Approaches to Controlling *Escherichia coli* O157:H7, a Foodborne Pathogen and an Emerging Environmental Hazard

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*Escherichia coli* is a ubiquitous bacterium that lives communally in the guts of most mammals, including man; however, some types of *E. coli* are pathogenic to humans, including the enterohemorrhagic *E. coli* (EHEC), of which *E. coli* O157:H7 is the most well-known member. *Escherichia coli* O157:H7 is a natural member of the gastrointestinal microflora of domestic ruminants (e.g., cattle, goats, sheep) and has been isolated from several wildlife species (e.g., deer, rabbits), and transmission may occur from any of these reservoirs. Although *E. coli* O157:H7 is primarily thought of as a foodborne pathogen, recent outbreaks have demonstrated other important routes that lead to human exposure, such as contaminated water and dust. Because *E. coli* O157:H7 represents a significant and widespread environmental health hazard, much effort has been directed toward the development of intervention strategies. Because of the relationship between ruminant animals, wildlife, and *E. coli* O157:H7, many of the strategies have focused on reducing levels in live animals. In this review article, we examine this virulent pathogen and explore several of the recently developed pre-harvest intervention strategies (including pro-biotic and anti-pathogen strategies, as well as environmental/management changes) and their potential to reduce zoonotic transmission of *E. coli* O157:H7 and other EHEC to man.


Ensuring the integrity of food and water has always been a major need of human populations. As the quantity and quality of food and water supplies have improved through the ages, the human species has prospered; malnutrition and disease have declined, and individual life expectancy has increased dramatically. Maintaining safe supplies of these essential commodities is one of the greatest achievements of human society, and it occurred more recently than most people realize. The idea that water could act as a vehicle by which pathogenic organisms could enter the human body was only proven in the 1850s through John Snow's investigation of the London cholera outbreak (Snow, 1849; Stanwell-Smith, 2002; Winkelstein, 1995). The regulation of food had a less scientific start. Published in 1906, Upton Sinclair's book *The Jungle* exposed the filthy conditions in United States slaughterhouses. The ensuing public outcry spurred the US government to pass in 1906 both the Food and Drugs Act and the Meat Inspection Act. Now, with food production and processing carefully regulated by the US Department of Agriculture (USDA) and Food Safety Inspection Service (FSIS) and water quality regulated at both the state and federal level by a number of organizations, it is generally conceded that the US has the safest food and water supply in the history of the world (Centers for Disease Control, 1999a). Both food and water safety, however, are again an important and growing concern in the medical and agricultural fields, and also in the eyes of the public and press.

Consumers expect and demand pure water and safe food and are increasingly wary of pathogens. The 1993 outbreak in the western United States associated with the Jack-in-the-Box fast food chain caused 732 illnesses, 195 hospitalizations, and four deaths, and it made *Escherichia coli* O157:H7 a household name (Centers for Disease Control, 1993; 1996b). In 1996, a multi-state outbreak traced to Odwalla brand unpasteurized apple juices

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resulted in at least 66 illnesses and one death, and it publicized the fact that *E. coli* O157:H7 is not just a "hamburger disease" (Centers for Disease Control, 1996b). Similarly, the 1999 Washington County Fair outbreak in New York State and the 2000 outbreak in Walkerton, Ontario, made clear to the public that, besides being a foodborne pathogen, *E. coli* O157:H7 is also a significant threat to the integrity of our water supplies. Ultimately, we need to remember that *E. coli* O157:H7 has traveled by unexpected vectors in the past, and it may do so in the future.

*Escherichia coli* strain O157:H7 annually causes an estimated 70,000 illnesses, 2,000 hospitalizations, and 60 deaths in the United States (Mead et al., 1999; United States Department of Agriculture/Economic Research Service, 2001). Although *E. coli* O157:H7 is classically considered a foodborne disease, transmission in water has recently been shown to be as critical. For example, of the 1,897 reported cases in *E. coli* O157:H7 outbreaks in the US in 1999, drinking water was implicated in 1,065 cases, swimming in 49 cases, and food (including fresh produce, ground beef, other meats, dairy, and other foods) in 604 cases (Centers for Disease Control, 2000; Peacock, Jacob, and Fallone, 2001). It has been estimated that *E. coli* O157:H7 costs the US economy $700 million per year in medical expenses, roughly $10,000 per sickened individual (Buzby et al., 1996). This average cost is a composite of patients who receive little or no treatment (<$10 per patient) to those that progress to the most serious forms of the disease, such as hemolytic-uremic syndrome (HUS), and require prolonged medical treatment ($50,000 to $100,000) (Buzby et al., 1996).

Enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC), which are primarily responsible for "traveler's diarrhea" and are a major cause of infant diarrheal disease and death in the developing world, have been reported to require $10^8$ to $10^9$ cells to infect healthy volunteers (Bell et al., 1994; Bolton, Crozier, and Williamson, 1996; Levine and Edelman, 1984). In contrast, the infective dose of *E. coli* O157:H7 is as low as 10 to 100 cells, a remarkably low number and similar to the infective dose of *Shigella* (Armstrong, Hollingsworth, and Morris, 1996; Nataro and Kaper, 1998). In addition, the complications of *E. coli* O157:H7 infections, which we discuss in detail later in this article, can be severe; for example, infection with *E. coli* O157:H7 is the most common cause of acute renal failure in children in the developed world (Fitzpatrick et al., 1991; Karch and Bielaszewska, 2001). The Centers for Disease Control (CDC) have estimated that 37% of *E. coli* O157:H7 patients are hospitalized, a rate comparable to the hospitalization rate for *Yersinia* (36%) and exceeded only by *Listeria* (88%) (Swartz, 2002). Together, the low infective dose and severity of the resulting illness have led to O157:H7 and similar *E. coli* strains being described as "the most dangerous enteric pathogens that clinical microbiologists in developed countries are likely to encounter" (Nataro and Kaper, 1998). Thus, it is not surprising that many research efforts are targeted at finding new ways to detect, control, and treat *E. coli* O157:H7. Despite the progress that has been made, outbreaks still take a significant annual toll in the US and worldwide and undermine public confidence in water supplies and food producers.

In this review, we address *E. coli* O157:H7 not only as a concern for the meat-processing industry, but as a widespread environmental pollutant. In addition to the history, identification, and biology of *E. coli* O157:H7, the difficulty of treatment of victims, and the known routes of exposure, we discuss current and prospective methods for controlling this pathogen in the environment and in the animal reservoir.

**History of *E. coli* O157:H7 Infection**

The first recognized outbreak of *E. coli* O157:H7 occurred simultaneously in Oregon and Michigan in 1982 and affected at least 47 people (Riley et al., 1983). After this outbreak, retrospective analysis of isolates revealed that O157:H7 had previously been isolated in 1975 from a California woman with bloody diarrhea, as well as in the United Kingdom between 1978 and 1982 and in Holland between 1974 and 1981 (Chart et al., 1991a; 1991b; Day et al., 1983; Johnson, Lior, and Bezanson, 1983; Riley et al., 1983). Sporadic cases of bloody diarrhea like those reported throughout the 1970s may represent early O157:H7 infection. Also, diarrhea-linked HUS was first reported in 1955, although there is no conclusive link to O157:H7 (Gasser et al., 1955). Because of this lag in recognizing O157:H7 as a new human pathogen, it is unlikely that the first O157:H7 case will ever be reliably identified. *Escherichia coli* O157:H7 infections have now been reported in over 30 countries on six continents (Cunin et al., 1999; Goldwater and Bettelheim, 2000; Irino et al., 2002; Mead and Griffin, 1998). Water was initially not considered a key vehicle that led to human O157:H7 exposure, although today it is increasingly recognized as such. The first recognized outbreak of waterborne O157:H7 occurred in 1989 in a rural water system in Missouri (Swerdlow et al., 1992). Because of the lack of awareness of water as a route of exposure, it is possible that earlier...
waterborne outbreaks occurred but were never identified. Since then, a number of highly publicized outbreaks, particularly those in Walkerton, Ontario, and the Washington County Fair in New York State, have highlighted this overlooked mode of transmission (Centers for Disease Control, 1999b; O'Conner, 2002).

In response to growing concern about *E. coli* O157:H7, in the early 1990s the FSIS established an end-product testing protocol for ground beef in particular as a rapid response to the high incidence of ground beef–related food poisoning that resulted in death. The implementation of the FSIS “zero tolerance policy” in 1994 has reduced large-scale outbreaks, but it has cost the meat industry dearly in both capital and public perception in the wake of repeated large-scale ground beef recalls. These stop-gap regulatory measures were hindered by our lack of basic understanding of the organism, its role in the gastrointestinal microbial ecology, the low numbers needed for infection, the lack of a sensitive detection method, and finally, the inability to test meat lots in a timely, quantitative manner, because procedures were poorly understood. More recently, the FSIS has implemented new, more precise protocols that can detect O157:H7 much more effectively; however, the zero-tolerance policy remains in effect for the foreseeable future.

**Detection of O157:H7 in the Patient and the Environment**

A number of methods of varying complexity and applicability are available to detect O157:H7 in the patient, in food, and in environmental samples. Detection of O157:H7 in the patient may at first seem extraneous, as there are no treatments available (as discussed later in this article); however, identifying its presence is key to tracking the causative agent and to controlling outbreaks. Perhaps more important than detection in the patient is the ability to detect O157:H7 quickly and precisely in food and environmental samples; however, low cell numbers in these samples result in more complex and less precise detection methods. The development of faster and more sensitive detection methods has been driven by the needs of epidemiologists and by the USDA-FSIS regulation that meat containing one colony-forming unit (CFU) of O157:H7 per 25 g of meat is adulterated and must be condemned or recalled. The intensity of work in this area is perhaps most easily demonstrated by the more than 50 posters presented at American Society for Microbiology conferences over the last two years.

Detection methods fall into a few broad categories: morphological examination of bacteria cultured on Petri plates to identify colonies with the characteristics of O157:H7; use of the polymerase chain reaction (PCR) to probe DNA for specific genes carried by O157:H7; detection of bacterial components, including the specific antigens and Shiga-like toxins (Stx) produced by O157:H7; and, in patients, the detection of antibodies to the bacteria. Until recently, the most used method was plate detection, because of its simplicity. This method is based on the fact that most O157:H7 strains are unable to ferment sorbitol, in contrast to most other *E. coli*. Therefore, O157:H7 strains form colorless colonies with smoky black centers on sorbitol-containing MacConkey’s agar supplemented with cefsulodin and tellurite; however, 6% to 25% of the sorbitol non-fermenting *E. coli* may be of other serotypes, many of them non-pathogenic (Farmer and Davis, 1985; March and Ratnam, 1986). Enhancements to the basic sorbitol-containing MacConkey’s medium have been developed that improve its selectivity (Okrend, Rose, and Latuada, 1990). Another plate method used extensively in laboratories is the β-glucuronidase catalyzed hydrolysis of 4-methylumbelliferyl-o-glucuronide. The absence of this activity (non-enterohemorrhagic *E. coli* fluorescence) is indicative of O157:H7 (Thompson, Hodge, and Borczyk, 1990). Although they are often used in clinical settings, plate detection methods have largely been superseded by newer, more precise techniques, including the PCR, Stx detection, or antigen agglutination tests. Environmental samples, however, pose another compounding problem: low cell numbers coupled with imprecise identification methods. To overcome this, the current USDA-FSIS method involves repeated enrichment steps followed by 10 biochemical steps that make the methodology slow (typically three days) and expensive (Dey and Lattuada, 1998). Two scientists for the Agricultural Research Service have developed an improved methodology to increase the sensitivity of plate detection methods specifically with water samples. Raw or pre-concentrated water samples are mixed with immunomagnetic beads coated with anti-O157:H7 antibodies. O157:H7 cells present in the sample specifically bind to the beads and are subsequently treated with a second antibody that allows the number of bound cells to be quantified by chemiluminescence. This methodology is sensitive enough to detect 25 cells per milliliter, and with a sample pre-concentration step, 25 cells per 100 ml (Shelton and Karns, 2001).
The most sensitive and specific methodology now available is the PCR. Specific PCR primers can be purchased to detect either of the Stx genes or to detect the genes for the O157:H7 surface antigens. These methods are rapidly improving. A recent step forward was the development of a PCR assay to detect the O157:H7 serotype and the eight critical toxin genes in one reaction (Wang, Clark, and Rodgers, 2002). Until recently the drawback of this method is that the direct PCR performed poorly in raw environmental or stool samples because of inhibitory components of the sample, and therefore its utility was effectively limited to pure cultures. Methods are improving, however, and a recent paper suggested that the PCR can be used directly with fecal samples (Belanger et al., 2002).

Today, a twofold approach using immunomagnetic bead separation and the PCR is the benchmark methodology being used. Immunomagnetic bead separation is considered the gold standard in identifying O157:H7 (or other enterohemorrhagic E. coli), with positive samples being subject to genotype analysis via pulse-field gel electrophoresis and the CDC's PulseNet system. The PCR is also used to confirm the presence of specific genes and virulence factors such as Stx 1 and 2, and eaeA (Chapman, Wright, and Siddons, 1994; Elder et al., 2000; Keen et al., 2003).

The presence of the O157 lipopolysaccharide and H7 flagella antigens can be detected by enzyme-linked immunosorbent assay (ELISA) from samples cultured on selective media such as sorbitol-containing MacConkey’s or by direct sampling of the suspected food product, patient stool, or environmental sample. Antiserum to both antigens is widely available commercially, as are many kits based on this immunoassay. Similar immunoassay techniques have been used to directly detect the Stx. Meridian Diagnostics (Cincinnati, Ohio) has recently developed an Immunocard Stat kit that can detect the toxin in feces quickly and at relatively low concentrations, as well as an ELISA-based toxin detection kit (Premier EHEC) (Kehl et al., 1997; Mackenzie et al., 1998).

One final assay that has been used to determine O157:H7 presence involves detection of the Stx activity. The sensitivity of kidney cells from green monkeys (vero cells) to Stx of O157:H7 has provided a highly sensitive method for toxin detection in both patient and fecal food samples, especially when the causative agent is difficult to isolate (Konowalchuk, Speirs, and Stavric, 1977). Its quantitative abilities are limited, however, and its use is restricted by its labor-intensive, slow, and extremely costly nature. As the Stx are rarely found at detectable levels in the environment, this method is not considered useful for these samples.

**Pathology of E. coli O157:H7**

Humans and other mammals carry thousands of bacterial species in their guts, including many E. coli strains. The vast majority of these bacteria, including both E. coli and other species, are harmless commensal organisms that supply us with a range of essential dietary nutrients such as vitamin K, biotin, and the B-complex vitamins; however, a few of these bacteria, such as O157:H7, are highly pathogenic to some of their host species.

Strains of *E. coli* that cause diarrhea are classified based on virulence, clinical symptoms, mechanism of pathogenicity, and the presence of particular cell-surface antigens. Confusingly, in much of the literature O157:H7 is interchangeably referred to as a type of STEC (Shiga toxin–producing *E. coli*) or VTEC (Vero toxin–producing *E. coli*). These describe the ability of a number of *E. coli* strains to produce two related and potent cytotoxins of the Shiga toxin family that kill African green monkey kidney cells (vero cells). This group of toxins is also called EHEC (enterohemorrhagic *E. coli*), because of their ability to cause bloody diarrhea in humans. First reported in the early 1980s, *E. coli* O157:H7 is differentiated from other EHEC strains based on the expression of two particular cell-surface antigens—the O lipopolysaccharide 157 and a particular flagellar protein, H7 (Riley et al., 1983; Wells et al., 1983). Since its initial recognition, O157:H7 has been identified as the dominant EHEC strain in the US (Armstrong, Hollingsworth, and Morris, 1996; Mead et al., 1999).

The Stx genes carried by EHEC strains are located in degenerate phage genomes integrated into the bacterial genome, and they were apparently originally "hijacked" by temperate lambdoid phages from *Shigella dysenteriae* (Plunkett et al., 1999; Wagner et al., 2001). The Stx proteins are n-glycosidases that bind to the Gb3 (Globotriaosylceramide) cell surface receptors, resulting in their endocytosis. Once inside the eukaryotic cell, the A1 component of each toxin acts upon the ribosome to remove an adenine from the 28S rRNA, thus halting protein synthesis (Boyd and Lingwood, 1989; Nataro and Kaper, 1998; Su and Brandt, 1995; Waddell et al., 1988). These toxins not only cause local physiological problems but also enter the bloodstream and can be transported throughout the body. In humans, both the intestinal endothelium and the kidney have high concentrations of
Gb3 receptors, and therefore these tissues are particularly vulnerable to the effects of the toxin. It has recently been found that infants have higher levels of Gb3 receptors in their brain tissues than do adults, suggesting another avenue of vulnerability in the very young (Kita et al., 2003). Shiga toxin production is not the sole virulence factor; other key components include (1) cell adherence, (2) survival in the intestine, (3) low infective dose, and (4) acid tolerance (Armstrong, Hollingsworth, and Morris, 1996; Griffin and Tauxe, 1991). In a 1994 outbreak associated with dry-cured salami, fewer than 10 cells may have sickened some individuals (Strachan, Fenlon, and Ogden, 2001; Tilden et al., 1996). Like many E. coli, O157:H7 can survive a pH as low as 2.0 and so pass through the natural antimicrobial barrier provided by the human stomach; this acid tolerance contributes to the low infective dose.

Once it is resident in the human gut, O157:H7 reproduces rapidly, with a typical incubation period of three days; however, incubation may be between two and eight days, depending on the ingested load and patient susceptibility. Although some people may show no ill effects from an O157:H7 infection, in others infection results in severe illnesses, including full hemorrhagic diarrhea (possibly progressing to HUS), thrombotic thrombocytopenic purpura (TTP), and death (Figure 1). Patients initially complain of non-bloody diarrhea and violent cramps, but within one to two days, up to 70% begin to pass blood, and 30% to 60% experience severe vomiting. Most recover in five to ten days and do not require specific treatment; however, up to 20% progress to HUS, with most of these cases seen among young children and the elderly. HUS comprises three individual physiological effects: (1) microangiopathic hemolytic anemia—the lysis of red blood cells within the vascular system, (2) thrombocytopenia—reduced levels of platelets and other clotting factors, and (3) renal failure. The mortality rate for patients who progress to HUS is in the order of 7% in children and as high as 87% among the elderly (Callaway et al., 2001). Severe complications, including stroke, seizures, coma, blindness, paralysis, pancreatitis, and diabetes mellitus, affect up to 50% of surviving patients (Mead and Griffin, 1998). Thrombotic thrombocytopenic purpura (bleeding from the capillaries of the skin and mucous membranes) is a syndrome that mainly affects the elderly. Some consider it a component of HUS, and others view it as a distinct syndrome.

At present, there are limited therapeutic treatments for victims of E. coli O157:H7 infection except to manage the complications of cellular damage with supportive therapy to maintain fluid and electrolyte balance, reduce hypertension, and control anemia (Nataro and Kaper, 1998; Su and Brandt, 1995). Patients with HUS require intensive medical care that may include blood transfusions and/or kidney dialysis. Chemotherapeutic intervention has not been proven effective and antibiotic use is contraindicated, especially in children, because antibiotic use has been reported to increase the likelihood of progression to HUS (Wong et al., 2000). The literature is contradictory on this issue, with the direct comparison of studies difficult because of variations in the antibiotics used, the severity of the infection, and the point in the infection process when chemotherapeutic intervention was begun. Some researchers have reported success in treating O157:H7 patients with the antibiotics fosfomycin, rifampicin, doxycycline, some macrolides, and aminoglycosides. For example, a retrospective study found that fosfomycin, if administered in the first two days of the infection, reduced the progression to HUS (Emiko et al., 2000; Wong et al., 2000; Yoh, Frimpong, and Honda, 1997). Other researchers have observed increased levels of Stx, increased progression to HUS, and increased mortality when broad-spectrum antibiotics such as β-lactams and quinolones have been...
used at various stages of infection (Carter et al., 1987; Kimmitt, Harwood, and Barer, 1999; Wong et al., 2000). The observed deleterious effects of antibiotic treatment can be explained by our current knowledge of bacterial physiology, toxin production, and the O157:H7 infection process. Stx are produced intracellularly, and when exported outside the cell often remain in close association with the cell surface, so antibiotic-induced cell lysis could lead to a concentration spike of Stx in the body and cause much greater systemic damage and a greater risk of HUS. Furthermore, when the bacterial “SOS” response is triggered (e.g., by antibiotics), previously silent regions of integrated bacteriophage genomes are expressed at high levels, including the regions encoding the Stx production genes (Neely and Friedman, 1998; Plunkett et al., 1999; Wagner et al., 2001). In vitro and in vivo animal studies have shown increased toxin production in response to various antibiotics (Carter et al., 1987; Kimmitt, Harwood, and Barer, 1999; Wong et al., 2000; Zhang et al., 2000). Based on all available findings, none of which are conclusive, “no action” is considered the best measure and the CDC now actively discourages the use of antibiotics in E. coli O157:H7 patients. The only clear therapeutic advice today, 20 years after the first patients were treated for O157:H7, is for doctors to avoid the use of intestinal antimobility agents; this is perhaps the most graphic indication of the complexity of the problem we are presented with.

Potential New Treatments for Patients

Some new technologies designed to limit the effects of Stx have been developed, but none have yet completed clinical trials. Synsorb Pk, a conjugate of Gb3 oligosaccharides and silica that specifically binds Stx in vitro, entered stage III clinical trials and was on the Food and Drug Administration (FDA) FastTrack (Armstrong et al., 1995; Armstrong, McLaine, and Rowe, 1998; Rafter et al., 1999). Initial clinical trials on 152 children aged 6 to 15 showed a great deal of promise; Synsorb reportedly reduced the rate of HUS from 17% (placebo) to 7% with no side effects when administered within three days of the onset of symptoms, but it only bound Stx still in the gut; any Stx already in the bloodstream were not inactivated. Subsequently, these data, as presented by Synsorb Biotech Inc., led to a warning from the FDA about their use of statistical analyses and presenting misleading statements (Food and Drug Administration, 2002a). Further, the most recent trial data suggest that the product has little therapeutic action in reducing the severity of the disease when supplied orally to sickened individuals; this probably led to the press release from Synsorb Biotech Inc. that the product will not see market in the near future (Trachtman et al., 2003). A product dubbed SUPER TWIG also uses Gb3 oligosaccharides to bind Stx and is specifically designed to be administered intravenously. A preliminary study has shown that it inhibits the uptake of the Stx into target cells and successfully protects mice from an otherwise fatal dose of O157:H7; however, further testing is needed to determine whether this product is safe and effective for human use (Nishikawa et al., 2002).

An alternative approach to human O157:H7 treatment has been to prevent the Stx from binding to the Gb3 receptors and so decrease their effects. It has been suggested that swainsonine, a toxin found in locoweed that inhibits glycosylation of the Gb3 receptor, could prevent binding of the Stx to their receptors (Droleskey et al., 2002). The inhibition by swainsonine is reversible; thus no permanent damage is caused by its consumption. No conclusive data on the use of this compound or similar methods involving the use of tunicamycin have so far been presented (Jacewicz et al., 1989).

Efforts at producing a human vaccine to prevent infection and the physiological damage caused by Shiga toxin–producing E. coli strains have focused on two main areas: vaccines targeted to the toxins, and vaccines targeted to the bacterium. Initially it was thought that vaccination would not work, because O157:H7 infection does not reliably protect the individual from subsequent infections (Karmali, 1998; Mead and Griffin, 1998; Robson, Leung, and Miller-Hughes, 1993; Siegler et al., 1993; Tarr, 1998). Recent research has shown that O157:H7-sickened individuals do produce antibodies to Stx, surface antigens, other proteins produced by the bacteria, and whole bacterial cells (Li et al., 2000); however, other work suggests that, as with tetanus, a single illness does not always stimulate enough antibody production to protect from subsequent infections, so “boosters” may be needed (Karmali, 1998).

One approach to vaccine therapy has been to raise antibodies to cell-surface antigens and thereby elicit an immune response in the intestine that would prevent gut colonization. For example, Konadu et al. (1998) tested three dead conjugate vaccines on 87 adults, with all showing a four-fold increase in IgG levels in four weeks (Konadu et al., 1999). Elevated IgG levels were maintained for a further 22 weeks. Live recombinant vaccines that express O157:H7 surface antigens have also been produced in the laboratory. These have been shown to stimulate an immune response and antibody production in mice and
we would then recommend 0157:H7 vaccinations to approach to eliminating the 0157:H7 problem. Because addressed before vaccination can be considered a viable suggested they would move quickly to begin human trials vaccine protects against the toxin; however, the researchers and inactivate the Stx to produce a toxoid vaccine. The volunteers to produce Stx antibodies. These antibodies could then be purified and administered to 0157:H7 infection in general, but specifically with application to young children. The human immune system does not have the ability to make antibodies to polysaccharide antigens in the first two years of life (Konadu et al., 1998). It does, however, produce antibodies in response to proteins. The researchers hope that by conjugating the polysaccharide antigen to a protein antigen, the vaccine will stimulate the infant immune system to produce anti-0157 antibodies. Sub-lethal strains of Salmonella that express the O157 antigen have also been suggested as an effective way to raise O157-specific antibodies in the gastrointestinal mucosa and so prevent gut colonization. In mice, these experiments have had some encouraging results (Conlan and Perry, 1998; Conlan et al., 1999; 2000).

Researchers are also attempting to create a vaccine that stimulates antibodies to the toxins themselves. Not only could such a vaccine be used to treat O157:H7 patients, but it could also be administered to stimulate healthy volunteers to produce Stx antibodies. These antibodies could then be purified and administered to O157:H7 patients to bind the Stx and reduce cellular damage. Researchers have also tried to develop methods to purify and inactivate the Stx to produce a toxoid vaccine. The challenge is to modify the toxin enough to render it harmless, while retaining its antigenic effects (Keusch et al., 1988). Preliminary tests in mice showed that the toxoid vaccine protects against the toxin; however, the researchers suggested they would move quickly to begin human trials in 2000. To date, no further details of the study have been presented.

Besides the technological hurdles, several issues need to be addressed before vaccination can be considered a viable approach to eliminating the O157:H7 problem. Because people of all ages are affected by O157:H7, targeting vaccination to a single age group would provide incomplete protection. The question also arises of whether we would then recommend O157:H7 vaccinations to visitors to the US. Although the US food supply is the safest in the world, a large-scale vaccination campaign would raise concerns about its safety in the eyes of both domestic and foreign consumers (Tauxe, 1998).

A number of researchers are working to develop antibody therapies, that is, Stx-binding antibodies that would neutralize the toxin in vivo. This work has centered on antibodies against Stx2 because of its lower lethal dose (400-fold compared to Stx1 in mice) and the fact that Stx2-producing strains have been suggested to be more clinically important in the progression to HUS (Boerlin et al., 1999; Ostroff et al., 1989; Tesh et al., 1993). Donohue-Rolfe et al. (1999) showed that antibodies against Stx2 administered to O157:H7-infected pigs reduced the death rate from 12/16 in the controls to 4/30 in antiserum-treated pigs. Concerns about using animal antibodies in humans and the associated potential human anti-animal antibody response, based on the differences in antibody structure, have led researchers to genetically engineer the animal antibodies to resemble human antibodies. In animal tests, “humanized” Stx antibodies produced by two strains of mice were still able to protect against a lethal dose of O157:H7 (Edwards et al., 1998). This approach has been adopted by other researchers and continues to show promise. Yamagami et al. (2001) showed that TMA-15, a humanized mouse anti-Stx2 antibody, markedly improved mouse survivability from 0/20 (0 mg TMA-15 kg−1) to 20/20 (1 mg TMA-15 kg−1) if administered 24 hours post-infection (Kimura et al., 2002; Yamagami et al., 2001).

In conclusion, while research continues to hunt for effective treatments to combat O157:H7 infection, we are still helpless to prevent severe illness once a person is exposed. We can, however, prevent illness by limiting exposure itself. To do this effectively, we need to examine the known routes of infection and consider how each might be blocked.

Routes of Exposure

Although there are many routes for O157:H7 to infect humans, more than 75% of US outbreaks of EHEC hemorrhagic colitis have ultimately been linked to cattle or products derived from cattle such as contaminated meat, and direct or indirect contact with cattle or other ruminants or their wastes (see Figure 2) (Centers for Disease Control, 1993; Chapman, 2000; Gage, 2000; Pritchard et al., 2000; Strachan, Fenlon, and Ogden, 2001; United States Department of Agriculture/Animal and Plant Health Inspection Service, 1997). O157:H7 and related strains are widespread and have been isolated not only...
Cattle O157 carriers

Environmental Reservoirs

O157:H7 Free Cattle

Other Domestic Reservoirs

Feces

Farm Run-off

Improper composting

Contaminated Crops

Water/Soil

Improper water chlorination

Uninfected Humans

Meat Products

Improper cooking

Dairy Products

Improper pasteurization

Infection

Diseased Humans

Direct contact

Exposure to contaminated human waste

Figure 2. Routes and reservoirs of E. coli O157:H7. The major reservoir that leads to human exposure is cattle feces, via five different routes.

from human outbreaks around the world, but also from a number of different animal species, including horses, pigs, wild rabbits, seagulls, pigeons, rats, flies, swine, dogs, and cats (Beutin et al., 1993; Johnsen et al., 2001; Nakazawa, Akiba, and Samegima, 1999; O'Brien and Kaper, 1998; Wallace, Cheasty, and Jones, 1997). O157:H7 is also often found in the environment, in both standing and running water as well as in the soil. While it is known that O157:H7 can persist in the environment outside of the mammalian gut, the duration of this persistence is not yet fully known and is probably highly variable. Various studies have found that O157:H7 survives for about 40 to 50 days in cattle feces kept at 20° to 30° C, but Kudva, Blanch, and Hovde (1998) found viable cells after at least 21 months in a manure pile from experimentally inoculated sheep (Fukushima, Hoshina, and Gomyoda, 1999; Jiang, Morgan, and Doyle, 2002; Kudva, Blanch, and Hovde, 1998; Wang, Zhao, and Doyle, 1996). Jiang, Morgan, and Doyle (2002) found that O157:H7 survived in manure-amended soil for more than 200 days when held at 15° C and at 21° C. In the laboratory, Gagliardi and Karns (2002) found that the presence of plant roots, a high clay content, and freezing temperatures all extend the survival of O157:H7 in soil, with frozen soil maintaining a viable O157:H7 population for at least 500 days. Furthermore, experimental results suggest that O157:H7 may survive longer in the field than in laboratory conditions meant to mimic field conditions, so lab results should be interpreted cautiously (Kudva, Blanch, and Hovde, 1998). McGee et al. (2002) showed that O157:H7 can survive in farm water for at least two weeks, and in some conditions it was still detectable at high levels for more than a month. Warburton et al. (1998) found survival times of more than 200 days in spring water, and greater than 300 days in sterilized water (both distilled and mineral water); however, the ultimate source of these organisms is the mammalian gut, and ruminant animals (cattle, sheep, deer) are considered the major reservoir (Besser, Griffin, and Slutsker, 1999; Bielaszewska et al., 2000; Chapman et al., 1993; Fischer et al., 2001; Gansheroff and O'Brien, 2000; Kudva, Hatfield, and Hovde, 1996; Rasmussen et al., 1993). A great deal of epidemiological research has focused on the incidence of O157:H7 in cattle. Initial studies employed a traditional, culture-based methodology and generally found from 1% to 3% of cattle to be colonized by O157:H7 (Faith et al., 1996; Hancock, Besser, and Rice, 1998; Meyer-Broseta et al., 2001); however, the recent introduction of molecular methods using immunomagnetic bead separation techniques have steadily increased the accepted incidence from tenfold to 100-fold (Chapman, Wright, and Siddons, 1994). Current estimates indicate that approximately 30% of all cattle in the US shed O157:H7 in their feces, and during summer months this level may be as high as 80% in feedlot animals (Elder et al., 2000; Zschock et al., 2000). In a recent study, as many as 80% of feedlot pens tested positive for E. coli O157:H7 (Khaitsa et al., 2003). In other recent studies, however, using slightly different methodologies the fecal incidence was much lower than the incidence of E. coli O157:H7 on the hides (6% versus 60%) (Barkocy-Gallagher et al., 2003). Therefore, it appears that O157:H7 is a more
common member of the microbial ecosystem than was previously thought.

From these and other data, it is believed that the ruminant colon provides a major site for the persistence and proliferation of *E. coli* O157:H7 (Grauke et al., 2002). It has been suggested that ruminant animals routinely inoculate each other with their gastrointestinal microorganisms via cud chewing and pasture grazing. Horizontal transmission of O157:H7 is thought to occur by the same process, as O157:H7 will appear sporadically in the feces of individual animals throughout a herd, and the hides and mouths of cattle have also been shown to be reservoirs of O157:H7 (Keen and Elder, 2002). Hancock et al. (2001) have suggested another explanation for the appearance of identical strains of O157:H7 on widely separated farms; they found that commercial cattle feed routinely contains *E. coli* O157:H7 (0.5% of samples). Further, in an earlier study Lynn et al. (1998) found that the bacteria can multiply in wet feed, such as certain grain mixes. If such environmental replication occurs more rapidly in warmer temperatures, this could contribute to the seasonal nature of O157:H7 prevalence.

Meat Contamination Route

The first recognized *E. coli* O157:H7 outbreak was linked to ground beef in 1982; since then, outbreaks have been linked to various meats including meatloaf, sirloin tri-tips, and venison jerky. Contamination has also been found in retail pork, lamb, and poultry, and in raw caribou meat; however, ground beef and other beef products still account for most of the meatborne outbreaks that occur in the US (Centers for Disease Control, 1999c; 2000; 2001; 2002; Keene et al., 1997).

Meat contamination begins at the slaughterhouse, but its roots are much earlier. Fecal populations of O157:H7 are positively correlated with carcass contamination levels, but this does not necessarily mean that feces are deposited directly on the carcass during slaughter; the hide can also act as a reservoir for pathogens (Elder et al., 2000). It has been suggested that bacterial transfer during hide removal is the major route that contaminates the carcass and subsequent meat products (Barkocy-Gallagher et al., 2003; Elder et al., 2000; Reid et al., 2002).

No matter which vehicle brings contamination into the slaughter plant, if *E. coli* O157:H7 is deposited on the carcass during slaughter, it can become thoroughly mixed into products during further processing, resulting in contamination of entire lots of ground beef that must be condemned (destruction of the matter) or reprocessed (thermally inactivated) (Koohmaraie, 2004). Recalls of ground beef have been on the scale of millions of pounds. The extent of these recalls is in part a consequence of changes over the last 50 years in the nature of meat production at both the farm and the processing plant. The number of individual farms producing beef, for example, has been massively reduced. From the 1970s to the 1990s, there was a marked consolidation of meat-producing feedlots, with the number of individual feedlots falling approximately 50% over that 20-year period, and a concomitant 300% increase in the size of individual feedlot herds, increasing the potential for cross-contamination (Good, 1997).

The industrial-level processing of beef has similarly changed in scale and structure. Modern meat processing is a high-technology, high-speed practice. As the demand to reduce costs increases, meat manufacturers have responded in three ways: (1) consolidation of plants, (2) increased line speeds, and (3) reduced worker training to reduce employee costs (Eisnitz, 1997). As a result of these changes, in the last 20 years the total number of plants has decreased 60%; 12 plants now process 54% of all cattle killed in the US (United States Department of Agriculture, 1999). It has been suggested that increased volume and line speeds coupled to a reduction in worker skill leads to increased contamination of the meat (Eisnitz, 1997). Meat cutting is a precise process that requires the rapid assessment of a carcass and then exacting cuts. As can be imagined, any error leads to the contamination of not only the meat cut from that carcass, but also subsequent carcasses if the tools are not cleaned properly as required by FSIS regulations.

In the large-batch ground beef processes operated today, lot sizes are on the ton (and often the tens of tons) scale. The raw materials are drawn from many initial points, and it is not uncommon for ground beef to come from three to four suppliers (Koohmaraie, 2004). The raw boxed beef received at a processing plant from those suppliers may also have originated from many different origination points. This problem used to be compounded by a procedure called reworking, where any leftover meat, such as misshapen patties, was reworked into the following day’s production—also carrying along any bacterial contamination. Thus, if the beef ground on day one came from a contaminated lot, beef produced down the line could also be contaminated. In many instances, the level of contamination is insignificant, but
the low infectious dose of \textit{E. coli} O157:H7 negates any advantages of dilution.

All these factors have combined in the past to create the perfect conditions for contamination on a massive scale. For example, in the summer of 1997, Hudson Foods began a recall of beef patties that had tested positive for an O157:H7 strain that was directly linked to the strain in an outbreak in Colorado (June 14 to July 14). Initially (August 15), the USDA called for Hudson to recall 20,000 pounds of meat, but this recall quickly expanded from a local single state incident to a national recall involving 25 million pounds of patties by August 21. The size of this outbreak illustrates many points—particularly the vast scale of processing and the potential for illness on a nationwide basis. The problem at Hudson was in part due to improper practices. The company had an onsite USDA inspector and followed most guidelines, but a lack of documentation and control in two areas was key: (1) the use of raw materials, and (2) carryover for reworking. The process of reworking did not cause the outbreak but was a factor in its scale by leading to the contamination of several days of processed meat. In light of the scale and scope of this outbreak, many meat processors discontinued reworking, and the USDA/Hazard Analysis Critical Control Point System (HACCP) developed new guidelines associated with this and other plant practices, which the beef industry adopted in their “Best Practice” documents (Anonymous, 1997; 1998a; 1998b). Today, misshapen patties are reworked within two hours of production and no ground beef is carried over from day to day (Koohmaraie, 2004).

Much of the research over the last few years has shown that cattle hides and feces are the predominant sources of \textit{E. coli} O157:H7 on beef carcasses. In spite of a significant investment in research by the government and the meat and livestock industry, there are currently no effective preharvest interventions being practiced. Hence, the meat industry has focused on intervention procedures to minimize the transfer of pathogens from hides onto carcasses during hide removal, as well as interventions that remove or kill pathogens if they are transferred to the carcasses. In spite of all of these efforts, outbreaks associated with ground beef that is improperly cooked prior to consumption continue to occur. Consequently, the meat industry has begun to implement a process called Test-and-Hold (Koohmaraie, 2004). Test-and-Hold is a process by which the processor samples raw ground beef materials and/or ground beef, and subsequently tests for \textit{E. coli} O157:H7. The product is held pending the results of this testing. If negative, the product is released. If positive, the product is diverted to a cooked product, but often the product is rendered and hence it does not enter commerce. The Test-and-Hold program is an expensive procedure for the beef industry, costing tens of millions of dollars each year. It is encouraging to see that through the first two-and-one-half months of 2004, USDA-FSIS verification sampling resulted in testing in excess of 1,000 samples of ground beef for \textit{E. coli} O157:H7, with no positives (Koohmaraie, 2004). Although testing alone is not (and never has been) the answer, it is a valuable tool as a component of an overall control program.

Other Food Routes

Fecal contamination of carcasses during slaughter is not the only route by which O157:H7 can enter the human food chain. In 1988, Morgan and co-workers found a link between an O157:H7 outbreak and handling of raw potatoes; since then, several other outbreaks have emphasized the potential for raw fruits and vegetables to carry the disease (Morgan et al., 1988). For example, in July 2002, an outbreak was linked to contaminated romaine lettuce. Most of the victims were children participating in a cheerleading camp in Washington State (Food and Drug Administration, 2002b). In total 32 people were sickened, seven were hospitalized, and one developed HUS. The FDA issued a nationwide alert warning consumers not to eat Spokane-brand romaine lettuce, but it was never determined how the lettuce became contaminated (Food and Drug Administration, 2002b).

A more puzzling outbreak in Maine in 1992 illustrates that the source of infection may not be initially obvious, and that any individual can become a victim of O157:H7. The first patient in this outbreak was a vegetarian whose diet consisted primarily of vegetables from her own garden. Subsequent investigation revealed that she fertilized her garden with cattle manure, and O157:H7 was isolated from the soil in her garden. Presumably, she contracted O157:H7 by eating inadequately washed, manure-contaminated vegetables. The other cases in this mini-outbreak (three children, one of whom died) probably occurred through person-to-person contact (Cieslak et al., 1993). As mentioned earlier in this review, O157:H7 can persist in manure and soil for months, perhaps years, and fertilizing with contaminated manure may be a significant factor in the contamination of produce. Disturbingly, recent research has shown that even thorough washing may not remove O157:H7 from produce. Solomon, Potenski, and Matthews (2002) found
that O157:H7 could migrate from contaminated soil or irrigation water, through the roots, to leaf tissues of lettuce, creating an internal reservoir that no amount of washing will eliminate. Itoh et al. (1998) showed that hydroponically grown radish sprouts similarly become internally contaminated when grown in contaminated water. It seems likely that this pathway may be common to many plants.

Produce may also be contaminated in other ways. Janisiewicz et al. (1999) showed that fruit flies can transmit E. coli to fruit. Although the amount initially carried may be too small to cause human disease, they also demonstrated that O157:H7 can grow rapidly on and in apple tissue, a finding that was later confirmed by Dingman (2000). Thus, even food that is kept from contact with manure, manured soils, and contaminated water can carry O157:H7, emphasizing again the importance of reducing O157:H7 generally in the environment.

In summary, although improperly cooked meat was the leading cause of outbreaks throughout the early 1990s, this has changed. Now other foods, including fresh produce, fruit juices, salted salmon roe, raw milk, and fresh cheese curds, are responsible for a significant number of the food-related outbreaks seen every year (Besser et al., 1993; Beuchat, 1995; Itoh et al., 1998; Jackson et al., 1998; Makino et al., 2000; Peacock, Jacob, and Fallone, 2001). The 1996 outbreak caused by Odwalla apple juice is one of the best-known examples, and it holds some clear lessons. Prior to the outbreak, managers at Odwalla had relaxed standards for sanitizing fruit, begun accepting more blemished fruit, and focused product testing on shelf-life rather than bacterial contamination (Henkel, 1999). Company executives said that, like many others in their industry, they simply did not realize that E. coli O157:H7 could live in apple juice. This oversight led to at least 66 illnesses, 15 cases of HUS, and one death. It also cost the company a $1.5 million criminal fine and a multi-million-dollar settlement with the families of the affected children (Belluck, 1998). Consumers, regulators, and the food industry cannot afford to dismiss O157:H7 as a concern of the beef industry alone.

Waterborne Contamination

Outbreaks of E. coli O157:H7 have also been traced to water: municipal water supplies, private wells, swimming and paddling pools, and lakes. The first identified waterborne outbreak in the US occurred in Burdine Township, Missouri, in the winter of 1989-1990. This outbreak, which sickened over 200 people and killed four, was traced to the unchlorinated municipal water system, which was probably contaminated by seepage from sewer lines or sewage overflows (Swerdlow et al., 1992). Contamination of an unchlorinated municipal water supply by deer and elk feces apparently caused a 1998 outbreak in Wyoming that sickened 157 people, four of whom developed HUS (Olsen et al., 2002).

Private wells have also been responsible for illnesses in North America and Europe. An isolated case on an Ontario farm in 1995 apparently resulted from manure contamination of the farm’s well (Jackson et al., 1998). A 1999 outbreak that sickened more than 900 people and killed two was linked to an unchlorinated well that supplied water to the Washington County Fair in New York State (Bopp et al., 2003; Centers for Disease Control, 1999b). This outbreak illustrated another key facet of foodborne infections, the presence of a second agent. Of the 15,921 persons who reported some form of diarrhea, stool cultures showed 116 persons with O157:H7, 32 that had only Campylobacter jejuni, and a further 13 who were co-infected with O157:H7 and C. jejuni (Centers for Disease Control, 1999b). An outbreak in the Highland Region of Scotland in 1999 that infected six people was linked to an untreated private well apparently contaminated by the feces of O157:H7-carrying sheep (Licence et al., 2001).

Paddling and swimming pools and swimming beaches at lakes have also been implicated in O157:H7 infection. In 1991, at least 21 people were infected with O157:H7 after swimming in a lake near Portland, Oregon (Keene et al., 1994). Seven people (six children and an adult) fell ill in a 1993 London outbreak linked to paddling pools (Hildebrand et al., 1996). In 1995, twelve children were sickened after swimming in a lake in Illinois, and in Georgia in 1996, 18 people fell ill in an outbreak associated with a private swimming pool (Centers for Disease Control, 1996a; Friedman et al., 1999). In May of 2000, an outbreak in Walkerton, Ontario, dramatically demonstrated the potential for contaminated drinking water to cause a major O157:H7 outbreak. For many years, the local Public Utilities Commission operators had violated treatment and testing guidelines; they routinely failed to perform daily residual chlorine tests, falsified records of those tests, deliberately mislabeled microbiological test samples, and knowingly used levels of chlorine below the standards of the Ministry of the Environment. In 1998, a Ministry of the Environment inspection had revealed some of the inadequacies of the...
Public Utilities Commission’s practices, but those practices were not significantly corrected as a result of the inspection report. In late April 2000, a farmer spread manure on his fields using commonly accepted best management practices. Heavy rains from May 8 to May 12 transported a heavy load of fecal contaminants from his fields to one of the wells supplying the town of Walkerton, but because of the practice of omitting chlorine residual tests the heavy contamination went unnoticed. On May 18, two children with bloody diarrhea were admitted to the local hospital, and twenty others were absent from school. The next day, a retirement home experienced an outbreak of enteric illness, and many other Walkerton residents began experiencing symptoms including bloody diarrhea, stomach pain, and nausea. The first death from the outbreak occurred on May 22. In all, the water contamination resulted in 2,300 illnesses, 27 cases of HUS, and seven deaths—nearly 50% of the town’s population fell ill during the outbreak, and the town had to have water trucked in for more than six months (O’Connor, 2002). These outbreaks emphasize that *E. coli* O157:H7 and other EHEC are not single point-source pathogens, but must be regarded as widespread environmental threats to public health.

Other Contamination Routes

There have also been several recent outbreaks of *E. coli* O157:H7 in children from urban areas that visited farms or petting zoos (Chapman, Cornell, and Green, 2000; Gage, 2000; Pritchard et al., 2000). In some of these cases, exposure to manure or soil contaminated with feces was determined to be the vehicle (Strachan, Fenlon, and Ogden, 2001). In other studies, viable *E. coli* O157:H7 were isolated from the hides and mouths of finished feedlot cattle (Keen and Elder, 2002). The presence of this pathogen in locations where people, especially children, routinely touch animals could explain many of the exposure-linked outbreaks (Gage, 2000; Pritchard et al., 2000). This explanation has led the CDC to propose mandatory hand-washing facilities and the controlling of food consumption at petting zoos and open farms (Centers for Disease Control, 2003).

There has been speculation about whether O157:H7 could be spread as an aerosol of manure dust. This transmission could occur in at least two ways: either the dust would directly interact with the human, causing infection by inhalation or ingestion, or the dust would land on some other item subsequently consumed by the human. It has long been known that farm dust carries pathogens, but in the age of modern feedlots where cattle are in high concentration, the increased concentration of manure dust has raised concerns to a new height. There is surprisingly little in the literature on this matter, although human illnesses have been attributed to this vehicle. A 1997 study of Mexico City found that many pathogenic *E. coli* strains could be isolated from household and street dust throughout the city (Rosas et al., 1997). In 2001, 111 people fell ill after they attended a county fair in Ohio, and 23 cases were confirmed to be O157:H7. There initially appeared to be no specific point of origin, but Ohio State Department of Health officials finally traced the outbreak to the Cow Palace, a show barn, where it was present from the floor to the rafters. Throughout the fair this barn was used to exhibit animals, except on the final evening when it hosted a teen dance. The investigators believe that O157:H7 present in some residual material and dust was stirred up by the dance and eventually consumed by individuals via their food (Reller et al., 2003; Varma et al., 2003). In 2002, Manitoba Agriculture and Food and Manitoba Health released a document highlighting to farmers and citizens that manure dust could carry farm pathogens, but because dust particles settle quickly they concluded such contamination would be limited by distance (Manitoba Agriculture and Food, 2002). Dust plumes from feedlots can travel more than 30 miles, however, so this potential transmission route would appear to require further research, especially in light of the recent study showing that in summer up to 80% of feedlot cattle may harbor O157:H7 (Callaway et al., 2003b).

**Current and Potential Ways of Controlling *E. coli* O157:H7**

Given the limited options for treatment of O157:H7 illness, with no current therapeutic treatments and human vaccines unavailable, avoiding exposure is currently the most viable option (Karmali, 1998; Li et al., 2000). Although individuals can largely protect themselves from O157:H7 at home by thoroughly cooking foods (such as hamburger) to the recommended temperature, avoiding uncooked or unpasteurized products, keeping uncooked parts of the meal (such as salads) away from raw meat, and taking care with meat-contaminated utensils, there are still areas of concern. For example, private water supplies are vulnerable to contamination, with the first sign of trouble often being diseased individuals arriving at healthcare facilities. Furthermore, individuals must rely on others to ensure the safety of municipal water supplies, restaurant
and institutional food, public swimming pools, and beaches. For this reason, it is critical for policy makers and managers to be aware of the risks of O157:H7 and the methods of disease prevention. Food workers need to scrupulously follow guidelines for sanitation and food preparation. All workers in municipal water districts and public swimming facilities should understand that waterborne diseases are potentially fatal and that it is vital they ensure the proper disinfection of water with chlorine, ozone, or ultraviolet light.

Most of the regulatory focus of O157:H7 prevention has been placed on meat, specifically on preventing contamination during slaughter and meat processing. Processing plants substantially reduce the entry of O157:H7 into the food chain using strategies such as pre-slaughter washing of the cattle and post-slaughter carcass washes. Elder et al. (2000) found that 28% of the cattle presented for slaughter shed O157:H7, and the level of carcass contamination immediately following evisceration reached 43%, but extensive post-harvest anti-microbial treatments reduced carcass contamination levels to less than 2%. It may be possible to reduce these numbers further by reducing the amount of O157:H7 shed by cattle at the time of slaughter. Diez-Gonzalez et al. (1998) reported that abruptly changing cattle from a high-grain diet (such as a feedlot diet) to a 100% hay diet for five days reduced E. coli populations 1,000-fold and reduced the population of acid shock–resistant E. coli 1,000,000-fold. These findings prompted a great deal of controversy and further research (Hancock et al., 1999; Keen, Uhlich, and Elder, 1999; Russell, Diez-Gonzalez, and Jarvis, 2000). In a large, well-controlled study, Keen, Uhlich, and Elder (1999) found that a switch from grain to hay reduced O157:H7 shedding in cattle for seven days. Other researchers have found no effect of diet on E. coli O157:H7 populations or its ability to survive an acid shock (Hovde et al., 1999; Kudva, Hatfield, and Hovde, 1995). A comprehensive review of the literature by Callaway et al. (2003b) suggests that cattle abruptly shifted from a grain diet to a hay diet do shed fewer E. coli and E. coli O157:H7 than do cattle fed high-grain diets, but the magnitude of the reduction varies.

Although modern meat-processing methods can reduce the levels of E. coli O157:H7 in post-eviscerated meat more than 20-fold, some EHEC still enter the food chain, and the resultant, repeated massive recalls have damaged consumer confidence in the safety and “wholesomeness” of ground beef. Controlling O157:H7 contamination in the slaughterhouse and afterward is important, and most of the time it is successful at preventing meatborne outbreaks. Nevertheless, efforts to reduce O157:H7 contamination at this stage of the process seem to have reached the point of diminishing returns. For example, in 1998, the CEO of ConAgra, one of the four largest meat-processing companies in the US, stated, “We spent twenty million dollars to get a 0.009 percent pathogen reduction from 99.999% to 99.9999%” (Gants, 1998). Even after these efforts, the June 10–24, 2002, ConAgra outbreak affected seven states and required the recall of 19 million pounds of beef. The meat industry is clearly determined to increase its safety, but it must do so under the restrictions of cost. Jordan and McEwen (1998) have suggested that efforts to reduce the levels of O157:H7 shed at the point of slaughter will have a greater impact on meat industry safety than will changing meat processing procedures within the abattoir. Furthermore, intervention within the slaughterhouse doesn’t address the issue of O157:H7 as an environmental contaminant, because manure produced throughout the animals’ lives can still affect crops, water, and air. Therefore, it is important that we address ways of eliminating O157:H7 in livestock and manure, not just meat.

It was generally assumed that the bacteria from manure spread on fields would become trapped in the soil, but it is now clear that they can be transported in runoff or migrate into groundwater. So far, only a few methods have been proposed for eliminating O157:H7 and other pathogens from manure. Composting is a traditional treatment, and is effective when aeration, moisture, particle size, and carbon-nitrogen ratio are all carefully controlled to ensure that adequate temperatures are reached throughout the composted material. Anaerobic digesters have also been used successfully, and the addition of various chemicals, such as lime, can also reduce pathogen levels (Duffy, 2003). Diez-Gonzalez (2004) found that treating cattle manure with 4 g kg⁻¹ sodium carbonate (Na₂CO₃) and 2 g kg⁻¹ sodium hydroxide (NaOH) reduced E. coli levels below detectable levels (10 cells grams⁻¹). This treatment would cost an estimated $10 per cow per year (Diez-Gonzalez, 2004). Though more expensive, potassium carbonate (K₂CO₃) can be used in place of sodium carbonate in areas where excessive sodium in soils is a problem. Methods to treat O157:H7-contaminated manure have many potential benefits; however, finding ways to eliminate O157:H7 from livestock would prevent the manure from ever becoming contaminated.

Potential Pre-Harvest Reduction Strategies

Researchers are currently investigating several approaches to eliminating O157:H7 from livestock, including antibiotics...
and anti-microbials, probiotics, vaccines, and bacteriophages. Unlike humans, cattle lack Stx receptors (Pruimboom-Brees et al., 2000) and are therefore insensitive to increased toxin production, so the use of antibiotics to control *E. coli* O157:H7 has been studied. *Escherichia coli* belongs to the family Enterobacteriaceae and is difficult to target with anti-microbial agents. Neomycin sulfate is an anti-microbial approved by the FDA for use in cattle that has a 24-hour withdrawal period. Cattle fed neomycin for 48 hours with a 24-hour withdrawal period shed significantly lower *E. coli* and *E. coli* O157:H7 populations in their feces (Elder et al., 2002). Although total *E. coli* populations had returned to near pre-treatment levels 120 hours after neomycin withdrawal, *E. coli* O157:H7 populations remained below the limit of detection (Elder et al., 2002).

Other anti-microbial strategies have been developed that target unusual physiological characteristics of *E. coli*, including the ability to use nitrate as an electron acceptor in anaerobic respiration. The nitrate reductase enzyme that catalyzes this reaction does not differentiate between nitrate and its valence analog chlorate, which is intracellularly reduced to cytotoxic chlorite, killing the bacterial cell. Chlorate treatment kills *E. coli* O157:H7 in pure culture, in mixed fecal culture in vitro, in experimentally infected swine, and in experimentally infected cattle, while not appearing to impair animal health, performance, or meat quality (Anderson et al., 2000; Anderson et al., 2001; Callaway et al., 2002). Experimental results have been promising, and the use of chlorate is currently being reviewed by the FDA. If it is approved, rapid development could lead to the use of chlorate in poultry, swine, sheep, and cattle rations immediately prior to slaughterhouse shipment (Callaway et al., 2003a).

Probiotics in the form of competitive exclusion cultures and direct-fed microbials have been used as a strategy to reduce O157:H7 in cattle. Direct-fed microbials are cultures of bacteria that are generally recognized as safe (such as *Lactobacillus* and *Bifidus*), and their use is not regulated. Competitive exclusion cultures have been isolated from healthy animals and are then administered to other individuals of the same species. Because these cultures contain a mix of various, sometimes unidentified, organisms, the FDA regulates their use. Both types of probiotics have shown promise as tools to fight O157:H7. Researchers isolated a defined population of multiple strains of non-O157:H7 *E. coli* from cattle that were naturally O157:H7 free and found this generic *E. coli* culture could displace established O157:H7 populations from cattle (Zhao et al., 1998). In very recent research, other groups found that addition of a *Lactobacillus acidophilus* culture to the diet of finishing cattle reduced *E. coli* O157:H7 shedding by more than 50% (Brashears and Galyean, 2002). Until now, the use of competitive exclusion has been limited in the cattle industry because of the confounding effects of antibiotics and the complexity of the ruminant gastrointestinal tract; however, probiotic products are being readied for market and, especially as antibiotics become more closely regulated as growth promoters, it appears that competitive exclusion could become an important tool to reduce pre-slaughter levels of O157:H7 in cattle.

Researchers are also investigating vaccination as a means of reducing ruminant colonization by *E. coli* O157:H7 (Gyles, 1998). In 2000, Potter and Finlay reported in their preliminary studies a recombinant vaccine that lowered the shedding of O157:H7 in treated cattle compared to the controls (Potter and Finlay, 2000). Their most recent trials (12,000-plus cattle), in which the vaccine was used in conjunction with a direct-fed microbial, demonstrated a significant reduction in fecal shedding. These data are highly promising; however, this method may run into major problems related to adverse immunological reactions in cattle and regulatory issues, and thus any product is still two to three years from entering the Canadian marketplace (Finlay, 2003; Moxley et al., 2003).

Another strategy to improve pre-harvest food safety involves the use of a natural inhabitant of the gastrointestinal tract: virulent bacteriophages, or “bacterial viruses.” The use of phages as anti-microbial agents has a number of advantages over other current methods of microbial control. Phages are highly specific and are often only active against a particular subset of strains within a species of bacteria. This specificity allows phages to remove the targeted microorganisms from a mixed population, with little harm to the rest of the microflora. Virulent phages attach to specific receptors on the surface of bacteria, inject their DNA, and then express genes that direct a transition from host to phage metabolism that leads to the synthesis of new phage DNA and new phage particles, ending with programmed cell lysis and the release of dozens or hundreds of new phage particles. The number of phages actually increases rapidly as long as enough susceptible bacteria are present, working their way deeper into pockets of infestation or infection in the process, rather than decaying over time and distance as do antibiotics. This exponential increase in phages means they increase in numbers until the supply of host bacteria is depleted, at which time the phage population gradually
diminishes (a self-limiting targeted antibiotic). Further, phages are living entities that adapt and evolve; if mutants of the pathogen evolve, the phage will similarly adapt. As phages can also pass from host to host, directly or indirectly, they have the potential of establishing an "infectious cure" to cope with infectious disease (Berkhier, Lovell, and Barrow, 1991; Smith and Huggins, 1983).

It should be noted that there are two main, discrete kinds of phages—the exclusively virulent or lytic phages, suitable for this sort of anti-microbial application, and temperate phages that can choose an alternative mode of infection, stably integrating into the host cell. There, they provide protection against other phages, particularly related ones, and generally retain the ability to excise themselves from the host genome many generations later to produce a new burst of phages. At that time, they sometimes carry with them adjoining pieces of the host chromosome; past incidents of this sort involving temperate lambdoid phages seem to be the origin of the Stx pathogenicity islands in E. coli O157:H7 strains. It is thus clear that while the virulent phage families lend themselves very well to anti-bacterial applications, the temperate phage families are not appropriate, both because of their potential ability to produce phage resistance and because of their ability to carry genes such as those involved in pathogenesis and integrate them into their new host.

The potential of phages for curing animals of microbial disease has repeatedly been demonstrated over the past century. In 1919, Felix d'Herelle successfully used phages isolated from sick chickens to control an outbreak of fowl typhoid (d'Herelle, 1917). More recently, phages were successfully used against an otherwise-fatal diarrheal disease caused by enteropathogenic E. coli (EPEC) in young calves, lambs, and piglets (Smith and Huggins, 1983; Smith, Huggins, and Shaw, 1987a; 1987b). The addition of two phages specific for EPEC strains typically led to a 10^5-fold reduction in EPEC cell numbers, and treated calves remained healthy, whereas untreated calves died or were near death within 48 hours of EPEC infection. Similar data have been presented by Reynaud et al. (1992) with rabbits and Barrow, Lovell, and Berkhier (1998) to control E. coli septicemia and meningitis in chickens and calves.

Phage biocontrol of O157:H7 in the laboratory was reported by Kudva et al. (1999). They isolated several O157-specific virulent phages, but unfortunately their phages only wiped out E. coli if substantial aeration was provided; as they said, these phages appeared useful for treating sprouts but not gut flora of animals, because the rumen and colon of cattle are highly anaerobic. Although this work demonstrates the potential of "phagebiotic" methodology for controlling O157:H7, it also shows that it is necessary to know the growth characteristics of phages selected for therapeutic purposes if this promising technology is to succeed.

The applications and advantages of phages as anti-O157:H7 agents have become of commercial interest, with patents filed and products in development. For example, researchers at the University of Guelph were granted a patent covering the use of a group of their own phages against O157:H7 in the gastrointestinal tract of ruminants. Interestingly, their in vitro data showed that these phages are selective for O157:H7 but the presented in vivo data do not clearly demonstrate their efficacy in removing O157 from calves (US Patent 6,485,902); there is no indication that these investigators have carried out anaerobic studies with their phages (Waddell et al., 2002).

Some promising new phage work by researchers at The Evergreen State College and the USDA has shown that resident bacteriophages may prevent gut colonization by O157:H7 in ruminants. Phage CEVi, isolated from the feces of sheep impervious to gut colonization by O157:H7, despite repeated attempts, infects most E. coli O157:H7 strains. Initial O157:H7 eradication trials, using CEVi in a ruminant gut model including the presence of normal gut flora, showed that CEVi efficiently eliminated two pathogenic O157:H7 strains in 11 days. Further, sheep treated with a single oral dose of CEVi had a 99% reduction in the gut O157:H7 population after two days. These results suggest that the protective effect of CEVi and other carefully selected phages against E. coli O157:H7 could be used as an effective management strategy to reduce the population of this pathogen in livestock (Dyen et al., 2003; Raya et al., 2003).

Other intervention strategies have been proposed to reduce E. coli O157:H7 contamination of meat by reducing the pathogens on the hide before dehiding (for example, simply by spraying an anti-microbial compound directly on the hide prior to an animal entering the abattoir), and such a procedure has been successfully demonstrated with cetylpyridinium chloride (CPC) (Bosilevac et al., 2004). Cetylpyridinium chloride, commonly found in human mouthwashes, was sprayed on hides of cattle prior to transportation to a slaughter plant. Use of a high-pressure wash lowered aerobic plate and Enterobacteriaceae counts on the hide by more than 10,000-fold (Bosilevac et al., 2004). Removal of the hair coat via chemical methods has also been shown to reduce E. coli O157:H7 populations on
the hide (Nou et al., 2003). These methods show promise for reducing pathogens entering the abattoir on the hide, and the simultaneous or consecutive use of several interventions should significantly reduce the pathogen load entering the food chain.

Conclusion and Outlook

Although food and water safety are at unprecedented levels in the US, E. coli O157:H7 highlights our continuing vulnerability to emerging diseases. Because of the organism’s distribution throughout various environments, the many modes of transmission, its low infective dose, and the current infeasibility of a vaccination program, the challenge of E. coli O157:H7 requires a combination of highly diverse approaches. This battle cannot be won solely by public health officials, the beef industry, public utility districts, or consumers; the problem needs to be addressed by all these groups and many others. Only through increased public awareness; careful regulation of the food, water, and livestock industries; and continuing research into new methods of treatment, control, and prevention will the risk of E. coli O157:H7 be curtailed. A multi-hurdle system is being advocated widely as the best solution to this complex problem.

Perhaps because the problem was first linked to consumption of beef products, the beef industry has become a leader in tackling the problem. In January 2003, more than 200 participants from all areas of the beef industry attended a two-day summit in San Antonio, Texas, titled “E. coli O157:H7 Solutions: The Farm to Table Continuum.” Their goal was to develop an “aggressive battle plan” to combat E. coli O157:H7. They concluded that E. coli O157:H7 remains a life-threatening risk to the public, despite the many advances in technology and knowledge in all areas of beef production since O157:H7 first came to prominence. A key “point of focus” identified in their battle plan was to reduce the number of cattle that reach market containing E. coli O157:H7, using methods based on sound scientific principles and data. As research continues, more such methods are becoming available.

As awareness of E. coli O157:H7 spreads among the public, in industry, in utilities, and in regulatory bodies, it is less likely that someone will be left in the position of the Walkerton Public Utility Commission operators or the Odwalla managers—culpable for illnesses and deaths because of ignorance of the potential severity of the problem. The legal cases against these companies has brought to the fore the reality that organizations can be held liable for negligence that leads to personal injury. This said, consumers must be vigilant, as they are the final step in this food safety process. Any efforts to control E. coli O157:H7 will require increased public education in the ways to handle and prepare the foods they purchase.

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