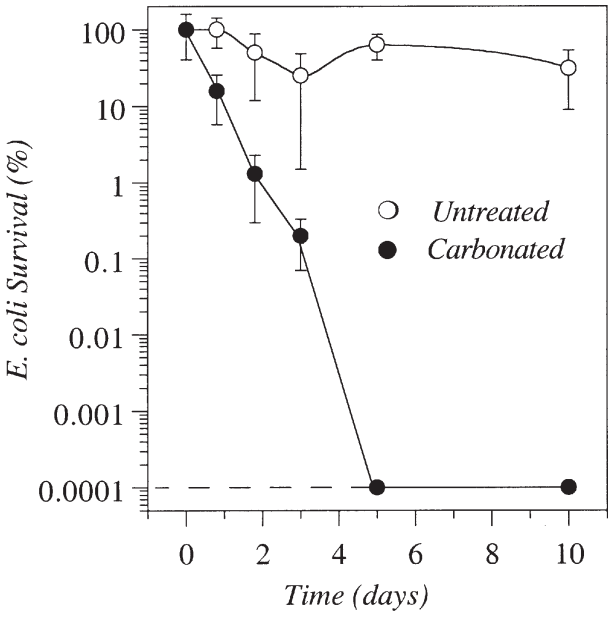

Figure 6. The survival of *E. coli* in cattle manure slurries (2.2 parts feces, 1 part urine, 6.7 parts water) that was incubated at 22° C in opaque sealed containers for 10 days. Closed symbols show samples that were treated with carbonate and alkali (4 mg sodium carbonate and 2 mg sodium hydroxide per gram manure, final pH 8.9). The error bars indicate standard deviations of the mean (n = 15 cows). The urine was a pooled sample (n = 6 cows). The dotted lines show the detection limit of the assays. Figure was re-drawn from the data of Diez Gonzalez *et al.* (2000).

Table 1. Antimicrobial Activity of Ammonium Chloride, Sodium Carbonate and Cattle Urine Against Pure Cultures of Bacteria

	Number of viable cells (log_{10} cells/ml)[a]		
Bacterial strain	NH_4Cl	Na_2CO_3	Urine
Escherichia coli 0157:H7 (ATCC 43895)	7.0	ND[b]	ND
Salmonella typhimurium (ATCC 14028)	7.0	ND	ND
Streptococcus pyogenes (ATCC 19615)	7.0	ND	ND
*Klebsiella pneumonia*e (ATCC 13883)	7.0	ND	ND
Staphylococcus aureus (ATCC 25923)	7.0	2.0 ± 0.8	1.3 ± 0.4
Listeria monocytogenes (10403S)	7.0	5.7 ± 0.5	6.3 ± 0.5

[a] Viable cell number after exposure to sodium carbonate or ammonium chloride (150 mM, pH 8.5, 24 hours). The ATCC numbers are those given by the American Type Culture Collection. The ratio of urine to culture was 1 to 1 (final carbonate 150 mM, pH 8.5). Cultures were grown in basal medium (approximately 10^7 viable cells/ml).
[b] ND indicates that the viable cell number was below the limit of detection (10 viable cells/ml).
[c] Data taken from Diez Gonzalez *et al.,* 2000

The mechanisms of carbonate killing is not yet clear, *but E. coli* cells that were incubated with a similar concentration of ammonia chloride at the same pH persisted (Table 1). These results indicated that the killing cannot simply be explained by the ammonia or alkalinity. Many bacterial enzymes are magnesium-dependent, and the outer membrane of *E. coli* is stabilized by magnesium cross bridges (White, 1995). Because magnesium and carbonate form insoluble complexes at alkaline pH, it is conceivable that the carbonate killing could be mediated via its effect on cellular magnesium. Preliminary experiments showed that *E. coli* can also be killed with EDTA, a divalent anion chelator, if the pH is alkaline, but the cost of this latter chemical is much greater than sodium carbonate.

Conclusions

In recent years, there has been heightened awareness of food and water contaminations, and "*E. coli* O157:H7" has become part of our modern vernacular. The CDC indicates other pathogens are more likely to cause food-borne illnesses than *E. coli O157:H7*, but *E. coli O157:H7* symptoms are severe. The cramping is very painful, the duration of the infection is relatively long (7 days or more), and the treatments can be very costly (kidney dialysis). Molecular biology has given us better detection methods and insights into the evolution and dissemination of *E. coli O157:H7* and other pathogens, but prevention has relied almost entirely on improvements in sanitation and cooking.

Because the prevalence of *E. coli* O157:H7 in cattle

and meats is 10-fold higher than previously thought, and sanitation procedures are not always effective, there is a need for the control of *E. coli* in cattle and cattle manure (Elder *et al.,* 2000). Recent work indicates that relatively simple procedures like adding sodium carbonate and alkali to manure and feeding cattle hay for a few days before slaughter can have a dramatic impact on total *E. coli* counts and the acid-resistance of *E. coli.* Because these procedures affect *E. coli* in general rather than specific serotypes, there is an increased likelihood of successful field application.

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