

FACTORS AFFECTING THE EMERGENCE OF NEW PATHOGENS AND RESEARCH STRATEGIES LEADING TO THEIR CONTROL¹

ARTHUR J. MILLER^{2,3}, JAMES L. SMITH and ROBERT L. BUCHANAN³

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ABSTRACT

Control of foodborne emerging or reemerging microbial pathogens has proven to be difficult. Even the Hazard Analysis Critical Control Point (HACCP) approach is intended to manage known hazards. We propose that each change occurring in the food chain — encompassing human, technological, and environmental factors — creates a new selection pressure that drives microbial adaptation and emergence potential. Escherichia coli O157:H7 is examined here, as a case study, to illustrate the multidimensional nature of pathogen emergence. While future emergence or reemergence events are expected, the fundamental questions of what, where, who, when, and how such events will unfold are unknown. Contingency planning can provide responses to probable hazard scenarios, with a goal of developing practical controls. Examples of potential microbial hazards and changes in the food chain are presented. Once a hazard, associated food, locale, and at-risk population are identified, critical acute research questions need to be answered. Longer term research will improve our ability to respond to the next inevitable emergence event. Such coordinated endeavors will permit rapid modification and deployment of a science-based hazard management system that will prevent or minimize human risks.

INTRODUCTION

Jensen's 1942 classic *Microbiology of Meats* (Jensen 1942) lists four bacterial agents responsible for foodborne illness: *Staphylococcus aureus*, *Salmonella*, *Clostridium botulinum*, and streptococci. During the ensuing fifty-five years we have uncovered additional microbial hazards, engaging the greater capabilities of medicine, diagnostic microbiology, and epidemiology. Despite this progress, for the period 1988 to 1992, a known cause for 58.2% of reported foodborne disease outbreaks could not be determined (Bean *et al.* 1996). Emerging and reemerging pathogens undoubtedly contribute to these undiagnosed illnesses.

The U.S. Centers for Disease Control and Prevention (CDC) has defined an emerging pathogen as an infectious agent whose incidence in humans has increased dramatically within the past 20 years, or one that has the probability of increasing in the future (CDC 1994). Smith and Fratamico (1995) listed foodborne pathogens that have emerged within the past 20 years (Table 1). More recent additions include *Salmonella typhimurium* DT104 (CDC 1997a), *Cyclospora cayatanensis* (Herwaldt and Ackers 1997), *Vibrio cholerae* O139 (Albert 1994; Mooi and Bik 1997) and non-O157 serotypes of enterohemorrhagic *Escherichia coli* (Johnson *et al.* 1996).

TABLE 1.
FOODBORNE PATHOGENS THAT HAVE EMERGED WITHIN THE PAST 20 YEARS^a

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|--|
| <i>Campylobacter jejuni</i> |
| <i>Clostridium botulinum</i> (infant botulism) |
| <i>Escherichia coli</i> O157:H7 |
| <i>Listeria monocytogenes</i> |
| <i>Salmonella enteritidis</i> |
| <i>Vibrio cholerae</i> (Latin America) |
| <i>Vibrio vulnificus</i> |
| <i>Yersinia enterocolitica</i> |
| Norwalk and Norwalk-like viruses |
| Rotavirus |
| <i>Cryptosporidium parvum</i> |
| <i>Giardia lamblia</i> |
| <i>Toxoplasma gondii</i> |
| Bovine spongiform encephalopathy prion |

^a Smith and Fratamico 1995

The control of these hazards in foods has proven to be difficult and the routes for control have generally been chosen haphazardly. Even the Hazard Analysis Critical Control Point (HACCP) approach, which is the premier system

for managing food safety hazards, is intended to manage *known* hazards. Successful hazard management is dependent upon the transformation of research-derived knowledge into practical production, processing, and food preparation practices. Considering the requirement for a preexisting and substantial body of knowledge, HACCP cannot be expected to control *unknown* hazards such as an emerging foodborne pathogen. Only the timely acquisition of critical research data can transform the hazard from the realm of the unknown to the known, and only the known can be controlled.

Thus, the aims of this paper are to classify and discuss the factors which appear to be important in the emergence or reemergence of foodborne illness and to propose a tiered approach to address research needs to control future microbial threats.

FACTORS CONTRIBUTING TO EMERGENCE OR REEMERGENCE

From the perspective of microbial ecology our sequential system of agricultural production, processing, distribution, storage, and meal preparation can be compared to a stack of micro-sieves, where each sieve selectively retains some microorganisms and lets others pass through to the next stage. In addition to hazards that are associated with raw agricultural commodities, some may be introduced during the various processing steps. Given the diversity of microorganisms on raw foods, this system reduces their numbers to a few well-adapted genera that are generally benign. Periodically, a change or failure in the control system will let a *bad bug* come through the stack.

The emergence or reemergence of an infectious disease is due to change. Table 2 lists the factors involved, and include: human (social, housing, behavioral, nutritional, demographic), technological (agricultural, medical, sanitation), environmental, and microbial adaptation elements. A few cases are elaborated to illustrate how changes in any of these factors may contribute to the emergence of new pathogens.

Agricultural Practice Changes

- (1) Extensive antibiotic use in animals has allegedly led to the emergence of a multidrug-resistant *S. typhimurium*, Designation Type (DT) 104 (Meslin 1997). In Europe and North America, the organism is quickly becoming the dominant phage-type of this *Salmonella* serovar. Its resistance genes reside chromosomally, which indicates that resistance is likely to be retained even in the absence of antibiotics. It is unclear if the resistance genes form a single block on the chromosome, i.e., a resistance island (Hecker *et al.* 1997), or if they are inserted randomly into the chromosome. Furthermore, it is unknown if these genes are associated with pathogenicity islands, which

have been described in *Salmonella* (Groisman and Ochman 1997). While we know that the organism exhibits enhanced virulence, compared to other salmonellae, we have little information about its survival and growth potential in food systems (Humphrey 1997). Transmission can occur when animals are raised in close proximity, since it is possible to isolate the drug-resistant pathogen from animals not subjected to antibiotic treatment (Meslin 1997). As with most salmonellae, humans can be infected via animal contact or by ingesting raw or undercooked meats from animals and poultry infected with the organism. The use of DT104-containing manures on crop lands also may contaminate vegetable crops.

- (2) Infection of the oviduct of laying hens with *Salmonella enteritidis* has led to outbreaks of human salmonellosis involving shell eggs (Humphrey 1994; St. Louis *et al.* 1988). Consumers have generally assumed that grade A shell eggs are safe and that egg contents are bacteria-free. The strains of *S. enteritidis* involved in these outbreaks have been shown to be invasive for chickens and the invasive strains causing foodborne outbreaks are closely related (Hinton *et al.* 1990; Stanley *et al.* 1992). Improper storage of grade A eggs allowing the growth of *S. enteritidis* (Bradshaw *et al.* 1990) combined with consumer preference for undercooked eggs or products containing raw eggs (Klontz *et al.* 1995; Mishu *et al.* 1991) are factors involved in outbreaks. Various poultry raising practices and industrialization of poultry breeding, poultry rearing, egg production and egg distribution have probably also contributed to the emergence and persistence of *S. enteritidis* (Smith and Fratamico 1995).

Technology Changes

- (1) Bovine spongiform encephalopathy (BSE) emerged as an infectious agent, mainly from adult dairy cattle in the United Kingdom. The evidence indicates that this prion-derived disease was a result of feeding cattle, meat and bone meal (MBM; derived from dead ruminants, such as sheep, and slaughter house by-products, infected with scrapie) as a protein supplement (Nathanson 1997). Reduction of temperature used in heat treatment and elimination of solvent extraction of animal tissue during MBM processing apparently led to a failure to inactivate the BSE prion and allowed its introduction into the cattle population (Collee and Bradley 1997; Nathanson 1997). The ban on the use of MBM has led to a steady decline in the number of BSE infected cattle in the United Kingdom (Collee and Bradley 1997). Will *et al.* (1996) has suggested that ingesting BSE-containing meat may lead to Creutzfeldt-Jakob disease in humans, but the evidence is tenuous.

CONTROL OF FOODBORNE PATHOGENS

TABLE 2.
FACTORS INVOLVED IN EMERGENCE/RE-EMERGENCE OF PATHOGENS^a

| Factor | Examples |
|--|--|
| Social | Economic impoverishment War Civil conflict or political upheaval Population demographics (growth or migration) Urbanization (decay, crowding) |
| Technological | Changes in medical technology (medical devices, immunosuppressive drugs) Industrialized food production and processing (consolidation, preservation technologies) Globalization of food supplies (Imports, uneducated workers) |
| Health care and public health infrastructure | Increased organ and tissue transplantation Antibiotic use Increased use of nursing homes for the elderly Reduction or elimination of disease prevention programs Inadequate infectious disease surveillance Insufficient numbers of trained public health personnel Inadequate treatment of potable water supplies and sewage |
| Demographics | Increase in number of immigrants Increase in number of elderly individuals Increase in number of immunocompromised individuals (malnourished, medically immunosuppressed, diseased, nursing home residents, organ transplant patients) |
| Human behavior | Changes in sexual behavior Increased use of recreational drugs (intravenous) Changes in diet (new foods, fewer meals eaten at home) Shift of women from home to workplace Increased use of child day care facilities (single parents or dual income families) Increase in number of pets (domestic and exotic) Increased international business travel and tourism Decrease in breast feeding |
| Environmental | Deforestation and/or reforestation Changes in water ecosystems (building of dams, irrigation) Climate (flooding or drought conditions, global warming) Famine Earthquakes Heat waves or cold spells Algae blooms |
| Microbial adaptation | Increase in or gaining of virulence factors and/or toxin production Development of drug resistance Microorganisms as cofactors in chronic diseases (toxoplasmosis in AIDS patients) Microorganisms crossing species barriers Introduction of pathogens into new geographic areas Ability to adapt to new environmental conditions |
| Vectors | Introduction to new geographic areas (climate changes; human migration) Development of resistance to pesticides Change in land or water ecology |

a) Centers for Disease Control and Prevention 1994; Hedberg *et al.* 1994; Lederberg *et al.* 1992; Levine and Levine 1994; Smith and Fratamico 1995

- (2) The emergence of *Listeria monocytogenes* as a major foodborne pathogen is directly related to increased use of long-term cold storage of foods. The psychrotrophic nature of *L. monocytogenes* allows its growth at refrigeration temperatures (Smith and Fratamico 1995). The predilection of the organism for immunocompromised individuals makes *L. monocytogenes* a particularly dangerous foodborne pathogen to a major segment of the population (Schuchat *et al.* 1991).

Public Health Policy Change

In order to reduce cancer risks, officials in Peru eliminated chlorination of drinking water (Anderson 1991). This decision resulted in a cholera epidemic occurring throughout much of Latin America. The poor infrastructure of potable water distribution and sewage disposal systems has made eradication of epidemic cholera in Latin America difficult (Swerdlow *et al.* 1992). The use of contaminated water to prepare food as well as preparation of food by infected food handlers has led to unsafe foods.

Microbial Adaptation

El Tor serotype O1 strains have been responsible for epidemic cholera in Asia, Africa and South America. Interestingly, in 1992, epidemic cholera due to serotype O139 *V. cholerae* emerged and has spread through southeast Asia (Albert 1994). A toxin-producing *V. cholerae* probably acquired the O139 antigen by horizontal gene transfer; the emergence of the new cholera toxin-producing serotype may have been facilitated by naturally acquired immunity to the O1 serotype (Mooi and Bik 1997). What is even more interesting, a new clone of El Tor O1 has displaced O139 as the cholera-producing organism in Calcutta. This new clone has a different ribotype from El Tor O1 present before the O139 outbreaks (Sharma *et al.* 1997). Faruque *et al.* (1997) have presented evidence that a new ribotype of Bengal O139 has recently appeared in Bangladesh. Cycling of successive ribotypes of O1 El Tor with O139 may well be the pattern of future cholera outbreaks in Asia.

The ability for bacteria to adapt to changes in their environment cannot be overemphasized. For many enteric bacteria, a single DNA change can convert a microorganism into a pathogen (Groisman and Ochman 1997). Moreover, as described below for *E. coli* O157:H7, hypermutable genetic sequences exist in bacteria, termed contingency loci, to facilitate rapid diversification (Deitsch *et al.* 1997). Indeed, for some pathogenic bacteria, it has been shown that an entire population of infecting organisms can be derived from a single clone (Moxon and Murphy 1978). Thus, in our efforts to keep food safe by developing adequate preservation systems, we are confronted with remarkably adaptable bacterial antagonists.

**ESCHERICHIA COLI O157:H7 AS A MODEL FOR THE
EMERGENCE OF A FOODBORNE PATHOGEN**

While a single change factor is frequently the direct cause of an outbreak by a new pathogen, a series of contributing underlying events have usually occurred prior to emergence in the human population. The emergence of *E. coli* O157:H7 as a major foodborne pathogen can provide insights to understand the multidimensional nature of emergence. For example, while undercooking was the primary factor contributing to beefburger outbreaks, the accumulation of virulence factors by the bacterium, shifts in livestock raising techniques, consolidation of the slaughter and processing segments of the industry, and modifications in human eating habits (Armstrong *et al.* 1996) also needed to occur. Each of these underlying components can be expanded.

Evolution of *E. coli* O157:H7 Pathogenicity

Clinical isolates from hemorrhagic colitis or HUS patients belong to a single genetic clonal group (Whittam *et al.* 1988). These strains are unrelated to those of the O157 serogroup, that lack the H7 antigen (Whittam and Wilson 1988). Instead, O157:H7 is genetically related to O55:H7 strains. (Whittam *et al.* 1993). Whittam *et al.* (1993) visualized the origin of O157:H7 as follows:

A O55:H7-like ancestor with the ability to cause disease via the attaching-effacing mechanism acquired the capability for producing Shiga-like toxin and adhesins via horizontal genetic transfer from other pathogens and by recombination. Acquisition of a new serogroup antigen (O157) led to the appearance of a new, highly virulent organism — *E. coli* O157:H7.

The initial localization sites for *E. coli* O157:H7 in cattle are the fore-stomachs where fermentation takes place (Doyle *et al.* 1997). Thus, the necessity to survive the acidic gastric environment probably created a selective pressure for *E. coli* O157:H7 to acquire acid tolerance (Foster 1995; Archer 1996). As a consequence, the pathogen can grow and/or survive in acidic foods (Conner and Kotrola 1995; Diez-Gonzalez and Russell 1997; Waterman and Small 1996). The ability to acquire such virulence genes may result from demonstrated increased mutation rates and enhanced recombination abilities (Moxon *et al.* 1994; LeClerc *et al.* 1996).

Hypermutability of *E. coli* O157:H7 suggests that the organism may acquire new factors that will render it even more virulent. In addition, Baur *et al.*

(1996) reported that *E. coli* became maximally competent for genetic transformation in freshwater environments when the bacterium was exposed to ≥ 2 mM Ca^{2+} , as temperatures increased from 10 to 20°C. In the broad sense, these results and those of Pupo *et al.* (1997) suggest that any *E. coli* strain which acquires virulence factors may give rise to a pathogenic type.

Changes in the Cattle Industry

There is likely a close relationship between animal management practices and the presence of *E. coli* O157:H7 (Armstrong *et al.* 1996; Hancock *et al.* 1997a,b). While *E. coli* O157:H7 was isolated from sheep, goats, horses, dogs, deer and wild birds feces (Chalmers *et al.* 1997; Keene *et al.* 1997; Kudva *et al.* 1997; Wallace *et al.* 1997), beef and dairy cattle appear to be the main reservoir in the United States. Changes in the cattle industry may have influenced emergence or persistence of *E. coli* O157:H7. For example, in the United States, there are fewer feedlots now with capacity of < 1000 head, and more with a capacity of $\geq 16,000$ cattle. In addition, feedlot preponderance has changed location from the upper midwestern states to the warmer lower midwestern states (Armstrong *et al.* 1996). Similarly, the dairy industry has shifted to fewer and larger facilities, with a large share of milk production shifted to western and southwestern states from northeastern and upper midwestern states (Armstrong *et al.* 1996). The concentration of larger numbers of animals in fewer facilities, combined with shifts to warmer locations, has perhaps led to increased prevalence of *E. coli* O157:H7 in slaughter cattle. Contributing factors include confinement and crowding, feed deprivation and trucking stresses associated with transport to slaughter. Such factors allow cattle to shed *E. coli* O157:H7 in feces (Rasmussen *et al.* 1993).

E. coli O157:H7 survives for long periods in animal fecal slurries (Kudva *et al.* 1995) which suggests that such manures could provide a source of contamination for cattle; however, Hancock *et al.* (1997a) demonstrated that prevalence of the pathogen in dairy herds was not correlated with the use of manure slurries on cattle forage crop land or pasture. Cattle manure on farmland is the probable source for the presence of *E. coli* O157:H7 in wild bird (mostly gulls) feces (Wallace *et al.* 1997). Thus, wild birds could be the vector for the transfer of the pathogen to cattle and other ruminants.

Changes in Cattle Slaughtering, Meat Processing and Meat Distribution

In the slaughterhouse, carcasses can become contaminated with feces containing the pathogen. Slaughter facilities have decreased in number, in the U.S., but those remaining have grown larger. These large facilities slaughter animals from multi-state regions, which involves stressful long-term trucking, feed deprivation, and stressful crowding. Stressful conditions can stimulate

pathogen excretion, increasing potential for carcass contamination during slaughter. Large processing and boning facilities take chilled carcasses and produce boned beef and trimmings that are co-mingled with meat from many carcasses. In turn, beef-grinding operations receive different types of raw material from multiple suppliers, further compounding the problem. Boneless beef and trimmings are blended without trace-back capabilities, if a problem should arise. The recent Hudson Food beefburger recall brought to light the common industrial practice of mixing the previous day's unused blends with today's product. Beef patties are shipped to food distribution centers which purvey the patties to food markets and to food service establishments (Armstrong *et al.* 1996; Levine and Levine 1994).

The increased industrialization of animal husbandry, slaughter, food processing and food distribution allows the consumer to buy foods readily and at reasonable prices. However, if a food is contaminated, the business concentration effect results in distribution of that pathogen to larger segments of the consuming population, which magnifies their potential risk (Smith and Fratamico 1995).

Changes in the Eating Habits of Consumers

Approximately half of all American's meals are eaten away from home, mostly in restaurants (Jensen and Unnevehr 1995); control over food preparation is rapidly being lost, perhaps with heightened risks. In addition, some consumers dislike the idea of pasteurization or heat treatment of certain foods. Thus, consumers can become infected with *E. coli* O157:H7 after consumption of unpasteurized apple cider (Besser *et al.* 1993; CDC 1997b) or apple juice (CDC 1996) or fermented but uncooked salami (CDC 1995; Tilden *et al.* 1996). Even when products are cooked there is frequently a preference for minimal cooking. For example, approximately 25% of respondents in a United States survey expressed a preference for rare or medium-cooked hamburgers (Klontz *et al.* 1995). Unlike other beef cuts where microbial contamination is confined to the surface, ground meat has microorganisms distributed throughout the product. In the preparation of a rare or medium steak, the cooking process destroys the surface organisms, but rare or medium-cooked hamburger will still contain a substantial microbial population. Since the infectious dose of *E. coli* O157:H7 is very low, the person who prefers rare or medium-cooked hamburgers is at risk (Armstrong *et al.* 1996; Tilden *et al.* 1996).

E. coli O157:H7 has probably emerged several times in the past, but its success as a pathogen persisted only when the changes discussed above occurred. It is critical to learn what factors can cause pathogens to emerge, and, attempt to predict a potential emergence.

ANTICIPATING THE NEXT EMERGING PATHOGEN

While future emergence or reemergence events can be expected, the complex questions of what, where, who, when, and how such events will unfold are unknown. The challenge becomes to plan for a microbial threat. Military contingency planning may be applied to this problem. This approach to preventing or minimizing consequences of potential outbreaks has four components: intelligence, personnel and facilities, rapid response, and strategic planning. Each is discussed below.

Intelligence consists of information gathering activities, in this case to permit an emergence to be identified. In the United States, the CDC serves as a clearinghouse for this activity, in direct cooperation with state public health agencies; however, CDC's surveillance activities are largely domestic, whereas an effective intelligence system for foodborne disease of necessity must be worldwide. Other potential sources of information include: The World Health Organization, other sovereign governments, the U.S. military's international network of laboratory and medical investigators, the medical and scientific literature, and the Internet. Intelligence gathering must include an awareness of changes and advances in food technology, agricultural practices and conditions, veterinary medicine, environmental and water microbiology, consumer trends, and general socioeconomic conditions. This information must be analyzed by experts to determine the potential impact on public health.

The second component of contingency planning is to ensure the availability of personnel and facilities to quickly characterize a new biological agent, then to develop control strategies. The broad range of capabilities and resources needed to deal with all contingencies will unlikely reside in a single organization. One approach is to establish "reserve" groups of subject-matter experts from various organizations that could coordinately mobilize as needed. This requires centralized planning, periodic drills, and inter-organizational cooperation to ensure that needed expertises and facilities are maintained.

The third component of contingency planning is rapid resource mobilization. Currently, the focus of rapid response efforts has been directed largely on the diagnosis of illness, identification of new agents, and the recall of suspect food. These are key initial steps; however, the initiation of critical research to prevent another occurrence of the emerging pathogen has been handled typically in a much less organized and timely manner, and should commence at this stage of response.

The fourth component, strategic planning, should be the first step to be conducted. Strategic planning involves simulating "what we would do if" scenarios, especially planning appropriate responses. This has received little attention in relation to emerging foodborne pathogens. This process relies on "futurist thinking" to consider changes in society, economics, technology,

agriculture, medicine, international trade, etc. in view of their likely impact on microbiological safety of the food supply. A broad view is needed since root causes of most disease emergences are general events or trends in society. Strategic planning is undertaken with the realization that the probability of a specific "what - if" scenario occurring is low, but the probability that one of many alternatives will occur is high.

FORECAST FOR PATHOGEN EMERGENCE

Nearly all microbial foodborne disease stems from consumption of either raw, undercooked, temperature abused, or cross-contaminated products, as the immediate cause (Bryan 1988). Thus, if these shortcomings were remedied, few outbreaks would occur. Yet, in the U.S., government agencies and the food industry have accepted as a challenge the reduction or elimination of the microbial hazards from the food supply. This is best demonstrated by the endorsement of HACCP by organizations, such as the American Meat Institute, the USDA Pathogen Reduction/HACCP rule, and, most recently, by the announcement of the Food Safety Initiative by President Clinton. These measures, however, primarily address known hazards. The emerging hazards remain in the realm of the unknown.

We can predict that new pathogens will emerge. We know they will emerge from either a failure of a traditional safeguard or from a change that creates an organism adapted to overcome the traditional safeguard. In this section, we offer some "intelligence" by providing examples of newer microbial hazards seen in the medical or veterinary community and of global trends in human demands, technology and trade that could affect food safety.

Pathogens with Potential for Emergence

Prion-Induced Variant Creutzfeldt-Jakob Disease. New variant Creutzfeldt-Jakob disease (nvCJD) has occurred in the United Kingdom and Europe. Unlike CJD, nvCJD presents with a pathology similar to BSE and thus this raised fears that the eating of BSE-infected meats may lead to nvCJD (Will *et al.* 1996). A disease similar to nvCJD has been induced in macaques by intracerebral inoculation of tissue from BSE-infected cattle (Lasmézas *et al.* 1996). However, *in vitro* experimental results suggest that transmission of the infectious agent of BSE and scrapie to humans is quite low (Raymond *et al.* 1997). Lantos *et al.* (1997) have demonstrated that there are many similarities between nvCJD and kuru. Kuru is a transmissible spongiform encephalopathy (TSE) associated with cannibalism. It is possible that the similarities in phenotypic presentation of kuru and nvCJD, both clinically and neuropathologically, reflect a common route of infection — oral ingestion of prion-infected

tissue which leads to disease. Since TSEs develop so slowly, it may be difficult to prove that an oral route of infection can lead to nvCJD.

If nvCJD arises from BSE ingestion, the factors that led to the emergence of nvCJD have been changed, i.e., elimination of feeding MBM derived from ruminants, destruction of BSE-infected cattle, elimination of ruminant central nervous tissue in meats. Since nvCJD probably has a long incubation period, 10-15 years, in humans, there may be an increase in nvCJD for the next few years before the disease disappears.

Viruses. It is certain that a portion of the outbreaks with unknown etiology are due to foodborne viruses. As virus detection technology improves, especially by the use of DNA-probes, more foodborne viruses will be recognized. It is probable that many of the factors that led to the emergence of *E. coli* O157:H7 also are important in viral emergence. Many of the foodborne viruses are also waterborne and the aging of potable water infrastructure will play a role in virus emergence.

Bacteria.

Citrobacter freundii. *C. freundii* is an intestinal commensal bacterium of humans and animals, which is present in water, sewage, soil and foods (Stiles 1989). The microorganism is an opportunistic pathogen in immunocompromised individuals (Lipsky *et al.* 1980; Samonis *et al.* 1991). Karasawa *et al.* (1990) found that *C. freundii* isolated from an infant with diarrhea produced a heat-labile toxin similar to cholera toxin and *E. coli* heat-labile toxin. Strains of *C. freundii* isolated from diarrheic children were shown to produce heat-stable toxin similar to that produced by enterotoxigenic *E. coli* (Guarino *et al.* 1987, 1989). Shiga-like toxin (SLT) II but not SLT I has been found in *C. freundii* strains isolated from diarrheic patients (Schmidt *et al.* 1993). Interestingly, a foodborne outbreak was reported by Tschäpe *et al.* (1995) in which SLT-II producing *C. freundii* were isolated from stools of patients with severe gastroenteritis and hemolytic uremic syndrome. Since *C. freundii* has the ability to produce and/or acquire a number of toxins, it seems likely that the organism could emerge as an important foodborne pathogen in the future. In the outbreak described by Tschäpe *et al.* (1995), green butter (parsley in butter) was the causative food. The parsley was grown organically in a garden fertilized with pig manure. SLT-II-producing *E. coli* O139 was isolated from the parsley in addition to *Citrobacter*. It is probable that the toxin-producing gene was transferred from *E. coli* to *Citrobacter*. With the increasing demand for organically grown food, more such outbreaks may occur unless the animal manures are sterilized to prevent transfer of toxin genes from one species to another.

Arcobacter butzleri. *A. butzleri* is present in a variety of foods including poultry, beef and pork (de Boer *et al.* 1996; Zanetti *et al.* 1996). The organism has been isolated from the feces of diarrheic animals and humans (Anderson *et al.* 1993; Kiehlbach *et al.* 1991). *A. butzleri* has been associated with severe diarrhea in immunocompromised individuals (Lerner *et al.* 1994) and bacteremia in a neonate (On *et al.* 1995). Essentially nothing is known about virulence factors in *A. butzleri*; however, Musmanno *et al.* (1997) examined 18 river isolates for virulence factors. Seventeen strains produced a cytotoxin active against Vero cells with production of cell rounding and nuclear pyknosis. One strain had cytotoxic activity against CHO cells with production of cell elongation; the strain also was adherent to intestine 407 cells. Cytotoxic distending effects or invasion was not observed for any of the 18 strains (Musmanno *et al.* 1997). Future studies should concentrate on virulence factors and the disease-inducing ability of *A. butzleri*. So little is known about *Arcobacter*, it is difficult to know what may be involved in its potential emergence. It is probable that many of the factors involved in the emergence of *E. coli* O157:H7 will be involved in the emergence of *Arcobacter*.

Salmonella. The recent identification of sub-populations of *S. enteritidis* PT4 (Humphrey *et al.* 1995) and *S. typhimurium* DT104 (Humphrey 1997) exhibiting enhanced survival characteristics suggests that this genus may be acquiring new survival genes. If true, this may necessitate reevaluating past practices, which were based on *Salmonella* inactivation.

Parasites. The demand for a year round supply of fresh produce creates opportunities for equatorial and Southern hemisphere countries that are fruit and vegetable producers. Similar to the emergence of *Cyclospora* in North America, we will in all likelihood see other parasites. The poor potable water infrastructure of developing countries (similar to that seen in Peru in the South American cholera outbreak) will ensure that even more parasites will emerge. As long as the water infrastructure of the developed nations remains intact, secondary cases of emerging parasites will be rare.

Human, Technology, and Environmental Factors

Trends in human demographics and consumer demands, technological breakthroughs, and the resultant infrastructure and land or water use practices, undoubtedly will create new niches for microbial hazards to emerge. The growing demand for a year-round supply of fresh foods, is offered as an example. The technology to satisfy this demand may be near, as FastShip container vessels — using water jet or gas turbine engines and alternative hull design — hold promise to cut transoceanic shipping time by nearly half (Giles

1997). Coupled with highly efficient loading and unloading systems, and land-based transportation systems keeping to tight schedules, massive quantities of fresh products will travel to foreign markets at high speed. This increased product availability, will heighten consumer demand, which will stimulate further international commerce in fresh fruits, vegetables, and seafood. One consequence may be the rapid dissemination of previously localized infectious organisms carried on contaminated products. Another consequence may be the spread of human pathogens from biofilms on the hull, and the bilge water carried within ships. As a case-in-point, the Peru *Vibrio* pandemic was disseminated by the dumping of *V. cholerae* laden bilge water into foreign harbors. Globalization of fresh food markets will undoubtedly create pressures to increase production, which, if implemented without considering potential public health effects, may lead to increased health risks. For example, intensive aquaculture, integration of hydroponics with aquaculture waste streams, and uncontrolled manuring of crop lands may offer attractive approaches to boost product yields, but also create new opportunities for pathogen emergence. Other trends, with health implications, include: consumer demand for nontraditional foods, without additional safety education; shipment of food during seasonal contamination cycles; and the demand for preservative-free foods. Any or all of these may result in an outbreak of foodborne illness from a heretofore unknown organism, or an old pest may reemerge.

TWO-TIERED APPROACH TO ADDRESSING RESEARCH NEEDS

Buchanan (1997) described two distinct classes of research needs on emerging pathogens, based on time constraints. Acute research needs provide knowledge and technology to control the emerging pathogen. It is specific to the microorganism and food and must be accomplished swiftly. This research is generally applied, though basic research may overcome deficiencies in knowledge, if little information exists. General areas include: diagnostics, food vehicles, contamination sources, pathogen growth and survival characteristics, microbial ecology, virulence characteristics, and at-risk human populations. In short, any data necessary to prevent a reoccurrence of the disease or to modify current HACCP, good production, or good manufacturing practices should be classified as an acute research need.

Buchanan (1997) described three components of longer term research associated with emerging pathogens. The first area is research to find improvements or alternatives to detect and control the emerging pathogen. The second area is research to reduce the response time between the emergence of a pathogen and its initial control. The third area is research that identifies factors that will allow new microbial threats to be anticipated.

WARS AVOIDED; BATTLES WON

Recent events demonstrate that we have been successful in identifying some potential hazards and reacting quickly to avoid or minimize adverse health consequences. Three examples are described.

Sous Vide

Precooked modified atmosphere or vacuum-packaged refrigerated entrees, introduced during the 1980's, are a potentially high risk food class that has not resulted in foodborne illness. Products, such as *sous vide*, are minimally pasteurized, lack conventional preservatives, and are maintained at refrigerated temperatures under an oxygen-free atmosphere. Potential hazards include the outgrowth of spores of nonproteolytic *Clostridium botulinum*, which can germinate at 3.3C, and the absence of microbial competition, primarily lactic acid organisms, to acidify a temperature-abused product, and inhibit pathogen growth. These concerns have been overcome by formulating acidified sauces which serve in the same role as the lactic acid organisms.

Fresh Mushrooms

Soon after oxygen barrier over-wraps were introduced into the U.S. market to extend the shelf life of fresh mushrooms, it was recognized that spores of *Clostridium botulinum* which are frequently associated with the product, could germinate and produce neurotoxin, after the aerobic microflora consumed residual oxygen. The problem was avoided by introducing a sufficient number of holes in the film to prevent anaerobic conditions from forming.

Listeria monocytogenes

Once it was recognized as a foodborne pathogen, and control measures were introduced, the annual incidence of human illness in the U.S. decreased by nearly 50% (Tappero *et al.* 1995). The record thus demonstrates that food safety can be ensured if proper controls are implemented.

CONCLUSIONS

For every change we impose on the food chain, we create a new selection pressure that increases the probability that a pathogen will emerge or reemerge. Yet, it is obvious to anyone associated with the food industry that change is unavoidable. Therefore, emergence of new or old pathogens can be expected to occur. When possible, we need to predict potential hazards and establish proactive control measures. When a pathogen emerges, we need to minimize its

human health impact by quickly establishing controls. If the technology or knowledge is lacking, a research effort is warranted. The overall goal needs to be hazard management throughout production, processing, distribution, and final food preparation.

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