IFT Expert Report on Emerging Microbiological Food Safety Issues

Implications for Control in the 21st Century

Microbiological food safety is a complex, fundamental issue of continuing concern. Contributing to this complexity and the emergence of food safety issues are ongoing changes in demographics, geographic origin of food, food production and processing, food consumption patterns, and microorganisms themselves. These host, environmental, and pathogen changes challenge our food safety policies and our ability to manage food safety throughout the food system.

Recognizing this, the Institute of Food Technologists (IFT), the 28,000-member nonprofit society for food science and technology, convened a panel of internationally renowned experts to review the science related to emerging microbiological food safety issues and implications for their control and to produce a comprehensive, scientific report. IFT's objective for this Expert Report is to increase the understanding—among IFT members, senior policy officials, and other interested groups—of the scientific information on emerging foodborne pathogens (from a broad ecological perspective) relative to public policy issues and strategies for preventing foodborne illness.

This report is the second Expert Report produced by IFT since the establishment of its Office of Science, Communications, and Government Relations, which led the production of this report and the IFT Expert Report on Biotechnology and Foods. In the seven sections of this report, the expert panel focuses on the complexity of emerging foodborne pathogens and factors influencing emergence; manifestation of clinical foodborne disease; human susceptibility; ecology of pathogens in pre-harvest and post-harvest environments; microbial virulence, evolution, selection, adaptation, stress, and driving forces; risk analysis, the Hazard Analysis and Critical Control Point system, Food Safety Objectives, microbiological performance criteria, microbial testing, and surveillance; and steps for managing food safety in the future.

Founded in 1939, the Institute of Food Technologists is a nonprofit scientific society with 28,000 members working in food science, technology, and related professions in the food industry, academia, and government. As the society for food science and technology, IFT brings sounds science to the public discussion of food issues.

IFT Expert Report Panelists

IFT is deeply grateful to the expert report panelists for the time and effort that each of them expended on this project, bringing their expertise and insight into the state-of-the-science on the numerous topics addressed in the report. Panelists traveled to Chicago to participate in full-day meetings and devoted considerable additional time to drafting the report, participating in conference calls to discuss drafts, and reviewing the drafts. IFT sincerely appreciates these experts' invaluable dedication to furthering the understanding of emerging microbiological food safety issues and food safety management.

Morris Potter, D.V.M., Panel Chair
Lead Scientist for Epidemiology
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration, Atlanta, GA

Douglas Archer, Ph.D.
Professor, Food Science and Human Nutrition
University of Florida, Gainesville

Andrew Benson, Ph.D.
Assistant Professor, Food Microbiology
University of Nebraska, Lincoln

Frank Busta, Ph.D.
Emeritus Professor, Food Science and Nutrition
University of Minnesota, St. Paul

James S. Dickson, Ph.D.
Dept. Chair and Associate Professor, Dept. of Microbiology
Iowa State University, Ames

Michael Doyle, Ph.D.
Director, Center for Food Safety
University of Georgia, Griffin

Jeffrey Farber, Ph.D.
Director, Bureau of Microbial Hazards
Health Canada
Ottawa, Ontario, Canada

B. Brett Finlay, Ph.D.
Professor, Biotechnology Laboratory
University of British Columbia, Vancouver

Michael Goldblatt, Ph.D.
Director, Defense Advanced Research Projects Agency
Defense Sciences Office, Arlington, VA

Craig Hedberg, Ph.D.
Associate Professor, Division of Environmental and Occupational Health
University of Minnesota, Minneapolis

Dallas Hoover, Ph.D.
Professor, Dept. of Animal and Food Sciences
University of Delaware, Newark

Michael Jahncke, Ph.D.
Associate Professor and Director, Dept. of Food Science and Technology
Virginia Seafood Agricultural Research and Extension Center at Hampton
Virginia Polytechnic Institute and State University, Hampton

Lee-Ann Jaykus, Ph.D.
Associate Professor, Food Microbiology
Dept. of Food Science
North Carolina State University, Raleigh

Charles Kaspar, Ph.D.
Associate Professor, Food Research Institute and Environmental Toxicology Center
University of Wisconsin, Madison

Arthur P. Liang, M.D., M.P.H.
Director, Food Safety Initiative Activity
Division of Bacterial and Mycotic Diseases
National Center for Infectious Diseases
Centers for Disease Control and Prevention, Atlanta, GA

James Lindsay, Ph.D.
National Program Leader, Food Safety
Agricultural Research Service
U.S. Department of Agriculture, Beltsville, MD

James Pestka, Ph.D.
Professor, Food Science and Human Nutrition
Michigan State University, East Lansing

Merle Pierson, Ph.D.
Professor, Dept. of Food Science and Technology
Virginia Polytechnic Institute and State University, Blacksburg

Peter Slade, Ph.D.
Director of Research & Technical Services
National Center for Food Safety and Technology, Summit-Argo, IL

R. Bruce Tompkin, Ph.D.
Vice President of Food Safety
ConAgra Refrigerated Prepared Foods, Downers Grove, IL

Mary Lou Tortorello, Ph.D.
Research Microbiologist
National Center for Food Safety and Technology
U.S. Food and Drug Administration, Summit-Argo, IL

The participants on the expert panel were chosen based on their scientific expertise. Their contributions represent their individual scientific perspective and do not represent the perspective of their employer.

IFT Staff

Mary Helen Arthur, M.T.S.C.
Lead Editor, Expert Report
Dept. of Science and Communications, Chicago, IL

Rosetta Newsome, Ph.D.
Director, Dept. of Science and Communications
Chicago, IL

Fred Shank, Ph.D.
Vice President, Office of Science, Communications, and Government Relations
Washington, D.C.

Table of Contents

Introduction 4
Trinity of Factors 4
Evolution of Controls 4
Fig. 1a-Foodborne Illness 4
Fig. 1b-Reducing One Factor 4
Fig. 1c-Reducing Multiple Factors 4
Table 1. Evolution of Food Processing 5
Evolution of Food Safety Policies 5
Microbiology 101 6
Incidence and Prevalence of Foodborne Illness 8
Emergence of Pathogens 8
Fig. 2. Foodborne Illness Identification 8
Table 2. Foodborne Disease in the United States 9
Complex Drivers of Change 11
Framework for Food Safety Management 12

Science of Pathogenicity 13
Nomenclature 13
Table 3. Classic Microbial Nomenclature 13
Nomenclature of *Salmonella* and Fig. 3 14
Virulence 15
Fig. 4. Virulence and Foodborne Illness 15
Quorum Sensing 16
Virulence of *Salmonella* 17
Pathogens Are More Than Just Bacteria 18
Evolution 19
Fig. 5. Contrasting Views of Pathogen Evolution 20
Fig. 6. Genetic Material in *E. coli* 20
Evolution of *Salmonella* 21
Selection 21
Stress 22
σ^{38} Regulated Proteins and Table 4 24
Driving Forces in Pathogenicity 25
Emergence of Viruses, Parasitic Protozoa and Marine Biotoxins as Foodborne Pathogens 25
Pathogenicity of *E. coli* O157:H7 27

Humans as Hosts of Foodborne Disease 28
Manifestations of Clinical Disease 28
Table 5. Causes of Foodborne Illness 29
Pfiesteria piscicida and *Pfiesteria*-like Microbes as Potential Foodborne Pathogens 30
Resistance to Microbial Foodborne Disease 31
Susceptibility to Microbial Foodborne Disease 34
Table 6. Factors That Increase Host Susceptibility 34
Cryptosporidiosis 35
Individual Choices that Affect Disease Risk 36
Table 7. Factors That Increase Risk of Foodborne Disease 37
Modification of Susceptibility 39

Microbial Ecology and Foodborne Disease 40
PRE-HARVEST ENVIRONMENT 40
Overarching Issues 40
Table 8. Sources of Imported Fresh and Frozen Produce 41
Table 9. Percentage of Total U.S. Consumption Provided By Imports 41
Typical Pre-Harvest Environment for Foods of Plant Origin 42
Production Practices and Mycotoxins 42
Typical Pre-Harvest Environment for Foods of Animal Origin 43
Development and Dissemination of Resistant Organisms 44
Wild-Caught Shellfish and Fish 45
The Role of Microbiological Indicators in Assuring Food Safety 46
Specific Production Methods 47
HARVEST ENVIRONMENT 48
Produce 48
Food Animals 48
Aquaculture and Wild-Caught Fish and Shellfish 49
POST-HARVEST ENVIRONMENT 49
Food Animal Slaughter and Meat Processing 49
Post-Harvest Processing of Other Commodities 52
Water 52
Alternative Processing Technologies 53
Table 10. Limitations to Alternative Processing Technologies Currently Under Development 54
Validation of Treatment Effectiveness Using Microbiological Surrogates 55
Transportation and Storage 60
Retail and Food Service 61
Outbreaks of *Shigella sonnei* Infection Associated with Fresh Parsley 61
Microbial Stress Responses to Processing 62
Table 11. Conditions That Can Produce Sublethally Injured Cells 64
New Tools for Pathogen Research 64
Ability of Pathogens To Survive in the Environment 66

Application of Science to Food Safety Management 67
Risk Assessment 67
Risk Management Using Food Safety Objectives 69
Fig. 7. Framework for Food Safety Management 70
Hazard Control and Monitoring 71
Table 12. FSOs in the Food Safety Management Framework 72
Fig. 8. Establishing Performance Criteria 74
Fig. 9. Unequal Levels of Food Safety 75
Table 13. Probability of Acceptance (P_a) of Defective Product Using a 2-class Sampling Plan 76
Table 14. USDA Monitoring Program for *Salmonella* 76
Value of Test Results and Fig. 10 77
Testing for Mycotoxins 79
Surveillance for Foodborne Hazards and Illness 79
Outbreak Investigations and New Foodborne Pathogens 80
Animal Surveillance for *E. coli* O157:H7 82

Next Steps in Food Safety Management 85
Strategic Prioritization to Reduce Foodborne Disease 85
Strategies for the Future 87
A Cooperative Approach to the Safety of Sprouts 89
Anticipating the Future: Food Safety Issues on the Horizon 92

Conclusions 93

References 97

On the Cover

The image on the cover is a scanning electron micrograph of *Listeria monocytogenes*, an emerging foodborne pathogen.

Introduction

The continued occurrence of foodborne illness is not evidence of the failure of our food safety system. In fact, many of our prevention and control efforts have been—and continue to be—highly effective. The U.S. food supply is arguably among the safest in the world, but significant foodborne illness continues to occur. Despite great strides in the area of microbiological food safety, much remains to be done.

Foodborne illness is not a simple problem in need of a solution; it is a complex combination of factors that must be managed on a continual basis. Aside from the inherent ability of pathogens themselves to evolve, pathogens' victims and the microbial environment play a role in the changing nature of foodborne illness, opening new niches and creating new vulnerabilities. No matter how sophisticated and complex a system is developed, food safety management is never finished or complete, because change is constant.

Recognizing that food safety is a fundamental and continuing issue, the Institute of Food Technologists commissioned an expert panel to review the available scientific literature related to emerging microbiological food safety issues. The experts were charged with identifying the factors that make a microorganism "emerge" as an important foodborne pathogen and identifying mechanisms that use this knowledge to improve the safety of our food supply. The objective of this report is to increase understanding, among IFT members and other interested parties, of the scientific information on emerging foodborne pathogens relative to public policy issues and strategies for preventing foodborne illness.

Trinity of Factors

At the simplest level, foodborne illness can be reduced to three factors: the pathogen, the host, and the environment in which they exist and interact (see Fig. 1a). Complex relationships exist among these factors, and all three factors are necessary for foodborne illness to occur. For example, a susceptible host may consume food that contains a significant amount of a microorganism, but if the microorganism does not possess the traits necessary to cause illness, foodborne disease does not occur. Similarly, adequately cooking a food to kill the pathogenic microbes can eliminate the exposure factor and render the food safe.

When one or more of the three factors changes, new foodborne pathogens "emerge." For example, host susceptibility can increase so much that existing microorganisms that do not cause illness in the general population achieve pathogen status in the newly immunocompromised individuals. The change also can be increased virulence, e.g., when a microorganism acquires characteristics that help it invade the human body. Or it can be new exposure, e.g., when fruit from one region carries a pathogenic microorganism to populations in a different geographic region that have never before been exposed.

This trinity is also the key to reducing foodborne illness. Prevention and control efforts often focus on the contribution of one of these factors, such as washing vegetables to remove surface contamination (Fig. 1b). In many cases, however, the most effective approach addresses more than one factor. Current technologies and production methods cannot provide a food supply that is completely free of all pathogenic microorganisms. Fortunately, even small reductions in several factors can have a substantial combined effect (Fig. 1c).

Evolution of Controls

New technologies, production practices and food manufacturing processes are developed to meet the needs of a changing society. Early food preservation methods—such as canning, cheese making, bread making, brewing, fermentation, pickling, salting, and drying—were used to provide sufficient year-round food availability. Later developments reflected a new focus on food safety, variety, convenience, and nutritive and sensory quality.

At the beginning of the 20th century, contaminated milk, meat, and other foods caused large outbreaks and many sporadic cases of foodborne disease, often with fatal consequences. The revolution in sanitation and hygiene related to food and water and the almost universal adoption of thermal pasteurization for milk produced tremendous improvements in food safety. New technologies with increasing sophistication have yielded continued improvements in microbiological food safety while delivering better quality foods with greater nutritional value and superior sensory characteristics (see Table 1).

Innovations in packaging have been integral to the developments in food processing and product development. Packages contain and protect their food contents and inform consumers; they also

Fig. 1a- Foodborne Illness

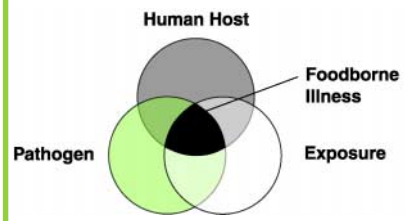


Fig. 1b-Reducing One Factor

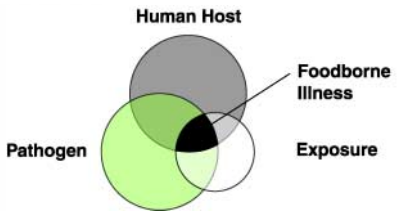


Fig. 1c-Reducing Multiple Factors

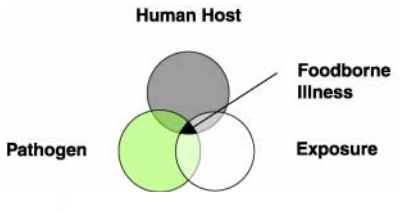


Table 1. Evolution of Food Processing (Goldblith, 1989; IFT, 2000a, 1989; Lund, 1989)

Early times	**Heating** Cooking food kills many foodborne pathogens
1770s-1800s	**Canning/Thermal Processing** Significant discoveries in response to industrialization forces and Napoleon's armies' need for less dependence on local provisions
1890s	**Pasteurization** Thermal treatment of raw milk to prevent milk from transmitting pathogens
1920s - 1930s	**Safe Canning/Processing Parameters** Calculation of product heat penetration curve and initial microbial contamination level to determine minimum time-temperature combination for commercial sterility
1940s	**Freezing** Mechanical quick-freezing methods to preserve while maintaining quality
1950s	**Controlled Atmosphere Packaging** Reduced oxygen levels, increased concentrations of carbon dioxide, or selective mixtures of atmospheric gases to limit respiration and ethylene production, delay ripening and decay, and increase refrigerated product shelf life
1960s	**Freeze Drying** Rapid deep-freezing followed by sublimation of water by heating the frozen product in a vacuum chamber. Best known applied to coffee, to preserve delicate aroma compounds and maintain flavor and odor
1940s-1980s	**Aseptic Processing and Packaging** High-temperature, short-time sterilization of food product independent of the container, container sterilization, and filling of product in sterile atmosphere, resulting in increased food quality and nutrient retention
	Irradiation Non-thermal process to kill pathogens, insects, and larvae, inhibit sprouting, and delay ripening and food spoilage
1990s	**Carcass Spray Treatments (e.g., water, acid), Steam Vacuuming, Steam Pasteurization** Carcass decontamination interventions to meet microbiological performance criteria
	High Pressure Processing Foods subjected to specified pressures and temperatures to preserve food while maintaining quality

preserve, perform, communicate, and sell (Downes, 1989). From the hermetically sealed containers for shelf-stable foods developed in the early part of the 19th century, to the metal cans for heat-processed foods, folding cartons, and milk bottles of the latter part of the 19th century, to the boil-in-bags, plastic tubs, high density polyethylene gallon milk jugs, aseptic cartons, and microwavable polymers of the 20th century, packaging has played a key role in the development of the food industry in the Western world in the 20th century (Downes, 1989).

However, changes in technology are not without risk. Conventional wisdom of decades ago held that properly refrigerated foods would remain safe because it was thought that pathogenic bacteria would not grow at refrigeration temperatures, but this is not always the case (Marth, 1998). Innovations in food processing, such as modified atmosphere packaging, can offer the benefit of greatly extending the shelf life of refrigerated foods but may present microbiological safety challenges. For example, modified atmosphere packaging of fresh packed, sliced mushrooms may allow the growth of *Clostridium botulinum* and potential toxin production (Doyle, 1998). Altering the package, incorporating microscopic holes to allow oxygen to permeate the interior of the packaged product, was another factor critical to ensuring the safety of modified atmosphere packaged fresh packed, sliced mushrooms. For extended shelf life refrigerated foods, strict temperature control and acceptable product shelf life are critical factors to consider (Doyle, 1998). As new technologies are introduced, they must be evaluated for their potential effect on microbiological food safety.

Despite all of the significant advances to date, our growing knowledge base continues to expose the role of various foods and technological innovations in foodborne hazards, and changes in the food, the consuming public, and the pathogens themselves continue to make foodborne disease an important and ever-changing challenge both for the industrialized and the developing world.

Evolution of Food Safety Policies

Current U.S. food safety policies are the accretions of decades of relatively independent efforts to address specific problems. Most are rooted in the sanitary revolution that occurred at the beginning of the 20th century, and they have characteristics that have served us well during the transition from an agrarian to an industrialized society.

Generally, these regulatory policies respond in one of three ways to obvious hazards that pose clear risk to human health. First, for hazards that have straightforward technical fixes, regulations require the application of the appropriate technologies. Regulatory standards frequently have been set at the performance limit of the technology or the detection limit for the analytical test used for process verification. However, technologies to mitigate hazards are not al-

ways apparent. In these cases, the regulatory response has been to either keep the hazardous food out of the marketplace or to forgo regulatory action and rely on prudent people to protect themselves.

Numerous food safety concerns have been successfully addressed by this regulatory paradigm. The promulgation of regulations for low acid canned food virtually ended the historic association of botulism with commercially canned food. Under the regulations, commercially canned foods undergo a minimum calculated destruction of 12D for *C. botulinum*. Another very successful example is the water quality standards for shellfish growing waters. These standards protected consumers from shellfish-associated typhoid fever at a time when typhoid was fairly common in coastal communities and contaminated shellfish was an important source of infection. Historically, the safety of foods without a pathogen elimination step earlier in the line has depended upon proper cooking.

The extraordinary complexity of contemporary food safety issues presents major challenges to food safety policy formulation. Factors like the global sourcing of products and ingredients, changes in land use, and evolution of science and technology have radically changed hazards associated with a particular food and the control options available.

These challenges present themselves in many ways depending on the particular hazard. For example, the indicator organisms used to predict the presence of *Salmonella* Typhi in shellfish growing waters poorly predict the presence of

Microbiology 101

The characteristics of the various microorganisms are part of what makes microbiological food safety issues so complex. One type of microorganism may thrive under conditions that are fatal to a different microbe. Some microbial pathogens cause disease by infecting the human host, while others produce toxins that cause illness. Some pathogens can multiply in food during storage while others cannot. Because most microorganisms can reproduce within a matter of minutes, these pathogens can evolve quickly when environmental stresses select for strains with unique survival characteristics.

Types of Microorganisms

Microorganisms are divided into three distinct categories: prokaryotes, eukaryotes, and viruses. Both prokaryotes and eukaryotes are highly regulated cells that possess elaborate "sensing" systems, enabling them to be aware of and react to their environment as it changes.

Prokaryotes—Bacteria

Prokaryotes are single-celled living microorganisms that have no nuclear membrane separating their genetic material from the cytoplasm within the cell. They are microscopic, in that they cannot be seen with the unassisted human eye. Bacteria are generally free-living in the environment, although some have complex nutrient requirements and can grow only in special niches.

Bacteria are arguably the world's most successful life form. Their diversity and "complexity through simplicity" have and will continue to assure their survival. Although the vast majority of bacteria are harmless or helpful to humans, some are pathogenic.

Eukaryotes—Parasites and Fungi

Eukaryotes are multicellular living organisms that possess a nuclear membrane that separates their nucleic acid from the cytoplasm. They are larger than bacteria and are sometimes able to be seen by the human eye. Eukaryotes are generally free-living in the environment.

Some protozoa can be foodborne pathogens (e.g., *Cyclospora, Giardia*). They usually exist in multiple forms, some of which are environmentally stable, but they seldom multiply in or on human food. Fungi such as molds and yeasts can multiply in or on human food and also can be pathogenic.

Viruses

Foodborne pathogenic viruses are comprised of a single type of nucleic acid surrounded by a protein coat. Viruses are not free-living. In fact, they are not living beings at all, but are obligate intracellular parasites. They are smaller than bacteria (10-350nm), and some viruses prey on bacteria (bacteriophages).

Types of Bacteria

Bacteria come in various shapes, such as rods, spheres (cocci), and spirals. As a response to certain adverse environmental conditions, some bacteria can form spores. The spores start as dense regions within the cell, but as the cell deteriorates, the spore is released into the environment. Spores are extremely impervious to physical and chemical harm, making them difficult to inactivate in the food processing environment.

In general, the bacterial kingdom can be divided into gram-positive and gram-negative cells. These designations are given based on the results of a staining procedure that separates the two divisions by color, which is reflective of the composition of their cell wall.

Growth and Life Span

Bacteria—Bacteria are the most adaptable life form on Earth. Bacteria have "optimal" (preferred) growth conditions, but some can grow and/or survive at extremes of temperature, pH, osmotic pressure, and barometric pressure. Bacteria are genetically programmed for maximum survival.

At optimal growth conditions, a bacterial cell may divide every 10-20 minutes. Assuming no death, a single cell could thus give rise to a bacterial mass equal to the Earth's mass in one or two days. Obviously death occurs, because of factors such as nutrient limitations or end product toxicity.

Viruses—In general, viruses reproduce more rapidly than bacteria, but they can only grow in an infected host cell, not in food. A single infected host cell may give rise to hundreds or thousands of new viruses within a few hours, each of which may infect a new host cell.

Roles of Foodborne Microorganisms

Not all microorganisms in foods are harmful. In fact, only a small proportion of foodborne organisms have been asso-

Vibrio, hepatitis A, or Norwalk-like viruses. Typhoid fever is now extremely uncommon in U.S. coastal waters, and it is now these other shellfish-associated pathogens from which we need to protect ourselves.

Pathogens on foods that are often consumed without cooking present a significant challenge. The pathogen *Cyclospora cayetanensis* is very likely of human origin, but our limited knowledge about its natural ecology does not enable us to assure its absence from fresh produce. Although adequate cooking will kill this parasite, the foods associated with human infection—raspberries, basil, and other fresh produce—are generally not cooked before consumption.

When microorganisms such as *Shigella* or the hepatitis A virus are found on fresh produce, it can be difficult to determine whether the contamination occurred during food preparation, during distribution, during processing and packing, or in the growing fields. Under these circumstances, the appropriate point of intervention is difficult to identify.

Modifications to thermal processing have made a wide array of food types possible, including a proliferation of "pasteurized" food products distributed in flexible plastic packages that require refrigeration. Although thermal processing inactivates a large majority of the vegetative spoilage organisms, in controlled atmosphere packaging, it also drives off oxygen and fails to inactivate sporeformers like *C. botulinum*. If the cold chain is not properly maintained, botulinum toxin can be produced before food spoilage is detectable.

ciated with disease in normal, healthy animals and humans.

Commensal Microbes

Virtually all raw food contains microorganisms. The source of these is the production environment, where a wide variety of organisms are environmentally ubiquitous. Processing and handling of foods can also contribute to the types and numbers of commensal organisms on foods. Most of these commensal organisms are harmless to animals and humans; in fact, they may actually be beneficial in that they provide high levels of "competitive" microflora that usually grow faster than contaminating pathogens. Although the purpose of many common food processing methods such as pasteurization and canning is the destruction of pathogens, commensal microorganisms are often destroyed in the process as well.

Spoilage Microbes

Spoilage may be defined as a condition in which food becomes inedible because of undesirable changes in color, flavor, odor, appearance, and texture. This condition occurs primarily because the organisms grow to high levels, producing enzymes that break down food components such as fats, proteins, and sugars. In most instances, spoilage is caused by commensal organisms that have been allowed to reach populations in the range of 10^5 to 10^7 CFU/g of food. Different classifications of foods (such as red meats, vegetables, fish, etc.) have different spoilage profiles because the food environment will dictate which organisms will grow, dominate, and cause spoilage. For instance, spoilage of raw meats is almost always associated with gram-negative psychrotrophs, the so-called cold-thriving organisms, because they grow at refrigeration temperatures. Fresh fruits are frequently spoiled by yeasts and molds that are able to thrive in acidic conditions. Most spoiled foods do not cause foodborne disease; in reality, the high levels of spoilage organisms have frequently "out-competed" the pathogens, keeping pathogen growth in check.

Beneficial Microbes

Perhaps the most widely recognized group of beneficial foodborne microorganisms are the members of the lactic acid bacteria (LAB) group. This is a functional name used to classify fermentative organisms that produce lactic acid as the primary by-product of metabolic activity, although other products, such as alcohol and carbon dioxide may be produced as well. These metabolic products are responsible for the characteristic flavor, odor and texture of fermented food products. The lactic acid bacteria are commonly used in dairy, vegetable and meat fermentations. Notable members of this group belong to the *Lactococcus*, *Lactobacillus*, *Pediococcus*, and *Leuconostoc* genera. Some species of yeasts and molds can also be used in the commercial production of fermented foods, including *Saccharomyces cerevisiae*, used frequently for producing bread, beer and wines, and *Aspergillus oryzae*, used in the fermentation of oriental foods such as soy sauce.

Foodborne Pathogens

Foodborne pathogens encompass a relatively small group of foodborne microorganisms that are associated with disease in humans and/or animals. Pathogenic microbes are capable of causing illness, either by infecting the host or by producing toxins that cause the host to become ill. Some microorganisms are pathogenic in one host species but not in another. For example, *Escherichia coli* O157:H7 causes illness in humans but not in cattle, its primary host.

Microbiological Indicators

An index or indicator refers to a single or group of microorganisms, or alternatively, a metabolic product, whose presence in a food or the environment at a given level is indicative of a potential quality or safety problem. Microbiological indicators are used in place of direct testing for a pathogen, largely because they are easier to work with. Indicators may be a specific microorganism (e.g., *E. coli*), a metabolite (e.g., lactic acid titration), or some other indirect measure (e.g., ATP bioluminescence as a measure of sanitation efficacy). Using a specific microorganism as an indicator is difficult, because appropriate indicator organisms are difficult to identify. An "ideal" indicator organism: (1) has a history of presence in foods at any time that the target pathogen or toxin might be present; (2) is present at concentrations directly related to that of the target pathogen or toxin; (3) is absent from food when the target is not present; (4) has growth rates equivalent to, or slightly greater than, the pathogen; (5) has rapid, simple, and inexpensive quantitative assays available; (6) has similar resistance profiles to the target; and (7) is nonpathogenic.

Non-thermal processes also have been modified over time. Consumers want foods with fewer preservatives, less salt, fewer calories, and better texture; the food industry has responded with many new formulations. However, substituting ingredients with gums or other fat replacers and reducing salt or sugar can require a reevaluation of food safety control measures. For example, the replacement of sugar with an alternative sweetener in hazelnut yogurt and failure to evaluate the impact of this change on food safety resulted in an outbreak of botulism (O'Mahony et al., 1990).

In addition to the impact of changes in processing, scientists have discovered that some foodborne pathogens survive traditional processes better than expected. For example, *Salmonella* have been found in 60-day aged cheese and on raw almonds, and newly recognized pathogens such as *E. coli* O157:H7 are more tolerant of conditions of low pH and other traditional barriers than anticipated. The resistance of pathogens to traditional treatments affects the safety of our drinking and processing water as well. We have relied on chlorination to rid drinking water of pathogens for decades, but recent waterborne outbreaks have been caused by parasites, such as *Giardia* and *Cryptosporidium*, that are not controlled by chlorine.

Handling during preparation in the home or foodservice establishment may affect the pathogens present in the food. For foods in which preparation should kill the pathogens, recurring outbreaks of foodborne illness highlight our limited ability to tightly control food preparation behaviors and practices. Certainly the incidence of foodborne disease would be significantly reduced if we could eliminate pathogens earlier in the food chain. However, the initial number of the pathogen in the food is only one factor in the risk of foodborne illness. At some point, improvements in sanitation will produce only small incremental gains. As the level of contamination becomes increasingly small, other food safety approaches will need to be adopted.

Incidence and Prevalence of Foodborne Illness

Food safety is a complex issue that depends on a number of interrelated environmental, cultural, and socioeconomic factors. More than 200 known diseases are transmitted through food, and more than half of all recognized foodborne disease outbreaks have unknown causes, indicating the real number of disease-causing agents is likely much larger than 200. The recognized causes of foodborne illness include viruses, bacteria, parasites, manmade chemicals, biotoxins, heavy metals, and prions. The symptoms of foodborne illnesses range from mild gastroenteritis to life threatening neurologic, hepatic, and renal syndromes.

In the United States, foodborne diseases have been estimated to cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths each year (Mead et al., 1999). A pathogen's ability to cause illness can be very different from the severity of the illness it causes. Some pathogens such as Norwalk-like viruses cause a great number of illnesses (9.2 million per year) but the fatality rate is very small (0.001%) (see Table 2). Others such as *Vibrio vulnificus* cause few illnesses (47 per year), but many of those illnesses are fatal (38.3%). *Salmonella*, *Listeria monocytogenes*, and *Toxoplasma gondii* are responsible for more that 78% of the deaths but only approximately 11% of total cases of foodborne disease. The issue is further complicated by pathogens, such as *L. monocytogenes*, that cause little or no illness in healthy individuals but cause severe illness and death in sensitive populations, including the immunosuppressed, the elderly and the developing fetus. Prioritizing food safety resources can be difficult.

Scientists gather data about the incidence and severity of foodborne illness through surveillance, both passive and active. Mild cases of illness often are not captured by surveillance programs because medical intervention is not required for recovery. Many steps are required for a foodborne pathogen to be identified as the cause of illness and for data to be gathered through surveillance programs (see Fig. 2). Furthermore, because many pathogens transmitted through food also may be spread by contaminated water and person-to-person contact, the role of food can be difficult to assess. Finally, some proportion of foodborne illness is caused by pathogens that have not yet been identified and thus cannot be diagnosed. In fact, many of the pathogens of concern today were not recognized as causes of foodborne illness just 20 years ago (Mead et al., 1999).

New scientific advances make it possible to approach foodborne illness from a different, broader perspective. More powerful diagnostic procedures and better communication technology allow improved tracking and surveillance for foodborne illness. Genetic identification methods allow scientists to link geographically distinct outbreaks of foodborne illness to a single source. Pathogens can appear to emerge simply because scientists develop methods to identify the presence of certain microorganisms and link them to foodborne disease.

New technologies based on recent advances in genomics also give scientists greater insight into pathogen virulence and evolution, opening the door to better controls and therapeutics. Future scientific advances will continue to enhance efforts to identify and understand foodborne pathogens, and these insights will contribute the data necessary for science-based risk assessment and food safety management.

Emergence of Pathogens

The terminology "newly emerging pathogen" has become somewhat overused, or perhaps it is merely ill defined. True pathogen "emergence" could be directly linked to evolution, whether that evolution occurs gradually or rapidly.

In a broader context, emergence can

Fig. 2. Foodborne Illness Identification

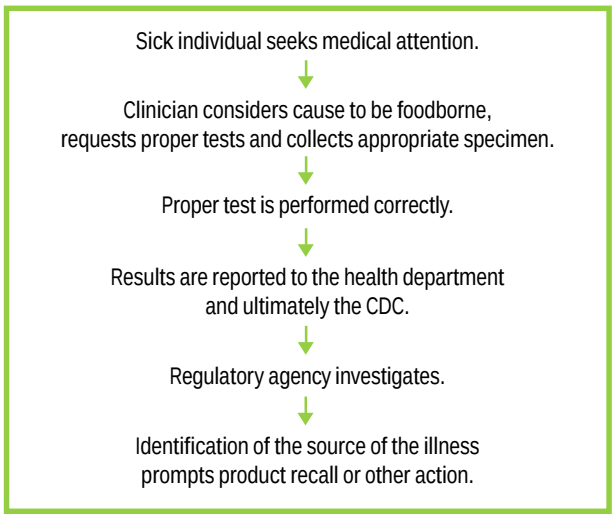


Table 2. Foodborne Disease in the United States, Including Estimated Annual Prevalence (FDA/CFSAN, 2002; Mead et al., 1999)

Microorganism	Principal Symptoms[a]	Onset[a]	Potential Food Contamination[a]	Illnesses[b]	Deaths[b]
Bacteria					
Bacillus cereus				27,360	0
	Diarrheal—Watery diarrhea, abdominal cramps and pain	6-15 hr	Meats, milk, vegetables and fish	n/a	n/a
	Emetic—Nausea and vomiting	0.5-6 hr	Rice products, starchy foods (e.g., potato, pasta, and cheese products	n/a	n/a
Brucella spp.	Sweating, headache, lack of appetite, fatigue, fever[c]	Days to weeks[c]	Raw or unheated processed foods of animal origin (e.g., milk, milk products, cream, cheese, butter)[c]	777	6
Campylobacter spp.				1,963,141	99
C. jejuni	Watery diarrhea, fever, abdominal pain, nausea, headache, muscle pain	2-5 days	Raw chicken, beef, pork, shellfish; raw milk	n/a	n/a
Clostridium botulinum	Weariness, weakness, vertigo, double vision, difficulty swallowing and speaking	18-36 hr	Improperly canned or fermented goods	58	4
Clostridium perfringens	Intense abdominal cramps, diarrhea	8-22 hr	Meat, meat products, gravies	248,520	7
Escherichia coli					
Enterotoxigenic	Watery diarrhea, abdominal cramps, fever, nausea, malaise	24 hr	Foods contaminated by human sewage or infected food handlers	55,594	0
Enteropathogenic	Watery or bloody diarrhea	n/a	Raw beef and chicken; food contaminated by feces or contaminated water	n/a	n/a
E. coli O157:H7	Severe abdominal cramps, watery or bloody diarrhea, hemolytic uremic syndrome	1-2 days	Undercooked or raw hamburger, alfalfa sprouts, unpasteurized juices, dry-cured salami, lettuce, game meat, cheese curds, raw milk	62,458	52
Enteroinvasive	Abdominal cramps, vomiting, fever, chills, generalized malaise, hemolytic uremic syndrome	12-72 hr	Food contaminated by human feces or contaminated water, hamburger meat, unpasteurized milk	n/a	n/a
Listeria monocytogenes	Nausea, vomiting, diarrhea; influenza-like symptoms; meningitis, encephalitis; septicemia in pregnant women, their fetuses or newborns; intrauterine or cervical infection that may result in spontaneous abortion or stillbirth	Few days-3 weeks	Raw milk, cheeses (particularly soft-ripened varieties), raw vegetables, raw meats, raw and smoked fish, fermented sausages	2,493	499
Salmonella spp.			Raw meats, poultry, eggs, milk and dairy products, fish, shrimp, frog legs, yeast, coconut, sauces and salad dressings	n/a	n/a
S. Typhi and *S.* Paratyphi	Typhoid-like fever, malaise, headache, abdominal pain, body aches, diarrhea or constipation	7-28 days[c]		659	3
Other *Salmonella* spp.	Nausea, vomiting, abdominal cramps, fever, headache; chronic symptoms (e.g., arthritis)	6-48 hr 3-4 weeks		1,341,873	553
Shigella spp.	Abdominal pain and cramps; diarrhea; fever; vomiting; blood, pus or mucus in stools; tenesmus	12-50 hr	Salads (potato, tuna, chicken, macaroni), raw vegetables, bakery products (e.g., cream-filled pastries), sandwich fillings, milk and dairy products, poultry	89,648	14
Staphylococcus aureus	Nausea, vomiting, retching, abdominal cramps, prostration	1-7 hr	Meat and meat products, poultry, egg products, salads (egg, tuna, chicken, potato, macaroni), cream-filled bakery products, milk and dairy products	185,060	2
Streptococcus spp.				50,920	0
Group A (*S. pyogenes*)	Sore, red throat; pain on swallowing; tonsillitis; high fever; headache; nausea; vomiting; malaise; rhinorrhea	1-3 days	Temperature-abused milk, ice cream, eggs, steamed lobster, ground ham, potato salad, egg salad, custard, rice pudding, shrimp salad	n/a	n/a

[a] n/a indicates FDA/CFSAN (2002) did not provide information.
[b] n/a indicates Mead et al. (1999) did not provide an estimate for this pathogen.
[c] As described by ICMSF (1996).

Table 2. Foodborne Disease in the United States, Including Estimated Annual Prevalence, continued

Microorganism	Principal Symptoms[a]	Onset[a]	Potential Food Contamination[a]	Illnesses[b]	Deaths[b]
Group D (other *Streptococcus* spp.)	Diarrhea, abdominal cramps, nausea, vomiting, fever, chills, dizziness	2-36 hr	Underprocessed or improperly prepared sausage, evaporated milk, cheese, meat croquettes, meat pie, pudding, raw milk, pasteurized milk	n/a	n/a
Vibrio cholerae				49	0
V. cholerae serogroup O1	Mild watery diarrhea, acute diarrhea, rice-water stools	6hr-5 days	Raw, improperly cooked, or recontaminated shellfish	n/a	n/a
V. cholerae serogroup non-O1	Diarrhea, abdominal cramps, fever, vomiting, nausea; blood or mucus-containing stools	48 hr	Raw, improperly cooked, or recontaminated shellfish	n/a	n/a
Vibrio parahaemolyticus	Diarrhea, abdominal cramps, nausea, vomiting, headache, fever, chills	4-96 hr	Raw, improperly cooked, or recontaminated shellfish and fish	n/a	n/a
Vibrio vulnificus	Fever, chills, nausea, septicemia in individuals with some underlying diseases or taking immunosuppressive drugs or steroids	16 hr	Raw or recontaminated oysters, clams, crabs	47	18
Vibrio, other	n/a	n/a	n/a	5,122	13
Yersinia enterocolitica	Fever, abdominal pain, diarrhea and/or vomiting	24-48 hr	Meats, oysters, fish, raw milk	86,731	2
Parasites and Protozoa					
Anisakis simplex	Tingling or tickling in the throat, vomiting or coughing up worm(s), abdominal pain, nausea	1hr-2 weeks	Raw or undercooked seafood	n/a	n/a
Ascaris lumbricoides	Exiting of roundworm, vague digestive tract discomfort, pneumonitis	n/a	Raw produce grown in soil contaminated by insufficiently treated sewage	n/a	n/a
Cryptosporidium parvum	Severe watery diarrhea (intestinal illness); coughing, fever and intestinal distress (pulmonary and tracheal illness)	1-12 days	Foods contaminated via food handlers and manure	30,000	7
Cyclospora cayetanensis	Watery diarrhea, explosive stools, loss of appetite, bloating, stomach cramps, vomiting, aching muscles	1 week	Water or food contaminated with infected stool	14,638	0
Giardia lamblia	Diarrhea, abdominal cramps, bloating, weight loss, malabsorption	1 week	Food contaminated via infected food handlers	200,000	1
Taenia spp.	Discharge of proglottids, anal itching, abdominal pain, nausea, weakness, weight loss, intestinal disorder	n/a	Raw or undercooked beef, pork	n/a	n/a
Toxoplasma gondii	Flu-like symptoms, swollen lymph glands, muscle aches and pains[d]	10-23 days[d]	Raw or undercooked meats, especially pork, lamb, venison[d]	112,500	375
Trichinella spiralis	Severe gastrointestinal distress, nausea, vomiting, headaches, weakness, muscle pain, chills, difficulty breathing, body swelling, visual deficiencies, fever, night sweating[c]	3-14 days	Raw or undercooked pork or wild game	52	0
Viruses					
Hepatitis A	Fever, anorexia, malaise, nausea, abdominal discomfort; jaundice may follow	10-50 days	Shellfish, salads, other foods contaminated via infected food handlers or water	4,170	4
Norwalk-like viruses	Nausea, vomiting, low-grade fever, diarrhea, abdominal pain	24-48 hr	Shellfish and salad ingredients contaminated by infected food handlers or fecally contaminated water	9,200,000	124
Rotavirus	Vomiting, watery diarrhea, low-grade fever; severe in infants and young children	1-3 days	Foods contaminated via fecal contaminated food handlers	39,000	0

[a] n/a indicates FDA/CFSAN (2002) did not provide information.

[b] n/a indicates Mead et al. (1999) did not provide an estimate for this pathogen.

[c] As described by ICMSF (1996).

[d] From CDC (2002).

be used to describe a recent significant change. Using this interpretation, a pathogen could be described as emerging when it is first linked to disease, when the illness it causes suddenly increases in frequency or severity, or even when a pathogen recognized for a significant amount of time suddenly "reappears." In terms of public perception, all these scenarios may be considered emerging microbiological food safety issues.

Public Perception of Emergence

C. cayetanensis, a recent arrival in the United States, is an example of the kinds of surprises that can occur as a result of increasing world trade in ready-to-eat foodstuffs. As foodstuffs are transported ever greater distances, pathogens can be transported to new areas as well. The United States also has experienced illness outbreaks caused by *Salmonella* serotypes either rarely or never before isolated here, apparently for the same reason(s) that *Cyclospora* "emerged."

Campylobacter jejuni and *L. monocytogenes* were well accepted as foodborne pathogens in the 1970s and 1980s, respectively, and, as such, they are hardly new. Prior to 1972, *C. jejuni* could not be cultured from feces, so it was not recognized as a common human pathogen, nor was the frequency of its presence in food, particularly raw poultry, realized. With improvements in both the sensitivity and rapidity of the available methodology, *C. jejuni* may appear to be an emerging pathogen. However, scientists cannot determine if the frequency of *C. jejuni* isolation from human stool or food is truly increasing, or if laboratory-induced bias gives the appearance of an increase. Similarly, methods for isolation of *L. monocytogenes* from foods and the environment have improved since its "emergence" as a foodborne pathogen in the mid-1980s. As a result, it appears that the frequency of *L. monocytogenes* in foods and the environment is increasing, but the influence of laboratory-induced bias is difficult to weigh. For both of these pathogens, increased awareness of analytical laboratory personnel and physicians have affected the frequency of isolation from foods and diagnosis of human illness.

Changes in foods serving as vectors have brought new attention to a long-recognized pathogen, *C. botulinum*, the causative agent of botulism. Botulism from commercially canned foods has been essentially eliminated; however, cases of botulism in the late 1980s and early 1990s led scientists to discover that *C. botulinum* could grow and produce toxins in foods such as twice-baked potatoes, grilled onions, and garlic-in-oil. When these new food associations were discovered, even *C. botulinum* was described by some as an emerging pathogen. Large outbreaks—although recurrences of situations that have happened in the past—can propel a pathogen to "emerging" status whether truly deserved or not. In many cases, it is the new food vehicle that is the surprise, and not so much the pathogen itself.

Emergence as a Function of Evolution

Evolution can produce pathogens that are truly emerging, in that the microorganisms have new characteristics that enable them to cause disease. Bacteria have evolved highly sophisticated signal transduction systems that allow the microorganisms to respond at a genetic level to environmental conditions in a coordinated manner. The many environmental stimuli that trigger such activity are collectively referred to as stresses.

The genetic response to stress(es) can activate certain virulence determinants, a microbe's "contingency plan" for surviving in hostile environments. Beyond activating virulence determinants, external stresses also accelerate the bacteria's rate of evolution, meaning new pathogens could "emerge" relatively quickly. Such events probably contributed substantially to the evolution of the highly virulent O157:H7 strain of *E. coli*.

Bacteria are constantly mutating, and environmental forces may select a mutation that confers an advantage in the face of the environmental challenge. The many and varied environmental stresses commonly include starvation, high or low pH, oxidation, heat, cold, and osmotic imbalance. The genetic response to one stress may protect the microbe from a different stress, a phenomenon known as cross protection.

Bacteria possess specific genetic loci, particularly in "contingency genes," that are highly mutable when compared with "housekeeping genes" that are relatively stable. Contingency genes help a microbe successfully interface with environmental change, while housekeeping genes run the routine cellular machinery. Genetic variation can occur in many ways, including increased mutation. Stresses can create microorganisms with greatly enhanced mutation frequencies (1000-fold or more). The large number of different mutations increases the chance of a mutation that will enable survival in the stressful environment. These hypermutable microorganisms also may more readily share DNA with other microorganisms, even remotely related species. Horizontal transmission of genetic material from one microorganism to another can result in quantum jumps in evolution. Gene transfer between separate lineages of a bacterial pathogen can lead to the emergence of altogether new pathogens. Recently sequenced bacterial genomes reveal more extensive exchange of genetic material between species than had been expected.

Complex Drivers of Change

No matter how emergence is defined, it becomes clear that the interrelationship of pathogen, host and environment plays a key role in microbiological food safety. A number of factors will drive the emergence of new food safety concerns, including changes in the characteristics of the consuming public, changes in the foods we manufacture and sell, changes in the hazards themselves, and changes in the ability of public health officials to identify illnesses as foodborne and to trace the illnesses to their food source.

Host Factors

Changing demographic characteristics of consumers affect the number of cases of foodborne illness. As the world's population continues to grow, constant rates of disease will increase the total number of cases. In addition, the proportion of the population that is at high risk of foodborne infections, illness, and death is rising. Factors that increase the impact of foodborne diseases include age, chronic diseases, immunosuppressive conditions, and pregnancy. The immune system functions less effectively in the elderly, putting them at greater risk. A growing proportion of our population is immunocompromised due to HIV infection, cancer chemotherapy, and drugs used to combat rejection of transplanted organs. Larger numbers of people with chronic diseases, like diabetes, now live longer and also are at increased risk of foodborne diseases.

Other consumers are at elevated risk of foodborne illness because of the in-

creased likelihood of exposure. This elevated risk is sometimes due to food preferences based on ethnicity, age, or gender. Young adult males, for example, are more likely to eat inadequately cooked ground beef.

Environmental Factors

Food industry economics and technical factors continue to drive consolidation in primary agricultural production and food processing. Although this helps reduce costs and assure uniform quality, when a large lot of a contaminated food enters distribution, the scope of the resulting outbreak is increased.

Global sourcing also carries the potential to move pathogens and toxins from areas in which the pathogen is indigenous to areas in which it has not previously existed. Unfamiliarity complicates diagnosis and containment and can result in outbreaks that become quite large before they are recognized. Hazards are truly mobile, and our food safety programs must be very agile to reduce our risk.

Even slight increases in environmental temperatures can significantly affect the risk of foodborne illness. The growth of algae in surface waters, estuaries, and coastal waters is sensitive to temperature. About 40 of the approximately 5,000 known species of marine phytoplankton (algae) can produce biotoxins, which may reach human consumers through shellfish. Warmer sea temperatures can encourage a shift in species composition of algae toward the more toxic dinoflagellates. Upsurges of toxic phytoplankton blooms in Asia are strongly correlated with El Niño, and in the United States, paralytic shellfish poisoning and other marine biotoxin-induced diseases have been associated with shell stock harvested from beds traditionally considered safe.

Consumer desires drive food product development. Food manufacturers respond to desires for "fresher" food, low fat products, or ready-to-eat foods by developing new processes or reformulating existing products. Changes in the food processing environment or product formulations can create a new niche for pathogenic microorganisms. Producing familiar foods in nontraditional sites also may lead to introduction of new food hazards; such was the case with the first outbreaks of cyclosporiasis associated with raspberries imported into the United States and Canada from Guatemala.

Pathogen Factors

Stable and accurate transfer of genetic information from parent to offspring is essential for the preservation of a species. However, keeping pace with an ever-changing environment also requires variability. When naturally occurring bacteria, for example, divide, most of the offspring look and act just like their parents, but a small proportion of the offspring mutate, increasing the chance that some might survive in a new, hostile environment. If the environment has not changed, these new strains may not survive, but this natural occurrence makes it almost certain that traditional food processes will fail to deliver their predicted level of safety at some point. This is part of nature and happens without human intervention.

In addition to this unstimulated hypermutability, the food production and processing environment can increase the rate of change in foodborne pathogens. Bacterial stress responses may increase pathogen virulence, and other actions can affect which microorganisms survive and dominate in a particular environment. For example, use of antimicrobial agents during livestock production may select resistant strains from a background of susceptible microorganisms, increasing the likelihood that the microorganisms in a food are resistant to those and related antimicrobials. Even if these microorganisms are not pathogenic, they can share the genetic material that enables them to resist antimicrobials with pathogenic microorganisms in the human gut, producing pathogens that cause infections that may be difficult to treat.

Through improved laboratory techniques, scientists are identifying adverse health effects associated with ever-smaller levels of exposure to natural and anthropogenic substances. New ELISA and radioimmunoassays for various mycotoxins are pushing tolerances for common mycotoxins down and are finding more poorly characterized mycotoxins in a broad array of commodities. Our understanding of biology, however, is not keeping up with our laboratory skills, and judging the public health significance of "positive" laboratory results is becoming more difficult. Unlocking the human genome and the genomes of pathogenic microorganisms, however, is beginning to clarify the very basis of the interaction between humans and the microorganisms that can make us sick.

Industrialized and developing nations have improved their ability to conduct surveillance and investigate outbreaks of disease in humans during the last two decades of the 20th century, and this progress is continuing. In addition, the combination of molecular biology and electronic information technology in centers around the globe is refining the quality of the data that links cases together around common exposures. National and multinational networks of collaborators are being pulled together with help and guidance from the World Health Organization and the Food and Agriculture Organization of the United Nations, to facilitate the rapid sharing of data.

The process of ensuring the safety of food is dynamic as well as complex. Changes in the types of food that are consumed, the geographic origins of food products, and the ways in which different foods are processed affect both the risk for contamination and the adequacy of safety measures. The processes used to control foodborne hazards to limit the potential for foodborne disease must be continuously reviewed and judged against new information and new hazards. Advances in risk assessment methodologies and availability of additional data make it possible to integrate information from the various stages in the food production process for those foodborne hazards we know about. This capability can be used to identify particular steps in the food supply system for targeted intervention to control hazards and prevent disease. It is more difficult to provide specific advice on how to prevent foodborne hazards that have not yet been identified.

Framework for Food Safety Management

Our existing approach to food safety management has given the United States an extremely safe food supply. However, estimates of the incidence of foodborne illness clearly show that, in some cases, the existing approach to control is inadequate. The complex, ever-changing nature of microbiological food safety guarantees that new challenges will continue to emerge.

Microbiological food safety is not an issue only for microbiologists. Just as the farm-to-table approach to food safety has provided an overall picture of food

safety management, many scientific disciplines contribute to our knowledge about food safety. The scientific community must pull together multidisciplinary teams that combine microbiology, epidemiology, genetics, evolutionary biology, immunology and other areas of expertise to enhance our understanding of the interrelated factors that drive emerging food safety issues.

Just as the issues change over time, so too must our management strategies and our regulatory framework. Regulatory programs must be flexible to address issues as they arise and to benefit from scientific advances. Continued research will improve our understanding of the complex factors that cause foodborne illness, and surveillance programs will gather data to document the effectiveness of our controls and identify new problems as they emerge. A science-based food safety management framework should use food safety objectives to translate data about risk into achievable public policy goals.

Microbiological food safety issues will continue to emerge. Although we cannot expect to accurately predict the details of complex changes such as pathogen evolution, scientific knowledge can be used to identify the areas of greatest concern, so that we may be ready to respond as issues arise.

Science of Pathogenicity

Pathogenicity is the ability to cause illness. Because pathogens are living organisms that rapidly adapt and evolve, the methods they use to cause illness are never static. Pathogen evolution is continuous and is driven by a variety of forces, only some of which relate to human activities. The continual evolution of foodborne pathogens forces us to change food production processes and products to maintain and improve microbiological food safety. Control strategies that were once effective may not remain so if the pathogens become tolerant.

Fortunately, genomic and improved molecular and imaging techniques have vastly expanded scientific understanding of the organisms that cause foodborne disease. These tools also have enabled scientists to attribute foodborne disease to microorganisms that had not previously been identified as pathogenic or as foodborne.

However, researchers still have many questions to answer: What makes one strain of a microbe pathogenic when other microorganisms within the same species are not? How do microorganisms become pathogenic? Understanding pathogenicity is not just necessary for developing methods to treat illness but is also needed for pathogen control.

Pathogen control includes prevention of food contamination, elimination from the food, reduction to an acceptable level, or prevention of multiplication and toxin formation. In addition, when scientists understand how a particular pathogen is able to cause illness, then they can look for ways to disrupt this process and render the microorganism harmless or find treatments that mitigate illness. The very factors that create pathogenicity are opportunities for control. Just as the pathogens adapt and evolve, so can our understanding and our response.

Nomenclature

Traditionally, the first step in understanding foodborne pathogens has been to develop a system of nomenclature and descriptions of microorganisms within this system. For the purposes of study, scientists try to classify microorganisms based on a set of common characteristics that sometimes include presumed pathogenic attributes. However, as our scientific understanding has improved, the initial classifications often no longer present a full and accurate picture. When nomenclature becomes outdated, questions are raised about the scientific validity of regulatory policies based on classification schemes that predict pathogenicity poorly or that cluster pathogenic and nonpathogenic microbes together under one name.

The names used to describe various microbiological foodborne pathogens are based on systematic nomenclature. It is common practice to identify an organism based on its genus and species. To provide additional detail, classifications such as subspecies, strain, serotype, pathovar, and toxin type may be used (see Table 3).

In the past, the classification of microorganisms has relied primarily on structural (morphological) and functional (physiological) characteristics. For example, shape is a morphological characteristic, and the pattern of enzymes produced is a physiological characteristic. The commonly used morphological distinctions of gram-positive and gram-negative are based on differences in cell wall composition. Morphological features remain the primary means of classification for molds. Although morphological characteristics can classify bacteria into broad categories (e.g., spherical, rod-shaped, or curved), bacteria generally have few morphological fea-

Table 3. Classic Microbial Nomenclature

Nomenclature	Example 1	Example 2	Example 3
Family	Enterobacteriaceae	Enterobacteriaceae	Mycobacteriaceae
Genera/genus	*Escherichia*	*Salmonella*	*Mycobacterium*
Species	*coli*	*enterica*	*avium*
Subspecies		*enterica*	*paratuberculosis*
Serovar	O157:H7	Typhimurium	

safety management, many scientific disciplines contribute to our knowledge about food safety. The scientific community must pull together multidisciplinary teams that combine microbiology, epidemiology, genetics, evolutionary biology, immunology and other areas of expertise to enhance our understanding of the interrelated factors that drive emerging food safety issues.

Just as the issues change over time, so too must our management strategies and our regulatory framework. Regulatory programs must be flexible to address issues as they arise and to benefit from scientific advances. Continued research will improve our understanding of the complex factors that cause foodborne illness, and surveillance programs will gather data to document the effectiveness of our controls and identify new problems as they emerge. A science-based food safety management framework should use food safety objectives to translate data about risk into achievable public policy goals.

Microbiological food safety issues will continue to emerge. Although we cannot expect to accurately predict the details of complex changes such as pathogen evolution, scientific knowledge can be used to identify the areas of greatest concern, so that we may be ready to respond as issues arise.

Science of Pathogenicity

Pathogenicity is the ability to cause illness. Because pathogens are living organisms that rapidly adapt and evolve, the methods they use to cause illness are never static. Pathogen evolution is continuous and is driven by a variety of forces, only some of which relate to human activities. The continual evolution of foodborne pathogens forces us to change food production processes and products to maintain and improve microbiological food safety. Control strategies that were once effective may not remain so if the pathogens become tolerant.

Fortunately, genomic and improved molecular and imaging techniques have vastly expanded scientific understanding of the organisms that cause foodborne disease. These tools also have enabled scientists to attribute foodborne disease to microorganisms that had not previously been identified as pathogenic or as foodborne.

However, researchers still have many questions to answer: What makes one strain of a microbe pathogenic when other microorganisms within the same species are not? How do microorganisms become pathogenic? Understanding pathogenicity is not just necessary for developing methods to treat illness but is also needed for pathogen control.

Pathogen control includes prevention of food contamination, elimination from the food, reduction to an acceptable level, or prevention of multiplication and toxin formation. In addition, when scientists understand how a particular pathogen is able to cause illness, then they can look for ways to disrupt this process and render the microorganism harmless or find treatments that mitigate illness. The very factors that create pathogenicity are opportunities for control. Just as the pathogens adapt and evolve, so can our understanding and our response.

Nomenclature

Traditionally, the first step in understanding foodborne pathogens has been to develop a system of nomenclature and descriptions of microorganisms within this system. For the purposes of study, scientists try to classify microorganisms based on a set of common characteristics that sometimes include presumed pathogenic attributes. However, as our scientific understanding has improved, the initial classifications often no longer present a full and accurate picture. When nomenclature becomes outdated, questions are raised about the scientific validity of regulatory policies based on classification schemes that predict pathogenicity poorly or that cluster pathogenic and nonpathogenic microbes together under one name.

The names used to describe various microbiological foodborne pathogens are based on systematic nomenclature. It is common practice to identify an organism based on its genus and species. To provide additional detail, classifications such as subspecies, strain, serotype, pathovar, and toxin type may be used (see Table 3).

In the past, the classification of microorganisms has relied primarily on structural (morphological) and functional (physiological) characteristics. For example, shape is a morphological characteristic, and the pattern of enzymes produced is a physiological characteristic. The commonly used morphological distinctions of gram-positive and gram-negative are based on differences in cell wall composition. Morphological features remain the primary means of classification for molds. Although morphological characteristics can classify bacteria into broad categories (e.g., spherical, rod-shaped, or curved), bacteria generally have few morphological fea-

Table 3. Classic Microbial Nomenclature

Nomenclature	Example 1	Example 2	Example 3
Family	Enterobacteriaceae	Enterobacteriaceae	Mycobacteriaceae
Genera/genus	*Escherichia*	*Salmonella*	*Mycobacterium*
Species	*coli*	*enterica*	*avium*
Subspecies		*enterica*	*paratuberculosis*
Serovar	O157:H7	Typhimurium	

tures that are readily discernible by light microscopy or that are stable under a broad range of environmental conditions. To create a system with more precision, taxonomists were forced to base classification schemes on both morphological characteristics and physiological characteristics that generally reflect the biochemical diversity among bacterial species.

As the available techniques and technology advanced, scientists found new ways to classify microorganisms. The advent of ribosomal RNA (ribonucleic acid) sequencing began a new era of taxonomy (Woese et al., 1990). rRNA is present in organisms in all kingdoms and performs the same essential functions in all organisms. rRNA evolves slowly so it serves as the ideal evolutionary clock. Scientists soon developed large databases of rRNA sequences used to classify new species (Olsen et al., 1992). This era also produced many of the current DNA (deoxyribonucleic acid) hybridization strategies used to detect and identify pathogenic microorganisms in food and clinical samples (King et al., 1989). Although the use of rRNA sequences as a chronometer to measure relationships among species has disrupted traditional groupings based on phenotypic characteristics, many of the taxa of importance to food microbiology remain intact, with some modifications among groupings of certain species. For example, *Campylobacter pylori*, initially named pyloric campylobacter for its similarity to *Campylobacter jejuni*, was subsequently renamed *Helicobacter pylori* (Dubois, 1995).

The era of microbial genomics reached full swing in the 1990s. Using several available microbial genome sequences, scientists have been able to compare the evolutionary histories of different bacterial "races" (phylogenies) to the universal phylogenetic tree predicted from rRNA sequences. These efforts resulted in the disappointing discovery that the genome-based phylogenies were frequently discordant with rRNA predictions (Brown and Doolittle, 1997).

Mining the sequences for the relative "time" that specific segments appeared within the genome revealed the reasons for the discordance: significant portions of microbial genomes have been acquired through gene transfer from other microorganisms (Lawrence and Ochman, 1998). Examining large sets of virulence genes on contiguous segments of DNA (known as pathogenicity islands) also demonstrated that genes conferring virulence characteristics are often some of the most recent acquisitions among the genomes of pathogenic species (Hacker and Kaper, 2000). Aside from the impact on nomenclature, this new information has profoundly changed scientific thinking about the evolution of virulence. The ability of a pathogen to suddenly obtain a critical virulence factor through genetic exchange is at odds with the idea of slow, gradual evolution of virulence.

Now that scientists know that microbial genomes change more rapidly than previously believed, the concept of bac-

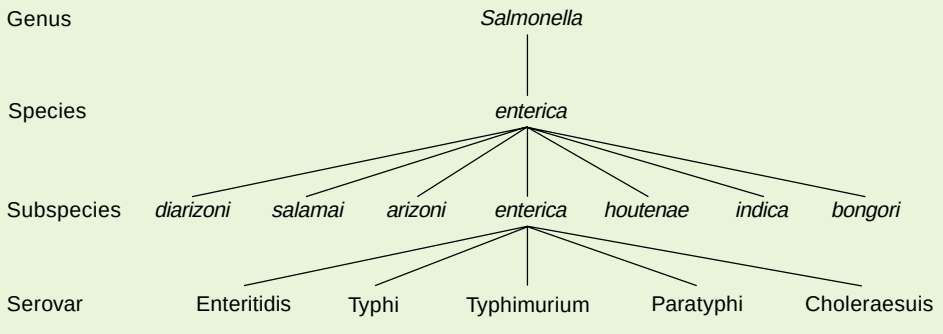
Nomenclature of *Salmonella*

Bacteria in the genus *Salmonella* are important contaminants in food and water. Recently, efforts have been made to simplify the nomenclature of *Salmonella*. Instead of using serotype designations (of which there are more than 2,000) incorrectly as species designations, most *Salmonella* species are now classified as *Salmonella enterica* and then further identified by serovar (e.g., *Salmonella typhimurium* becomes *S. enterica* serovar Typhimurium, see Fig. 3). For convenience, the species (*enterica*) designation is frequently eliminated, leaving *Salmonella* Typhimurium.

The diverse population of organisms sharing the same name has complicated efforts to study *Salmonella*, but recent advances illustrate that most *Salmonella* are actually quite similar to each other, especially at the virulence factor level.

Salmonella typically cause three diseases in humans: gastroenteritis (caused by *S.* Typhimuriuim, *S.* Enteritidis, and others); enteric fever (*S.* Typhi and *S.* Paratyphi); and an invasive systemic disease (*S.* Choleraesuis). In the United States, nontyphoidal *Salmonella* account for an estimated 1.3 million illnesses annually, with several hundred deaths (Mead et al., 1999). The U.S. incidence of typhoid fever is relatively low—approximately 700 cases annually, mainly as a result of international travel. Worldwide, the incidence of typhoid fever is declining (due, in part, to better distribution of safe water and successful vaccines), while the incidence of nontyphoidal salmonellosis is increasing rapidly. A portion of this increase correlates to changes in the food production environment that may have given *Salmonella* the opportunity to spread and to contaminate foods that are distributed through large complex networks.

Fig. 3. Nomenclature of *Salmonella*



terial "species" is in flux. In terms of diversity, genome size may vary by 20% among subpopulations of a single species (for example, see Bergthorsen and Ochman, 1998). This variability can cover as many as 1-2 megabases of DNA that code for thousands of genes, which can confer unique characteristics—such as virulence—on specific subpopulations. Still, a subset of genes that define signature characteristics of the species must be shared among all members of that species.

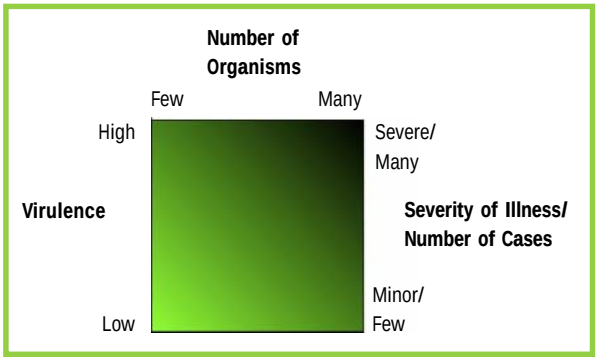
These contrasting views of what constitutes a bacterial species challenge the concepts that underlie microbiological criteria and testing for the control of pathogens in food manufacturing. In some cases, the potential to cause foodborne disease can be characteristic of an entire species, such as *S. enterica*, but in other cases it may be a consequence of recently evolved virulence characteristics among specific subpopulations of a species, such as *Escherichia coli* O157:H7. The sensitivity of genome-based fingerprinting methods has even called into question the equality of virulence in genetically distinct subpopulations of *E. coli* O157:H7 (Kim et al., 1999). As another example, it is questionable whether all microorganisms in the *Listeria monocytogenes* species are capable of causing disease in humans. Even though each distinct genetic lineage of the species appears to harbor the known virulence genes, mounting phylogenetic evidence indicates that virulence characteristics differ (Rasmussen et al., 1995; Wiedmann et al., 1997).

New scientific information provides the opportunity to better target the pathogenic subpopulations within a species. Present day approaches to control must be modified to be consistent with the information presently available. As improved detection and identification methods enable scientists to differentiate between virulent and avirulent organisms, we should be able to allocate our risk management resources more efficiently, focusing only on pathogenic organisms in our food supply.

Virulence

If pathogenicity is a microorganism's ability to cause disease, then virulence can be considered the degree of pathogenicity. Some pathogens are particularly efficient at causing clinically significant disease (highly virulent), while others may cause only minor effects in a small number of cases (less virulent). Virulence is related to the severity of disease and the number of ingested pathogens (infectious dose) required to cause illness (see Fig. 4). Within the *Escherichia coli* species, for example, there is great contrast in virulence. Enterohemorrhagic *E. coli* (e.g., O157:H7) can cause significant and severe illness even if very small numbers of cells (10 or less) are ingested, whereas enterotoxigenic *E. coli* require an estimated 100 million to 10 billion cells to cause a relatively mild set of symptoms (FDA/CFSAN, 2002). The underlying reasons for observed differences in virulence among various pathogens, and even within a single species of pathogen is difficult to state with absolute certainty, as at least two dynamics are operative, the microorganism and the host. Even less virulent pathogens can cause serious illness in debilitated hosts. Likewise, it is probable that fewer pathogens need to be ingested by a debilitated host than a healthy host to cause infection if all else is equal.

Fig. 4. Virulence and Foodborne Illness



The infectious dose is different for each pathogen. As stated above, *E. coli* O157:H7 is infectious in very low numbers. There may be several possible explanations for this, including: (1) O157:H7 is more acid tolerant and therefore fewer cells are killed by gastric acidity, (2) O157:H7's virulence may be influenced by intestinal flora via quorum sensing (see sidebar, p. 16), (3) its combined virulence factors simply make it more adaptable to the intestinal lumen, or (4) its virulence factors are more potent.

The situation among *Salmonella* is more complex. Prior to the 1980s, conventional wisdom held that large numbers of *Salmonella* were necessary for infection. Human feeding trials of volunteers from penal institutions with several different *Salmonella* subtypes certainly suggested that numbers >100,000 cells were required for illness (D'Aoust, 1985). However, outbreaks involving cheddar cheese and chocolate were apparently caused by very few cells (<10 total cells for cheddar and 50-100 total cells for chocolate). It has been theorized that the lipid content of cheddar cheese and chocolate facilitated the pathogen's transit through the stomach acid. Very little is known about specific food matrices and the effect on microbial survival and virulence. Some salmonellae appear to be acid susceptible, and others appear acid tolerant, but additional research will be required to better answer this question. Antacid consumption has been shown to affect the apparent virulence of certain pathogens such as *Salmonella* and *L. monocytogenes*, and this suggests that stomach acid, and a microorganism's tolerance to stomach acid, plays some role in defining the infectious dose.

Nowhere in current food microbiology is the issue of infectious dose as contentious as in the case of *L. monocytogenes*. Currently, the detection of *L. monocytogenes* in ready-to-eat foods is grounds for removing the foods from the marketplace. *L. monocytogenes* only very rarely affects healthy individuals, but immune-compromised individuals are susceptible to illness and sometimes death. Of all the known pathogens, *L. monocytogenes* has one of the highest mortality rates, approaching 20% (Mead et al., 1999).

The question is whether there is an ingested dose of *L. monocytogenes* that is tolerable by the vast majority of the population (i.e., is there a realistic dose that can be tolerated in food?). Studies on virulence gene and ribotype pattern have separated *L. monocytogenes* into three lineages, and each lineage appears to differ in its pathogenic potential for humans (Wiedmann et al., 1997). Studies on *L. monocytogenes* isolated from smoked fish using the same methods further suggest that some subtypes of *L. monocytogenes* in ready-to-eat foods may have limited pathogenic potential for humans (Norton et al., 2001). If these variations in pathogenic potential exist as suggested, it implies that more special-

Quorum Sensing

Not long ago, bacteria were thought to lead a solitary existence and either live or die as a single cell. It has been established that intercellular communication is fairly common among bacteria, and that this intercellular communication can lead to coordinated activities once thought to be the exclusive domain of multi-cellular organisms. It was not surprising then that researchers asked whether intercellular communication was involved in virulence, and it was also not surprising that genetically well characterized foodborne pathogens such as *Salmonella* and *Escherichia coli* would be investigated.

Intercellular communication among bacteria is carried out by small molecules called autoinducers. The theory is that at very low population densities, there is insufficient autoinducer in the environment to be detected by those bacteria present, but that when some "threshold" number of bacteria is reached, autoinducer is present in sufficient concentration to trigger some activity in bacteria capable of detecting the autoinducer. The terminology to describe these events is quorum sensing.

Quorum sensing has been demonstrated in *E. coli* and *Salmonella* Typhimurium (Surette and Bassler, 1998), and more recently, the role of quorum sensing in the virulence of enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *E. coli* has been elucidated (Sperandio et al., 1999). The hallmark intestinal lesion caused by EHEC and EPEC is called attaching and effacing, and is coded by the Locus of Enterocyte Effacement (LEE). This pathogenicity island codes for a type III secretion system, as well as other virulence factors such as the intimin intestinal colonization factor and the translocated intimin receptor protein. It is possible that the low infectious dose of *E. coli* O157:H7 is in part because the pathogen is induced to colonize the intestine by quorum sensing of signals from resident, nonpathogenic *E. coli* in the intestine of the host.

Quorum sensing appears to regulate the virulence factors of a wide variety of plant and animal pathogens (Day and Maurelli, 2001). Unlike other enteric pathogens, the signalling system in *Shigella flexneri* does not regulate virulence. There may be sound ecologic reasons why some enteric pathogens regulate virulence with quorum sensing systems and others do not. Quorum sensing systems are not limited to the gram-negative bacteria, and notable gram-positive bacteria, such as the pathogen *Staphylococcus aureus* and its numerous virulence factors are under the control of intercellular signals (de Kievit and Iglewski, 2000).

Obviously it is important that we gain a better understanding of the regulation of virulence via quorum sensing systems. One obvious question is: Does quorum sensing occur in or on foodstuffs, and if so, is it a factor in the virulence of foodborne pathogens? Many pathogenic bacteria have evolved a chemical language, and it would behoove us to learn and understand that language. It may be possible to exploit these intercellular communication pathways to reduce virulence, or use them as targets of novel antimicrobial substances (de Kievet and Iglewski, 2000).

ized testing might be prudent before foods are rejected for the presence of *L. monocytogenes* that may not be pathogenic.

The inherent ability to cause disease is the result of virulence factors encoded at the genetic level (Finlay and Cossart, 1997; Finlay and Falkow, 1997). Many diverse characteristics are considered virulence factors. If these factors are missing, the microorganism would be expected to be less virulent or avirulent. Some examples of virulence factors include:

- toxins (molecules secreted by the bacteria that affect host cell processes), such as cholera toxin;
- adhesins (molecules that enable pathogens to adhere to host surfaces), such as fimbriae; and,
- invasins (molecules that enable pathogens to actively enter into a host cell (invasion) where they can exist as an intracellular pathogen), such as those used by *Shigella* and *Salmonella*.

Most pathogens have a variety of virulence factors that assist in host colonization and disease. The repertoire of functional virulence factors will dictate which host a pathogen will colonize, which tissues will become infected, and ultimately which disease symptoms will occur.

Many bacterial pathogens are genetically similar to common bacteria that inhabit the host but do not cause disease under ordinary circumstances (commensal organisms). Thus, a pathogen is often a "genetically enhanced" organism: a common organism that contains a specialized collection of virulence factors. This complicates efforts to control foodborne pathogens by diffusing resources across a large group of microorganisms when only a subset can cause foodborne illness. The ability to share the genetic material that encodes virulence factors within food-producing animals, the environment, or the human gastrointestinal system significantly complicates control (see Evolution, p. 19). Each of the several steps in the progression of pathogenic foodborne disease requires one or more virulence factors.

Toxins are perhaps the best understood family of bacterial virulence factors, presumably because they are often secreted into the area surrounding the bacteria where they can be isolated and studied. Toxins have specific mechanisms to recognize host cell surface receptors enabling them to transport themselves into the host cell. Some toxins can modify specific cellular targets to affect fluid secretion, cytoskeletal structure, or even nerve functions. Others insert themselves into the host cellular membrane, resulting in cell disintegration or dissolution (lysis). Despite the vast number of cellular targets, toxins can be classified into families based on their structure and function.

To cause disease, most pathogens require the ability to adhere to host surfaces following ingestion. Mammalian hosts have several nonspecific defenses designed to prevent colonization by inhibiting pathogen attachment, such as peristalsis, the mucocilliary system, and even cell sloughing (see section on human host, p. 28). Bacterial pathogens

have a wide variety of adhesins, usually glycoproteins or glycolipids, on their surface that target specific host cell molecules. These adhesins are usually filamentous- or hair-like structures (fimbriae or pili) or proteins without the hair-like fimbriae (afimbrial adhesins) on the bacterial surface. Pathogens usually encode several adhesins that help dictate tissue and host specificity. For example, *Salmonella* use a combination of at least six different adhesins that contribute to intestinal colonization. Many pathogens also have the ability to adhere to the area outside and surrounding the cell (extracellular matrix), which contributes to host colonization. Recently, it has become apparent that some pathogens, such as *E. coli* O157:H7, actually insert their own receptor into host cells, which then enables them to adhere to the outside surface of the host cell (Kenny et al., 1997).

Many foodborne pathogens have the additional capacity to invade host cells. For example, *Salmonella, Shigella, Yersinia,* and *Listeria* are invasive organisms, capable of penetrating the intestinal epithelial barrier as part of their pathogenicity. The ability to invade provides the pathogen with additional environments to colonize that protect the pathogen from host defenses present within the lumen of the intestinal tract. The virulence factors that facilitate invasion may be as simple as a single surface protein, such as those used by *Yersinia* (invasin) or *Listeria* (internalin). In other cases, the factors may be complex, requiring the delivery of several proteins into the host cell and resulting in profound cell surface changes such as membrane ruffling and macropinocytosis before bacterial uptake occurs.

Numerous bacterial pathogens, including *Salmonella* and *Shigella,* have a complex secretion apparatus, known as a Type III secretion system, that delivers numerous bacterial proteins into the gel-like fluid inside the host cell (cytosol) to mediate invasion. Type III secretion systems are major virulence mechanisms for many gram-negative pathogens (Hueck, 1998). *Salmonella* actually have two systems (one for invasion, one for intracellular survival), while *Yersinia* use one to avoid isolation and destruction within the cell (phagocytosis).

Although invasion into a host cell may protect the pathogen from some host defenses such as antibodies, it also exposes the pathogen to another mechanism that kills most bacteria: fusion with the lysosome. Lysosomes are small sacks within the cell that contain enzymes that "digest" substances—such as old DNA, proteins, or lipids—into small pieces for reuse by the cell. Invasive pathogens, including bacteria and protozoan parasites, have devised several strategies to avoid lysosomal fusion following invasion. For example, a second Type III secretion system alters the targeting of the membrane-bound vacuole surrounding *Salmonella*, causing the vacuole to diverge from the standard pathway that would deliver it to a lysosome. The salmonellae then live in the protected intracellular environment of the vacuole (see sidebar below). In contrast, *Shigella* and *L. monocytogenes* use enzymes to degrade the vacuolar membrane, thereby releasing the pathogen into the host cytosol. Once present in the cytosol, the pathogens use the structural proteins in the cell around them to propel themselves within the cell and into a neighboring cell. Finally, pathogens such as *Yersinia* and enteropathogenic *E. coli* use their Type III systems to

Virulence of *Salmonella*

Recently, scientists have made significant advances toward understanding the molecular mechanisms of the pathogenicity of *Salmonella*. Similar to pathogenic *E. coli, Salmonella* have a collection of virulence factors, including factors needed to adhere to intestinal surfaces, invade host epithelial cells, and survive within phagocytic cells. It has been estimated that *Salmonella* contain more than 200 virulence factors, encoded in at least five pathogenicity islands in addition to a virulence plasmid and many pathogenicity islets.

The complex mechanisms used by *Salmonella* species to adhere to intestinal cells continue to be studied and debated. Many adhesins have been reported, and because they appear redundant, defining the role of each in the disease process has been difficult.

Significantly more is known about the mechanisms *Salmonella* use to enter epithelial cells. These intestinal cells are not phagocytic, and thus do not normally ingest microorganisms. However, *Salmonella* use a Type III secretion system to inject several bacterial factors into the host cell. These molecules affect normal cellular processes, including those that control the actin cytoskeleton and other signal transduction pathways. The end result is significant actin rearrangement beneath the adherent bacterium, membrane ruffling, macropinocytosis, and engulfment of the bacterium into a membrane-bound vacuole. Unlike *Shigella, Salmonella* remain within the vacuole to survive and proliferate. *Salmonella* redirect vacuole movement within the host cell so that the vacuole it inhabits does not fuse with lysosomes.

Salmonella also form specialized structures that enable it to survive and replicate within phagocytic cells. *Salmonella* species have another Type III secretion system that encodes several factors needed for survival in this intracellular compartment. Finally, *Salmonella* have a virulence plasmid that provides the factors needed for prolonged survival within the host.

S. Typhi is different from most *S. enterica* serovars in that it has a capsule and does not encode the virulence plasmid. Because of its systemic infectious nature, *S.* Typhi presumably has additional virulence factors that contribute to the different nature of the disease. Although *Salmonella* species are fairly close relatives of *E. coli*, they have many additional genes that presumably account for their virulence. Scientists know little about the role for most of these additional genes and the factors they encode.

Significant progress has been made with vaccines for *S.* Typhi. Two current vaccines—one live-attenuated strain, the other a component vaccine—have significantly decreased the levels of typhoid fever worldwide. However, little success has been made toward controlling nontyphoidal salmonellae, which continue to cause increasing numbers of illnesses worldwide.

avoid being pulled into the cell and therefore blocking phagocytosis, allowing them to remain attached to the exterior of the cell (extracellular pathogens).

Because expression of virulence factors at the correct time and place is critical, bacterial virulence factors are tightly regulated by a variety of control mechanisms and regulatory circuits. Successful pathogens are very adept at sensing their microenvironment, and virulence factor expression often relies on environmental parameters to ensure coordination and appropriate expression.

The continual genetic exchange between bacteria ensures that pathogens will continue to evolve as they acquire different combinations of virulence factors. This evolution is a natural process, although it can be enhanced and facilitated by human actions.

Pathogens Are More Than Just Bacteria

Although bacteria are perhaps the first type of microorganism that come to mind when discussing microbiological food safety, they are by no means the only pathogenic foodborne microorganisms.

Human Enteric Viruses

Viruses of concern to human health that are known to be transmissible through foods are shed in the feces of infected humans and transmitted via the fecal-oral route. Of these viruses, hepatitis A virus causes the most serious recognized foodborne viral infection, whereas the Norwalk-like gastrointestinal viruses (NLVs) are the most prevalent. According to recent epidemiological estimates, the NLVs account for over 60% of cases, 33% of hospitalizations, and 7% of deaths among all of the illnesses that are attributable to known foodborne pathogens (Mead et al., 1999). Human enteric viruses have properties that make them quite different from the common bacterial agents of foodborne disease. As obligate intracellular parasites, they require live mammalian cells to replicate. To protect the viral genome from inactivation outside of infected cells, virus particles have properties that make them environmentally stable to the extremes of pH and enzymes present in the human gastrointestinal tract. This stability enables virus particles to survive a variety of food production, processing, and storage conditions making virtually any type of food product a potential vehicle for transmission of viral pathogens (Jaykus, 2000a). The inability of human enteric viruses to replicate in foods and the fact that they are generally present in low numbers does not ensure product safety, as the infectious doses (10^0-10^2 infectious units) are presumed to be low (Iversen et al., 1987; Jaykus, 2000b; Moe et al., 1998).

Three major routes for viral contamination of foods have been recognized and include: (1) shellfish contaminated by fecally polluted marine waters; (2) human sewage pollution of drinking and irrigation waters; and (3) ready-to-eat and prepared foods contaminated as a result of poor personal hygiene of infected food handlers (Jaykus, 2000b). In addition, the NLVs have been shown to be spread by aerosolization of vomitus and through fomites (Marks et al., 2000; Patterson et al., 1997).

Protozoan Parasites

Like viruses, parasitic protozoa replicate in the intestines of infected hosts and are excreted in the feces. However, their host range is wider than viruses, being able to replicate in human and non-human animal hosts. Since they are transmitted primarily by the fecal-oral route, the major source of contamination for foods and water is through contact with human and animal fecal pollution. This contamination may occur directly, through contaminated meat carcasses or poor personal hygiene practices of infected food handlers, or indirectly, via contact with fecally contaminated waters or other cross-contamination routes. Like the viruses, the parasitic protozoa (in the cyst or oocyst form) are environmentally inert, they do not replicate in foods, are extremely environmentally stable, resistant to many of the traditional methods used to control bacterial pathogens, and have notably low infectious doses (DuPont et al., 1995; Haas, 1983). Although transmitted by the fecal-oral route, direct person-to-person transmission of parasitic protozoa is unlikely because excreted oocysts require days or weeks under favorable conditions to sporulate and become infectious.

Since 1981, enteric protozoa have become the leading cause of waterborne disease outbreaks for which an etiological agent could be determined (Moe, 1996). Although considerably less information is available about their importance in foodborne disease, their potential for transmission by foodborne routes is increasingly recognized (Bean et al., 1990). For instance, from 1988-1992, seven food-associated outbreaks of giardiasis, comprising 184 cases, were reported in the United States (Bean et al., 1996), and protozoan parasites such as *Giardia* and *Cryptosporidium* species may be present in shellfish-growing waters as a result of contamination with animal farm runoff or as a result of treated and untreated sewage input. The ability of bivalve molluscs to concentrate *Giardia* and *Cryptosporidium* has been demonstrated by Toro et al. (1995). *Cryptosporidium* oocysts have been isolated in Eastern oysters harvested from commercial sites in the Chesapeake Bay (Fayer et al., 1998).

The most common human enteric parasitic infections in the United States are caused by *Cryptosporidium parvum* and *Giardia lamblia*. *Cyclospora* is also an emerging enteric protozoan that has recently been associated with the consumption of contaminated fruits (Ortega et al., 1993). Large, community-wide waterborne outbreaks of parasitic protozoa are usually associated with surface water supplies that are either unfiltered or subjected to inadequate flocculation and filtration processes (Moe, 1996). Two large waterborne outbreaks have occurred in the United States within the last 10 years (Hayes et al., 1989; MacKenzie et al., 1994), including the largest recorded waterborne disease outbreak in U.S. history (MacKenzie et al., 1994).

Marine Biotoxins

Marine biotoxins are produced by several dinoflagellate and diatom spe-

Although foodborne pathogens have developed a vast repertoire of virulence factors, several common themes enable scientists to place many of these factors in related families based on structure, function, and other characteristics. Understanding why a pathogen is virulent and how it overcomes host defenses and causes illness creates opportunities for preventing the infection or for mitigating the illness. The use of similar kinds of Type III secretion systems among many diverse pathogens is one example of a common mechanism that might be exploited for potential therapeutics and control strategies.

Evolution

Pathogen evolution is a continuous biological process that is influenced by

cies (Epstein, 1998; Plumley, 1997), most of which are produced by the proliferation of algae in the form of harmful algal blooms (HABs) (Steidinger and Penta, 1999). Of the several thousand identified marine microalgae, at least 80 species are known to be toxic or harmful (Baden et al., 1995). Reports of seafood toxicity due to these biotoxins date back to the 1600s, have occurred worldwide, and recent evidence suggests that HABs are increasing. This indicates that marine biotoxins may indeed be considered emerging pathogens (Anderson, 1994; Shumway, 1989).

The toxins produced by dinoflagellate and diatom species are often classified according to their mode of action and resulting disease syndromes. The most common marine HAB toxins are chemically characterized as alkaloids, polyethers, or substituted amines and are the end products of elaborate biochemical pathways (Plumley, 1997). Most have been classified as neurotoxins although some hemolytic substances have also been identified (Baden et al., 1995). Although marine toxins are pharmacologically diverse, most exert toxic effects through perturbations of voltage-gated sodium channels located in excitable membranes of neurons (Catterall, 1985; Manger et al., 1995). Binding of these toxins to receptor sites leads to conformational changes of the ion pore of the channel, thus altering the flow of ions controlling nerve signaling (Baden et al., 1995).

In general, the marine biotoxins are small, chemically complex, and highly potent substances that tend to accumulate in finfish and/or shellfish. When the seafood is consumed by humans, it acts as a passive carrier of the toxins. The toxins are tasteless, odorless, and most often heat and acid stable, which means that routine food safety inspection and food preparation techniques will not detect contamination, inactivate the toxins, or prevent human disease upon consumption (Baden et al., 1995). Symptoms of seafood-borne intoxications may include gastrointestinal distress, vomiting, headaches, neurologic dysfunction, paralysis and muscular pain. The degree of human toxicity is affected by the health of the victim, the amount of toxin ingested, the rate of toxin elimination, and the biotransformation of toxins by enzyme systems within the body (Steidinger and Penta, 1999).

Mycotoxins

A large group of mycotoxins, known collectively as trichothecenes, have been associated with acute human illness (Pestka and Casale, 1990). The most severe manifestation is alimentary toxic aleukia. Initial symptoms after a single contaminated meal include throat inflammation, vomiting, diarrhea and abdominal pain; with continued ingestion, the illness can progress to oral hemorrhaging, pneumonia, bone marrow depletion, and potentially death. Human outbreaks of gastrointestinal illness have been linked to millet contaminated by a variety of *Fusarium* that was capable of producing T-2 toxin and other trichothecenes and also wheat and barley infected with *Fusarium* that produced the trichothecenes deoxynivalenol (vomitoxin), nivalenol and fusarenon-x (Bhat et al., 1989; Luo, 1994).

Other mycotoxins have been associated with acute disease. Aflatoxin ingestion has caused acute liver inflammation in Kenya (Ngindu et al., 1982) and India (Krishnamachari et al., 1975). In the early 20th century, cardiac beriberi—an acute cardiac disease that produces a rapid pulse, abnormal heart function, and low blood pressure leading to respiratory failure and death—that occurred in Japan and other Asian countries was linked to contaminated rice containing the mycotoxin citreoviridin (Ueno, 1974).

In general, except when large numbers of people are affected, medical professionals are unlikely to recognize mycotoxicoses because of insufficient knowledge about these diseases and the lack of appropriate diagnostic methods. Unlike microbial agents that can be detected by culturing and/or PCR amplification, mycotoxins are metabolized and may no longer be present in the tissues of patients shortly after the onset of acute disease.

Mycotoxins produced by a wide variety of molds including *Aspergillus*, *Penicillium* and *Fusarium* may cause chronic toxicosis (Coulombe, 1993). Chronic effects often result from prolonged ingestion of low to moderate levels of toxin that do not produce symptoms of an acute illness, making the chronic effects difficult to attribute to contaminated food. The link between aflatoxin and human liver cancer has been well established through the use of clinical biomarkers (aflatoxin adducts), but other organs (kidney, spleen and pancreas) also may be affected. Another chronic disease link that has been the subject of considerable study is those diseases induced by the fumonisins, zearalenone, and trichothecene mycotoxins. Fumonisin levels in corn-based foods have been statistically associated with an increased risk of human esophageal cancer. Zearalenone has been associated with premature puberty. Trichothecenes modulate immune function, meaning that over time mycotoxicosis could reduce immune resistance to infectious diseases, facilitate tumor growth through reduced immune function and cause autoimmune disease (Bondy and Pestka, 2000; Jackson et al., 1996). Ochratoxin A is becoming closely linked to the kidney disease Balkan endemic nephropathy as well as tumors in the urinary tract and kidney.

environmental factors. Historically, scientists thought that pathogens arose from sequentially cumulative mutations, gradually changing from avirulent to pathogenic. In the past few years, new evidence has shown that evolution of pathogenicity progresses in quantum leaps that are driven by acquisition of foreign DNA (see Fig. 5). Analysis of some pathogen genomes has supported this new understanding and even extended it by demonstrating that genetic exchange plays a larger role than previously thought in genome composition. Comparing genomes of related pathogens reveals many recently acquired genes scattered throughout the genome.

As discussed above, the ability to cause disease is attributed to specific virulence factors. The genes encoding these virulence factors are often found on pathogenicity islands, that is, clustered together at specific loci on the chromosome or plasmids (Hacker et al., 1997). Evidence indicates that these genetic regions have evolved independent of the rest of the microbe's genetic information, i.e., they developed in a different organism and were acquired as a set. These genetic regions usually have a different G+C content of DNA, often have repetitive ends, and are often inserted into or near tRNA genes. Particular pathogenicity islands encode specific virulence factors that in turn dictate which disease the pathogen may cause.

In gram-negative bacteria, Type III secretion systems are often encoded within these pathogenicity islands. For example, enteropathogenic (EPEC) and enterohemorrhagic *E. coli* (EHEC), which both cause diarrhea, contain the Locus of Enterocyte Effacement (LEE) island, which encodes a Type III system and other virulence factors essential for disease (McDaniel et al., 1995). However, uropathogenic *E. coli* (which cause urinary tract infections) have a completely different pathogenicity island inserted at exactly the same site (Hacker et al., 1997), which encodes an adhesin (P fimbriae) and a toxin (hemolysin), virulence factors needed for urinary tract colonization. *Yersinia* and *Shigella* species encode Type III systems on their virulence plasmids rather than in their chromosomal DNA, but they have other virulence attributes that are chromosomal and not in islands. In addition to pathogenicity islands, smaller pieces of DNA (pathogenicity islets) also appear to move between bacterial pathogens.

Bacteriophages, viruses that infect bacteria, also play a major role in the movement of virulence factors between pathogens. For example, Shiga toxin is a key virulence factor for *Shigella dysenteriae*, which causes dysentery. The genes for toxin production are encoded on a phage that has been incorporated into the chromosome. *E. coli* O157:H7 also contains genes for Shiga-like toxin(s), which cause hemorrhagic colitis and contribute to disease progression to hemolytic uremic syndrome, sometimes characteristic of infection with this pathogen (Kaper and O'Brien, 1998). It is thought that the phage encoding this toxin infected an EPEC strain of *E. coli* and created a new pathogen, an EHEC. Under certain circumstances, the phage DNA replicates and breaks out, forming a new phage and releasing the toxin. Another example of the role that phage play in the evolution of pathogens is found within *Vibrio cholerae* (Waldor and Mekalanos, 1996). The cholera toxin is encoded within a bacteriophage capable of moving between strains, and, in this case, the receptor for this phage is one of the pathogen's major adhesins (a pilus). This arrangement ensures that the phage encoding the toxin only infects bacteria that already possess an essential adhesin, thus ensuring virulence.

Fig. 5. Contrasting Views of Pathogen Evolution

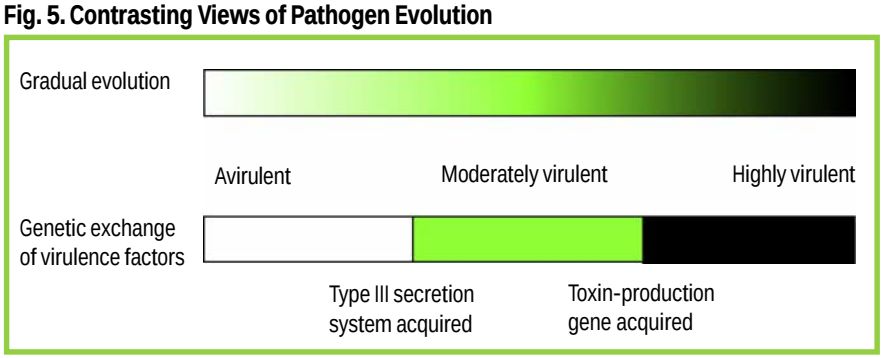
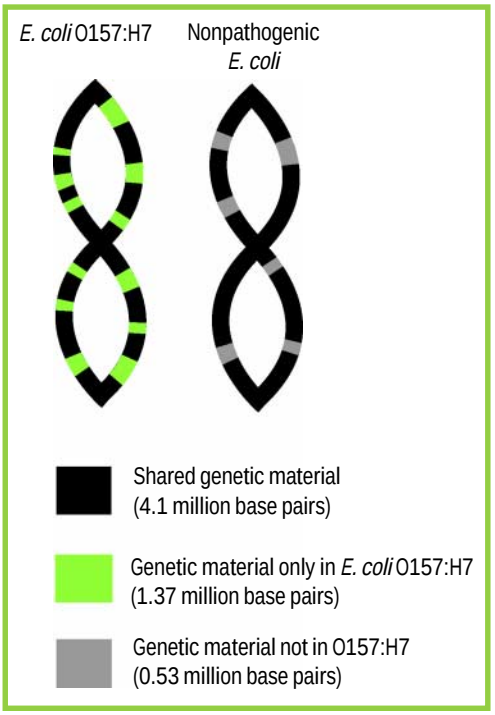


Fig. 6. Genetic Material in *E. coli*



Genomics has greatly facilitated our understanding of pathogen evolution. For example, comparing the recently sequenced *E. coli* O157:H7 genome to a nonpathogenic *E. coli* reveals some surprising information (Perna et al., 2001). As expected, both strains share a common genetic "backbone" of about 4.1 million similarly arranged base pairs (see Fig. 6). However, the O157:H7 genome contains an additional 1,387 new genes in 1.37 million base pairs. Furthermore, the nonpathogenic *E. coli* has 0.53 million base pairs (528 genes) that are not in O157:H7. Perhaps the most significant finding is that the additional DNA in the pathogenic *E. coli* is distributed among 177 different packets, each of which was likely inherited through an independent event. Researchers calculated that these two *E. coli* strains shared a common ancestor approximately 4.5 million years ago. Although scientists know the function of two pathogenicity islands (the LEE and the Shiga toxin phage) within O157:H7 that encode virulence factors, the function of the additional genes within O157:H7 remains to be determined, as does their contribution, if any, to virulence. Even among O157:H7 strains, significant variability in virulence can be detected, indicating that genome diversification

Evolution of *Salmonella*

S. enterica shows significant diversity in its host specificity, but the reasons for host specificity remain undefined. Some serovars, such as *S.* Typhi, are very human specific while others are animal specific (e.g., *S.* Pullorum infects chickens). Others, such as *S.* Typhimurium and *S.* Enteritidis, have a wide host range. *S.* Typhimurium typically causes a diarrheal disease in humans, but a typhoid-like disease in mice. Because many strains of *Salmonella* cannot tolerate the low pH of the gastric environment, the infectious dose for ingested exposure to *Salmonella* is usually (but not always) quite high (greater than 100,000 bacteria required to produce illness). However, if delivered intravenously, a mere 10 organisms of strains that require a large oral infectious dose can kill a mouse.

The incidence of antibiotic resistance among *Salmonella* and some other foodborne pathogens continues to increase. In some Asian countries, more than 90% of *Salmonella* isolates are resistant to the most commonly used human antibiotics. Globally, the three main causes of antimicrobial resistance have been identified as use of antimicrobial agents in agriculture, overprescribing by physicians, and misuse by patients. The combination of increased antibiotic resistance and an apparent increase in virulence has resulted in strains, such as *S.* Typhimurium DT104, that continue to cause much concern. *S.* Typhimurium DT104, which has a broad host reservoir, is usually resistant to five antibiotics (i.e., ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracyclines) and can be resistant to others (e.g., fluoroquinolones). Reporting on an outbreak of quinolone-resistant *S.* Typhimurium DT104, Molback et at. (1999) stated that because fluoroquinolones remain a standard treatment for suspected extraintestinal *Salmonella* infections and serious gastroenteritis, the occurrence of quinolone-resistant *Salmonella* in animals is a great concern.

Although the genomic sequences of *S.* Typhimurium and *S.* Typhi have been recently completed, scientists still have more questions than answers about *Salmonella.* It is not known how they cause diarrhea, or why some are constrained by host specificity. Because of the complexity of their virulence factors, little progress has been made in converting the available knowledge into therapeutics. Good agricultural and manufacturing practices, appropriate food handling, and adequate water treatment remain our best preventive measures for most *Salmonella* infections, although the typhoid vaccines are effective against *S.* Typhi in humans, and vaccines for several other serovars have shown promise in food animals.

occurs rapidly.

Genetic exchange between bacterial species is obviously frequent and can significantly affect the evolution of pathogens. The acquisition and spread of antibiotic resistance has been an easy way to follow genetic exchanges, and the efficiency of this spread is demonstrated by the pervasiveness of resistance in many bacteria not previously resistant to antibiotics. As discussed above, the genetic material that encodes virulence factors can move between bacteria, with the potential to rapidly create a new pathogen, even from a commensal organism. A major unanswered question is often where these virulence factors originated. Despite the prevalence of Type III secretion systems within several bacterial pathogens, scientists have not found the system's ancestor.

As in all organisms, the evolution of pathogens is continuous. In some cases, classifying pathogens based on their virulence factors is a more logical method than classification based on serotype or some other trait unrelated to virulence. The evolution of pathogens will become clearer as scientists sequence and study additional genomes of related species. However, even seemingly related organisms can contain significant diversity overlaid on a common genetic backbone. Also, many avirulent bacteria can carry inactive forms of genes common to virulent strains.

Selection

Evolution and selection are closely related. Evolution produces microorganisms that are distinctly different from previous generations; selection gives these mutant strains an advantage and causes them to become prominent. Together, these forces have a profound impact on the emergence of foodborne pathogens.

The proposed step-wise model (Feng et al., 1998) for evolution of *E. coli* O157:H7 (as opposed to a gradual evolution) points out some rather interesting features of the process by which new pathogens emerge. First, mobility of gene segments appears to be a limiting factor in the rate at which microorganisms can test new gene combinations. New high-throughput genome sequencing methods will produce data to develop a much better estimate of the frequency of such events and an improved understanding of their underlying mechanisms. Second, the common virulence genes shared by the O157:H7 and EHEC O26:H11/O111:H8 lineages of enterohemorrhagic *E. coli* demonstrate that acquisition of the same virulence gene elements has occurred on multiple occasions, even through parallel paths (Reid et al., 2000). This discovery raises a significant question regarding the selective pressures that cause the abrupt rise to dominance of particular virulence gene combinations within a species.

In infectious diseases that are primarily carried by humans and transmitted person-to-person, the appearance of new alleles of virulence genes is associated with the rise and spread of more virulent clones (Musser, 1996). This process likely also occurs among foodborne pathogens. However, foodborne pathogens must overcome unique hurdles, including survival in the pre-harvest environment, as well as survival during food processing, storage, and preparation.

Many of the hurdles in food production and processing are a different set of selective pressures than those exerted by the human host's gastrointestinal tract. Successful foodborne pathogens, such as *Salmonella* and *L. monocytogenes,* have acquired not only virulence characteristics, but also physiological and ecological characteristics that

allow them to propagate in food production and processing environments and overcome hurdles. Using the new tool of genomics, researchers are examining how changes in food production, processing, storage, and preservation methods can impose new selective pressures on foodborne microorganisms and how such pressures might affect virulence in known pathogens or emergence of new pathogens.

Shifts in Known Pathogens

A cause-effect relationship has been documented between changes in food production methods and shifts in populations of a pathogenic species favored in the food production environment. Perhaps the best example is the displacement of *Salmonella* serovar Gallinarum by the Enteritidis serovar in poultry production environments. Scientists theorize the shift was caused by programs to eradicate *S.* Gallinarum, a poultry pathogen (Bäumler et al., 2000). Combinations of mathematical modeling, epidemiologic investigations, and population genetic studies suggest that the population shift and spread of *S.* Enteritidis took independent but parallel paths in Europe and North America. Genetically distinct subpopulations of *S.* Enteritidis rose to dominance on the two continents, in theory, due to competitive advantages over *S.* Gallinarum in occupying the poultry environment (Rabsch et al., 2000).

Although this displacement likely did not involve biological changes in the poultry host, it illustrates the capacity for changes in veterinary practices to have significant impact on the populations of microbial species that inhabit the production environment. In addition, it demonstrates that such population shifts have the potential to change the relative risk of foodborne illness to humans. Bioinformatics uses computational methods to analyze large sets of biological data, such as genome sequences, or to make predictions, such as protein structures. Given the tools of genomics and bioinformatics, it may be possible to understand why such shifts occur when epidemiologic studies fail to identify the actual selection pressure.

Emergence of New Pathogens

Scientists know relatively little about how the microbial controls imposed during food production and processing affect the emergence of new pathogens. However, hurdles imposed in food processing—such as pH, osmolarity, and temperature—are all known to affect physiological characteristics and, in some cases, virulence characteristics of pathogenic microorganisms. A good model from which to begin drawing conclusions might be to examine the distribution of Shiga toxin-converting phages among strains of *E. coli*. Clearly, the Shiga toxins play a pivotal role in the pathogen's ability to cause illness. However, Shiga toxin-producing *E. coli* (STEC) can be found in many environments, and only certain serotypes of STEC appear to commonly cause disease in humans, indicating that the Shiga toxins alone are insufficient to confer virulence. The convergence of Shiga toxin genes and genes conferring other virulence characteristics—such as the ability to attach to host cells—is necessary for the emergence of a new and successful pathogen. Considering the hurdles imposed in the food production and processing environment, it seems likely that convergence of such genes onto physiologically robust genome backbones favored the spread of EHEC lineages. Research is needed to identify how selective pressures in the food production and processing environment affect the potential for new pathogens to emerge or for subpopulations of known pathogens to increase in dominance.

By focusing on the results of selection in recently emerged foodborne pathogens, scientists can begin to address these issues. The approach is an adaptation of the method used by geneticists to identify the genes involved in a particular biochemical pathway. First, scientists induce mutations at random, and mutants of interest are selected based on phenotypic traits. Mutations that block the pathway under study are traced back to specific genes. Once the genes are marked and identified, scientists use biochemical and molecular techniques to understand how the genes function in the pathway.

In the case of foodborne pathogens, the strategy is similar but reversed. Here, the goal is to identify the genes or alleles that have been selected in food production environments, elucidate their function, and pinpoint the selective advantage conferred. The combination of genomics and population genetics will provide methods for identifying genetic alterations that correlate with the descent of specific populations of foodborne pathogens. However, scientists must begin to devise strategies for identifying which genetic alterations, among the many different gene sequences that define a subpopulation, confer selective advantage. Identifying and understanding these genes may enable scientists to pinpoint the selective forces at work.

Stress

Each microbe prefers a specific set of environmental conditions. When environmental parameters are significantly different from the desired range, the microbes undergo stress. To be more specific, stress is defined as chemical or physical parameters that impair the function of the macromolecular machinery of the microorganism. Examples of stress for certain microorganisms might include high and low temperature, acidic pH, low water availability, and presence or absence of oxygen. Specific genes are activated, producing proteins that protect the bacterium from stress. This process, known as an adaptive response, improves the microorganism's ability to survive under the stressful conditions. Although the responses improve the range of conditions the microorganisms can tolerate, these responses also require energy, so they are only expressed when needed. Under normal conditions, bacteria that do not turn off their stress responses would be outgrown by those that reroute that energy to other cellular processes. Stress responses are of particular interest in foodborne pathogens because they can render bacteria tolerant to traditional food processes or the intrinsic parameters of the food.

Bacteria have evolved elaborate networks to protect against or repair damage caused by detrimental conditions. Bacterial responses to stress are varied and complex, including both structural and physiological changes. For most bacteria, these responses are modulated by specific sigma (σ) factors (Grossman et al., 1984; Lange and Hengge-Aronis, 1991) or regulators (Christman et al., 1989) that direct the activation of specific genes that comprise regulons (large numbers of coordinately controlled genes) and encode for the proteins re-

sponsible for cellular protection. The proteins produced in response to stress enhance bacterial survival in the environment outside the host, including in foods (Cheville et al., 1996; Humphrey et al., 1993; Jenkins et al., 1988; Leyer and Johnson, 1993; Miller and Kaspar, 1993; O'Neal et al., 1994).

Studies of adaptive responses to stress can be classified into two distinct areas: the response itself and the ability to generate the response. Of particular interest in the response is how it mitigates the physiological consequences of stress conditions. On the other side is the perception of stress, specifically how cells communicate the physical and chemical signals of ensuing stress conditions to the regulatory machinery that governs the response. Understanding the response mechanics will provide the information necessary to finely tune processing conditions to avoid triggering the stress protection mechanisms. In addition, this knowledge can be used to develop rational targeting strategies to identify novel antimicrobials for use in pre-harvest settings.

Responses to Stress

The cellular responses to stress can generally be divided into two categories—general and specific stress responses. In many instances, a certain stress response gene may be part of both specific and general stress response pathways. This is usually because the gene has multiple regulatory elements that are recognized by the distinct machinery that coordinates the general or specific response. It is therefore difficult to separate the contribution of general and specific stress response pathways to cellular viability under any given stress condition. Rather, the combination of the two, and specifically the combined fine-tuning of each pathway, dictates many of the survival characteristics of the species.

General Stress Response

The general stress response (GSR) regulon is a large group of genes that collectively comprise several different functions that facilitate growth and survival under different conditions, such as osmotic shock, thermal stress, pH stress, oxidative stress, and nutrient depletion (Hengge-Aronis, 1996; Hengge-Aronis, 2000; Lee et al., 1995). The GSR is a complex system; more than 50 genes in *E. coli* are responsible for its GSR and are coordinately regulated by the product of the *rpoS* gene encoding σ^s, an alternative sigma-subunit of RNA polymerase (Loewen et al., 1998). Many of the genes in the GSR regulon appear to have obvious functions for mitigating specific types of stress, such as compatible solute transporters that facilitate transport of solutes in response to osmotic stress (Loewen et al., 1998), while others may confer general protective properties under multiple stress conditions.

The GSR for one stress may induce changes that improve the organism's survival under other stress conditions, a phenomenon known as cross protection. For example, it has been demonstrated in laboratory media that heat-shocking *Salmonella* Enteritidis (shifting the temperature from 20 C to 45 C) results in an approximate 3-fold increase in the D values (the time required to inactivate 90% of the organisms) at a pH of 2.6 and a greater than 10-fold increase when the temperature is raised to 56 C (Humphrey et al., 1993). In this example, the original stress (exposure to heat) increases protection to both heat and acid even when the bacteria had not been previously exposed to low pH.

Specific Stress Response

One of the best-characterized examples of specific stress response systems is the heat shock response. Like the GSR, the heat shock response involves an alternative sigma factor, σ^{32}, as a primary regulator. When the temperature increases, σ^{32}, which is normally degraded rapidly, becomes more stable and is translated at a higher rate, resulting in a transient accumulation of the σ^{32} protein and a corresponding increase in the rate of transcription from heat shock promoters that are recognized by σ^{32} RNA polymerase (Morita et al., 2000). Approximately 30 proteins belong to the heat shock regulon. Basal levels of the heat shock proteins are produced at all temperatures, but at higher temperatures the microorganism needs a greater concentration of these proteins to remain viable (Gross, 1996). Induction of the heat shock response is somewhat more specific than the GSR; however, there are other triggers of the response, such as exposure to ethanol (Gross, 1996). Heat shock and the production of associated proteins protects the cell from the detrimental effects of heat, and, as noted above, results in cross protection to other stresses. In *E. coli*, a second heat shock system, controlled by σ^E (σ^{24}), also has been identified (Erickson and Gross, 1989, Wang and Kaguni, 1989). This system recently has been shown to comprise a mechanism for sensing and coordinating responses to the effects of thermal stress in the periplasm (Mecsas et al., 1993). Thus, with σ^{32} and σ^E, *E. coli* has separate and highly specialized systems for adapting to thermal stress in the cytoplasmic and periplasmic compartments.

Spore Formation

In addition to general and specific stress response pathways, some bacteria and other microorganisms also have evolved highly sophisticated pathways for stress adaptation, such as forming spores. Spores are metabolically inactive or dormant and are much more resistant to adverse environmental conditions, e.g., extremes of temperature, low water activity, and radiation.

In organisms that form spores, the adaptive response pathways are somewhat hierarchical and, depending on the environmental conditions, can be triggered alone or in combination. *Bacillus subtilis*, the model organism for studying spore formation, relies on the general stress response, several stationary phase and transition state pathways, the development of competence, and differentiation into the dormant endospore. The pathways for spore formation, competence, and normal growth are mutually exclusive, but each one can be used in combination with the general stress response. Before the organism commits to a major step such as normal growth, competence, or spore formation, sophisticated signal transduction pathways measure environmental nutritional and chemical signals, as well as the state of cellular processes such as DNA replication.

Role of Stress Adaptation

Scientific interest has recently focused on determining the role of stress adaptation pathways not only in the food matrix but also in a host or host cell. To some extent, virulence genes can be considered an adaptive response to the stresses encountered during entry into the host. Studies have shown that components of specific and general stress responses are sometimes necessary to survive entry into a

host cell. Specific examples include the role of the general stress response regulatory protein *rpoS* in survival of *Salmonella* inside phagosomal vacuoles (Fang et al., 1992), the role of protease/chaperone proteins in the intracellular survival of *S. enterica* and *L. monocytogenes* (Buchmeier and Heffron, 1990; Johnson et al., 1991; Rouquette et al., 1996), and the role of acid tolerance genes in intracellular survival of *L. monocytogenes* (Marron et al., 1997). Because these molecules facilitate survival in both the food matrix and entry into a host cell, inducing these responses in the food matrix could therefore "prime" the pathogenic microorganisms, increasing their capacity to survive entry into a host cell and establish infection.

Moreover, conditions in food processing environments that subject pathogens to sublethal stress may further select pathogen subpopulations with increased survival efficiency (see sublethal injury, p. 63). Over time, these mechanisms could increase the relative potential for a species to cause disease. Using genome-based methodologies in food processing research centers, future scientists will be able to examine the populations of species that survive food processing conditions.

Signal Perception and Induction

In addition to different stress responses, microorganisms have evolved multiple and unique mechanisms (pathways) for sensing and transducing physical and chemical signals to the regulatory machinery that coordinates stress responses. It should be noted, however, that distinguishing between the impact of the regulatory machinery and slight variations in the actual responses is difficult. Examining the mechanics of signal perception and transduction can yield new insights into optimizing the safety of food production processes and can provide specific targets for design of antimicrobial agents.

Detailed analyses of distantly related bacteria reveal that similar stress responses may be modulated by very different regulatory machinery. For example, gram-negative enteric bacteria and low G+C gram-positive organisms use different mechanisms to trigger similar stress responses.

In gram-negative bacteria, the GSR pathway is modulated primarily by a protein called σ^{38} or RpoS, which is an RNA polymerase subunit. σ^{38} accumulates during several different stress conditions and activates the target genes that produce the general stress response. The rate of synthesis for the RpoS protein increases little during stress conditions; it accumulates rapidly after stress because the degradation rate is slowed. Unlike most response regulators, which directly modulate gene transcription by increasing the amount of signal protein produced, this response regulator functions by controlling the stability of the protein and thereby changing its rate of degradation.

In the instance of the GSR of gram-positive bacteria, much is known about *B. subtilis*. As in the gram-negative bacteria, the GSR in *B. subtilis* is modulated primarily by an alternative sigma subunit of RNA polymerase, in this case known as σ^{B}. However, σ^{B} is controlled primarily by its accessibility, not by its rate of synthesis or degradation. When under stress, the organism produces another protein (the anti-sigma factor) that binds with the σ^{B} protein, making it no longer accessible (Benson and Haldenwang, 1993). The anti-sigma factor protein is controlled by a complex cascade of signal transduction proteins, which appears to form branched pathways of signal flow and provides several points of entry for different types of signals. Because many of these signal transduction proteins appear to bind to ribosomes, scientists have hypothesized that stress in the cytoplasm is measured by increases in ribosome dysfunction.

Despite differences in regulatory machinery, there is striking similarity between the stress protection system(s) of distantly related bacteria such as *E. coli*

σ^{38} Regulated Proteins

Initially identified and characterized in *E. coli* (Hengge-Aronis, 2000), σ^{38} homologues with analogous functions have been identified in other enteric and nonenteric gram-negative bacteria (Fujita et al., 1994; O'Neal et al., 1994). The *rpoS* gene encoding for σ^{38} was initially identified as the master regulator of the phenotypic properties associated with stationary phase-reduced size and tolerance to a variety of physical and chemical challenges (Hengge-Aronis, 1993; Jenkins et al., 1988; 1990; Matin et al., 1989). The general stress tolerance induced by stationary phase/starvation is primarily due to the effects of σ^{38}-regulated proteins, although the concomitant morphological and physiological changes likely contribute to the stress-tolerance phenotype (Hengge-Aronis, 1993; Kolter et al., 1993; Matin et al., 1989).

These protective proteins are likely involved in the ability of a pathogen to survive the gastric acidity and other host defenses (Fang et al., 1992; Price et al., 2000). Moreover, σ^{38} mediates expression of the *SpvR* virulence operon in *Salmonella* (Robbe-Saule et al., 1997) and the *esp* genes of pathogenic *E. coli* that encode for a Type III secretory system (Beltrametti et al., 1999) and consequently the virulence of these bacterial pathogens. Considering the important functions of σ^{38}-regulated proteins, the finding of variations in the *rpoS* allele in stationary-phase cultures of *E. coli* (Zambrano and Kolter, 1996) is of particular importance to the emergence of new strains of pathogens with enhanced survival or virulence properties. These changes could result in enhanced production of these protective proteins and greater tolerance to stress.

Table 4. Functions of σ-Regulated Proteins

Function	Example	Reference
Metabolic changes	*otsBA* operon, trehalose metabolism	Hengge-Aronis, 2000
Protection	Oxidative stress protection by *dps*	Altuvia et al., 1994
	Oxidative stress protection by *katE*, catalase HPII	Mulvey et al., 1990
Repair	*aidB*, repairs methylation damage of DNA	Landini et al., 1996

and *B. subtilis*. Several genes in the RpoS-mediated stress protection system in *E. coli* have homologues in *B. subtilis* that are part of the σ^{32} stress protection system regulon. The shared genes of the respective stress protection systems likely constitute a core collection of important functions that confer the general protective properties needed for exploiting soil and intestinal environments.

Despite the similarity of the *E. coli* and *B. subtilis* stress protection system regulons, there are also some clear differences. For example, several heat shock genes in the *B. subtilis* stress protection system are governed independently of the stress protection system in *E. coli* where they are modulated primarily by the heat shock regulons.

Comparing the σ^{B}-mediated stress protection system in the closely-related species *B. subtilis*, *L. monocytogenes*, and *S. aureus*, reveals clear instances of divergent evolution despite the similarity of the regulatory machinery. In *B. subtilis* and *L. monocytogenes*, the seven regulatory genes that modulate σ^{B} activity are collinear and comprise an operon including the σ^{B} gene itself. Despite the collinearity in position, there is not a corresponding collinearity in structure; the distal gene of the operon shares only distant similarity with its homologue in the other species although the upstream genes are generally much more similar to one another (Becker et al., 1998). Given that this gene, known as rsbX, is the first regulator in the signal transduction pathway, the divergence could be intimately associated with the specialized physiology of the two species. A second example of the divergence is observed in *S. aureus*, which lacks three of the seven collinear regulatory genes (Kulick and Giachino, 1997; Wu et al., 1996). Although the precise meaning of these examples of divergence in regulatory molecules is not known, one might generally conclude that the differences reflect fine-tuning of the regulatory machinery to the physiological needs of the species.

The function of the regulatory machinery also is likely to be fine-tuned in both signal perception and the target genes of the regulons. Studies with *B. subtilis* and *L. monocytogenes* have shown that the magnitude of the stress protection systems to different types of signals is species-specific. For example, osmotic shock triggers a relatively minor response in *B. subtilis* but in *L. monocytogenes* is one of the most potent inducers (Becker et al., 1998). Consequently, the stress protection system contributes little to the growth of *B. subtilis* in conditions of high osmolarity, but for growth of *L. monocytogenes* the contribution is significant.

Collectively, stress protection systems play pivotal roles in the survival, dissemination, and virulence of bacterial foodborne pathogens. Understanding them may enable scientists to predict future foodborne pathogens with greater accuracy.

Driving Forces in Pathogenicity

Primary drivers of microbial pathogenicity are the growth in the human population and the proportion of the population that is immunocompromised either because of age, pregnancy, underlying disease, or immunosuppresive treatments. With higher densities of humans, microbes can exploit a multitude of routes to transfer from an infected person to other humans. The concentration of humans in urban settings can select for microbes that are transmitted from person-to-person or through contaminated air, food, and water. The same case can be made for high-density farms raising meat animals or food crops. Worldwide human travel and the global distribution of foods facilitate the introduction and flow of pathogens and exotic microbial genes into human and animal populations.

In the absence of a properly functioning immune system, microbes that are harmless to a majority of the population can cause life-threatening infections in immunocompromised individuals. People older than 65 years of age generally have reduced immunity that

Emergence of Viruses, Parasitic Protozoa and Marine Biotoxins as Foodborne Pathogens

Although pathogenicity of some of the marine biotoxins has been characterized, there is a paucity of information available regarding the disease mechanisms or virulence factors for enteric viruses and parasitic protozoa. The reasons for this are several-fold; for many of these agents, in vitro cultivation and/or animal models are nonexistent. Because they have had relatively less scientific emphasis over the last few decades, fewer research dollars have gone into understanding these agents as compared to the emerging bacterial agents such as *L. monocytogenes* or *E. coli* O157:H7. Finally, the ability to work with many of these agents has been restricted by methodological limitations that have been partially overcome by the introduction of routine molecular biological techniques.

New knowledge has emerged that highlights the unique nature of these agents. This information has been obtained largely through: (1) increased epidemiological surveillance; (2) improved detection methods; and (3) increased research funding in food safety. Such initiatives have helped scientists understand infectious doses, the role of ever-increasing internationalization of the food supply and the increased impact of environmental pollution as a contamination source.

The prevalence of viral gastroenteritis in the United States and worldwide has been drastically underestimated for many years. For instance, public health officials have consistently failed to report and investigate outbreaks of mild gastrointestinal disease, in part because of a lack of resources. In the absence of reliable laboratory methods, there has been a general reluctance on the part of public health officials to classify foodborne outbreaks as viral solely on the basis of epidemiological criteria (Bean et al., 1990). Today, clinical labs are using molecular biology techniques such as the polymerase

Continued on next page

Continued from previous page

chain reaction to facilitate diagnosis of infected patients, although these methods are not yet adapted to the routine detection of viruses in contaminated foods. These same methods are being used to identify genetic relatedness between human caliciviruses and similar viruses detected in stool samples obtained from farm animals, sparking the debate that animals may be a reservoir for the NLVs and concern over the potential for zoonotic transmission (van der Poel et al., 2000). Other investigators have focused their efforts on tracking epidemics in both space and time, concluding a winter-spring seasonality of NLV outbreaks; the presence of many genetically different variants, suggesting that most outbreaks are independent events; and on occasion, the presence of a common, predominant strain, without obvious epidemiological link, that emerges, spreads and then disappears (Fankhauser et al., 1998). There are also ongoing research initiatives to ascertain the infectious dose of representative NLVs such as the Norwalk agent and the Snow Mountain agent. Taken together, these factors will continue to contribute to the emergence of this important group of foodborne pathogens.

We also do not yet understand the importance of foods as vectors for parasitic protozoan disease, although it is likely that this will be better defined in the coming decades. Unlike the viruses which are only transmitted by humans, animal fecal pollution, and associated runoff from farms may contribute substantially to the contamination of water and subsequently crops. International trade issues have certainly impacted the emergence of *C. cayetanensis*, but the importance of poor water quality as opposed to direct contamination by local wildlife has not yet been determined. This does, however, bring up the critical issue of water quality and food handler hygiene, which is likely to be less advanced in developing nations and hence may contribute directly or indirectly to the safety of foods imported into the United States. As with the viruses, human challenge studies are currently underway in an effort to better understand the infectious doses of the parasitic protozoal pathogens. Although detection methods exist and refinements are being reported, the routine implementation of these screening methods requires highly trained personnel, and scientists are unable to detect parasitic protozoa in contaminated foods at the present time.

With respect to the marine biotoxins, much has yet to be learned. While the mechanisms of pathogenesis of some of the known biotoxins has been elucidated, the emerging agents such as *Pfiesteria* have not been characterized. In fact, the purified toxins cannot always be isolated. In many instances, scientists do not fully understand the stimulation required for the production of HABs. While many HABs may be associated with normal fluctuations in nutrient input and water temperature in the estuarine environment, it is likely that nutrient loading associated with organic and inorganic pollution may contribute to their increased prevalence and perhaps to the emergence of new toxic algal species. In the southeastern United States, some have cited intensive animal agriculture practices and/or increased land development with associated population density increases as providing the necessary environmental forces. Certainly, ongoing epidemiological studies will help ascertain the true public health impacts of these new toxic algae. In all instances (viruses, parasitic protozoa, and marine biotoxins), continued emphasis on research and vigilant surveillance will likely result in reports of increased prevalence, and hence "emergence," of these agents as associated with human foodborne disease.

continues to decline with age. By 2050, the U.S. human population will reach an estimated 400 million, and, of this population, 80 million will be 65 years of age or older. This growth in immunocompromised populations will certainly affect the number of cases of foodborne illness associated with opportunistic pathogens, which will become much more prevalent.

In terms of the environment, microbes continue to evolve to gain a competitive advantage, and, as advances are made to eliminate or control one pathogen, another organism will quickly occupy the niche that has been vacated, known as "niche filling". In some cases, the new organism that occupies this niche may be more pathogenic than the original pathogen or may employ a different mechanism of virulence that is more detrimental to human hosts (see shifts known pathogens, p. 22).

In addition, microbiological ecology involves the interplay of climate changes, pollution, and genetic exchange that selects for and perhaps drives the generation of new microbial strains. Changes within an ecosystem whereby the micro- and macro-populations of organisms are out of balance, selecting for new variants with a competitive advantage, is one theory that has been proposed to explain the emergence of new variants of existing microbes.

In terms of increased virulence in pathogens, two themes should be emphasized. First, many of the stress response systems that contribute to survival in the food matrix also contribute to survival during passage through the gastrointestinal tract and the invasion of host cells. If this phenomenon proves to be a significant feature of "virulence" for a species, then pre-harvest environments and food processing conditions should be designed to avoid imposing sublethal stress and hence selection of resistant bacterial populations. Secondly, the diversity of stress regulatory response systems and regulatory molecules holds promise for rational design and targeting of antimicrobial agents that eliminate pathogen populations while minimizing the disruption of the total bacterial population. Such agents could be used in pre- and post-harvest settings, such as feeds and carcass washes to facilitate elimination of unwanted species.

Pathogenicity of *E. coli* O157:H7

E. coli O157:H7 is typical of what might be expected in terms of an emerging pathogen.

Nomenclature

E. coli O157:H7 belongs to a group of *E. coli* (enterohemhorrhagic *E. coli*, EHEC) that cause hemorrhagic colitis (severe bloody diarrhea) and, in a small portion of the cases, hemolytic uremic syndrome (HUS). These strains are within a larger group of Shiga-toxin-producing *E. coli* (STEC), but EHEC possess virulence factors that other STEC do not. EHEC have been the source of many food- and water-borne outbreaks. It has been estimated that these pathogens cause about 75,000 cases of diarrhea and several hundred deaths annually in the United States (Mead et al., 1999). Young children and older adults are particularly susceptible to HUS, while people of all ages can get the diarrhea.

The designation O157:H7 is based on serological analysis: lipopolysaccharide (O) type 157, and flagellar (H) type 7. However, scientists have since discovered that several related *E. coli* strains with different O serotypes cause similar disease, such as O26:H11. Although O157:H7 is the most predominant EHEC serotype in North America, Japan and the United Kingdom, different serotypes of EHEC such as O26:H11 and O111:NM dominate in other areas of the world, notably central Europe and Australia. Because of the conservation of virulence factors, but not serotypes, classification schemes of EHEC strains should probably be based on virulence factors rather than the variant serotype. However, O157:H7 (EHEC 1) and O26:H11/O111:NM (EHEC 2) clearly comprise two distinct genetic lineages.

Virulence

E. coli O157:H7 and related strains have the capacity to persist in cattle without causing disease because cattle lack a receptor for the illness-producing Shiga toxin (Pruimboom-Brees et al., 2000). As many as half of all cattle carry O157:H7 at some time in their lives, and some observers suggest that nearly all cattle have been exposed to EHEC. The organisms are introduced into the environment through the feces, including manure used as fertilizer for food crops. Rainwater runoff can then spread them to water reservoirs and wells. Alternately, fecal contamination at slaughter may result in meat contamination. In addition to cattle, other ruminants, such as goats and sheep, and wild ruminants, such as deer, can carry this organism, and it has now been found in birds, flies, and in the food-producing environment.

Tolerance to low pH facilitates passage through the stomach, making it possible for *E. coli* O157:H7 to cause disease at a low infectious dose (10-100 bacteria). The organism's acid tolerance also allows it to survive within acidic food, which was a major factor in outbreaks of illness traced to unpasteurized apple juice, a product with a pH of approximately 3.5 that usually inhibits the less virulent strains of *E. coli*.

In the past few years, scientists have made significant advances in understanding the underlying virulence mechanisms of EHEC strains. Two major virulence pathways contributing to disease have been identified, although there are probably many others yet to be discovered. To cause disease, EHEC must possess a Shiga toxin gene and genes within the LEE pathogenicity island that enable the bacteria to adhere to epithelial cells and form a pedestal on the epithelial surface upon which the bacteria reside. The attaching and effacing genes also are found in enteropathogenic *E. coli* (EPEC), a common cause of watery diarrhea in the developing world, but these *E. coli* lack the Shiga toxin gene and, possibly, other virulence factors present in EHEC.

The Shiga toxins are comprised of two components, A and B subunits, that structurally resemble other toxins such as cholera toxin. The B subunit confers tissue specificity, enabling the toxin to adhere to a specific glycolipid receptor, globotriaosylceramide (Gb3), on cell surfaces. The active (A) portion of the toxin is then delivered into the host cell where it inhibits protein synthesis, ultimately killing the host cell. The toxins target certain cells, including the endothelial cells of blood vessels; the dead cells accumulate and plug the kidney, causing HUS. Shiga toxin is encoded within a mobile genetic element, a bacteriophage, that enables it to move to different strains of bacteria. As discussed below, it is believed that the acquisition of this toxin by *E. coli* is a relatively recent genetic event.

In addition to producing Shiga toxin, EHEC adhere to the large bowel, the pathogen's preferred site in the body, using a variety of virulence factors contained within the LEE pathogenicity island. This 38-kb region of DNA, inserted near a tRNA gene, contains all the genes necessary for binding to epithelial cells and causing a pedestal to form on their surface. Pedestals are created when actin in the cytoplasm is accumulated and polymerized beneath the pathogen. In addition, effacement of the microvilli occurs, giving rise to the term "attaching and effacing *E. coli*". Pedestal formation is a complex process that utilizes a Type III secretion system, several Type III *E. coli* secreted proteins (Esp's), and a key molecule, Tir (Translocated intimin receptor), that is delivered to host cell membranes. Once in the host cell membrane, Tir binds to intimin, a bacterial outer membrane protein, resulting in intimate bacterial adherence. Because Tir spans the host membrane, it is also able to recruit host cytoskeletal proteins to cause actin accumulation and pedestal formation.

Emergence

E. coli O157:H7 is believed to have arisen from a series of fairly recent genetic events. O157:H7 was first reported as a foodborne pathogen following an outbreak associated with contaminated hamburgers in 1982. Subsequent studies of diarrheal samples from prior outbreaks and sporadic cases revealed only a single *E. coli* O157:H7 isolate in the Center for Disease Control and Prevention's collection. This isolate had been obtained from an individual in the mid-1970s. A closely related strain of *E. coli* called enteropathogenic *E. coli* (EPEC) causes diarrhea in children (but does not

Continued on next page

Continued from previous page
cause HUS). EPEC contains the LEE locus, but not the Shiga toxin. It is believed that EHEC arose when an EPEC-like organism (containing the LEE island) acquired a Shiga toxin via a bacteriophage. This event has occurred on more than one occasion, leading to the two distinct EHEC lineages. Experimental evidence comes from studies done in rabbits, where a LEE-encoding *E. coli* was altered to also encode the Shiga toxin. The resulting pathogen produced a diarrhea that resembled hemorrhagic colitis, which was an EHEC-like disease.

Treatment

Therapies against EHEC infections are extremely limited. Treatment with antibiotics is thought to worsen the illness, presumably by breaking up the bacteria, which releases more toxin and increases toxin expression. Kimmitt et al. (2000) reported that some antimicrobial agents, particularly quinolones, trimethoprim, and furazolidone, were shown to induce toxin gene expression and should be avoided in treating patients with potential or confirmed STEC infections. These investigators also reported, however, that results of available studies conflict with regard to the influence of antibiotics, noting that age group, timing of antibiotic therapy, and range of agents used complicate the analyses. Further, Kimmett et al. (2000) reported that their observations suggest that the complex interplay of infection stage, number of organisms present at the time antibiotics are administered, and the environmental conditions of those microorganisms, coupled with time-concentration profile, and bactericidal effect of the drug, could render an antibiotic clinically beneficial, neutral, or disadvantageous in different situations.

One potential therapeutic is currently in phase III clinical trials. New therapies use an inert substance that mimics the toxin's glycolipid receptor. Ingestion of the mimicking substance should bind excess toxin and thereby limit disease progression. Experiments indicate it may be effective, but only when taken very early after infection. Alternate therapies are being explored.

In addition, significant effort is focused on developing treatments such as vaccines and probiotics to reduce carriage of O157:H7 by cattle. Decreasing the level of O157:H7 in cattle would significantly decrease the potential for food and water contamination. Similarly, childhood vaccines are being developed, but given the low incidence of disease, questions remain about whether universal vaccination should be employed, were an effective vaccine to be developed. Experimental approaches to block virulence factors such as the Type III secretion system also are being studied.

Humans as Hosts of Foodborne Disease

A number of factors that relate to the human host have a major impact on the occurrence and severity of foodborne disease. The host's age, gender, place of residence, ethnicity, educational background, underlying health status, and knowledge, attitudes, and practices related to health and diet all have important bearing on foodborne illness. The health of the host affects the individual's susceptibility to infection and illness, and the host's dietary and hygiene practices affect exposure to pathogens. From medical and behavioral perspectives, human host factors can be altered by modification of susceptibility or elimination of exposure. For example, assuring proper handling and cooking of ground beef contaminated with *Escherichia coli* O157:H7 could eliminate that food safety risk even in the presence of continuing contamination of raw ground beef. Obviously, risk communication is an essential tool for preventing foodborne illness. However, successful control requires effective interventions at all stages of the food system.

Manifestations of Clinical Disease

As the name implies, foodborne diseases—including intoxication, infection, and toxicoinfection—are illnesses acquired by consumption of food containing pathogens or their toxins. The pathogens or their toxins can damage or destroy host cells or processes, or they can induce a host response to their presence that is harmful to the human host. Foodborne illness is caused by: viral, bacterial, or parasitic infections (e.g., Norwalk-like viral gastroenteritis, *Campylobacter* enteritis, toxoplasmosis); toxins produced during microbial growth in food (e.g., *Clostridium botulinum*, *Staphylococcus aureus*, *Bacillus cereus*, and *Aspergillus flavus*); and toxins produced by algal and fungal species (e.g., ciguatera fish poisoning) (see Table 5).

Foodborne infections occur when pathogenic microorganisms are ingested, colonize the intestine, and sometimes invade the mucosa or other tissues. Foodborne toxicoinfections arise when a microorganism from ingested food grows

Continued from previous page
cause HUS). EPEC contains the LEE locus, but not the Shiga toxin. It is believed that EHEC arose when an EPEC-like organism (containing the LEE island) acquired a Shiga toxin via a bacteriophage. This event has occurred on more than one occasion, leading to the two distinct EHEC lineages. Experimental evidence comes from studies done in rabbits, where a LEE-encoding *E. coli* was altered to also encode the Shiga toxin. The resulting pathogen produced a diarrhea that resembled hemorrhagic colitis, which was an EHEC-like disease.

Treatment

Therapies against EHEC infections are extremely limited. Treatment with antibiotics is thought to worsen the illness, presumably by breaking up the bacteria, which releases more toxin and increases toxin expression. Kimmitt et al. (2000) reported that some antimicrobial agents, particularly quinolones, trimethoprim, and furazolidone, were shown to induce toxin gene expression and should be avoided in treating patients with potential or confirmed STEC infections. These investigators also reported, however, that results of available studies conflict with regard to the influence of antibiotics, noting that age group, timing of antibiotic therapy, and range of agents used complicate the analyses. Further, Kimmett et al. (2000) reported that their observations suggest that the complex interplay of infection stage, number of organisms present at the time antibiotics are administered, and the environmental conditions of those microorganisms, coupled with time-concentration profile, and bactericidal effect of the drug, could render an antibiotic clinically beneficial, neutral, or disadvantageous in different situations.

One potential therapeutic is currently in phase III clinical trials. New therapies use an inert substance that mimics the toxin's glycolipid receptor. Ingestion of the mimicking substance should bind excess toxin and thereby limit disease progression. Experiments indicate it may be effective, but only when taken very early after infection. Alternate therapies are being explored.

In addition, significant effort is focused on developing treatments such as vaccines and probiotics to reduce carriage of O157:H7 by cattle. Decreasing the level of O157:H7 in cattle would significantly decrease the potential for food and water contamination. Similarly, childhood vaccines are being developed, but given the low incidence of disease, questions remain about whether universal vaccination should be employed, were an effective vaccine to be developed. Experimental approaches to block virulence factors such as the Type III secretion system also are being studied.

Humans as Hosts of Foodborne Disease

A number of factors that relate to the human host have a major impact on the occurrence and severity of foodborne disease. The host's age, gender, place of residence, ethnicity, educational background, underlying health status, and knowledge, attitudes, and practices related to health and diet all have important bearing on foodborne illness. The health of the host affects the individual's susceptibility to infection and illness, and the host's dietary and hygiene practices affect exposure to pathogens. From medical and behavioral perspectives, human host factors can be altered by modification of susceptibility or elimination of exposure. For example, assuring proper handling and cooking of ground beef contaminated with *Escherichia coli* O157:H7 could eliminate that food safety risk even in the presence of continuing contamination of raw ground beef. Obviously, risk communication is an essential tool for preventing foodborne illness. However, successful control requires effective interventions at all stages of the food system.

Manifestations of Clinical Disease

As the name implies, foodborne diseases—including intoxication, infection, and toxicoinfection—are illnesses acquired by consumption of food containing pathogens or their toxins. The pathogens or their toxins can damage or destroy host cells or processes, or they can induce a host response to their presence that is harmful to the human host. Foodborne illness is caused by: viral, bacterial, or parasitic infections (e.g., Norwalk-like viral gastroenteritis, *Campylobacter* enteritis, toxoplasmosis); toxins produced during microbial growth in food (e.g., *Clostridium botulinum*, *Staphylococcus aureus*, *Bacillus cereus*, and *Aspergillus flavus*); and toxins produced by algal and fungal species (e.g., ciguatera fish poisoning) (see Table 5).

Foodborne infections occur when pathogenic microorganisms are ingested, colonize the intestine, and sometimes invade the mucosa or other tissues. Foodborne toxicoinfections arise when a microorganism from ingested food grows

Table 5. Causes of Foodborne Illness

Type of Causative Agent	Example(s)	Frequency (in the U.S.)
Bacterial infection	*Campylobacter jejuni*	Common
Viral infection	Norwalk-like viruses	Very common
Parasitic infection	*Toxoplasma gondii*	Relatively common
Bacterial toxin	*Clostridium perfringens, Bacillus cereus*	Relatively common
Algal toxin	Ciguatera fish poisoning	Less common
Mycotoxin	Aflatoxin	Less common
Prions*	BSE	None
Inorganic contaminants*	Heavy metals	Less common
Organic contaminants*	Pesticide residues	Less common

* Not addressed within this report.

in the intestinal tract and elaborates a toxin(s) that damages tissues or interferes with normal tissue/organ function. Foodborne microbial intoxications occur by ingestion of a food containing harmful toxins or chemicals produced by the microorganisms, usually during their growth in the food.

Despite our best efforts, foodborne diseases remain common. Based on the available data, the Centers for Disease Control and Prevention (CDC) has estimated that 76 million cases of foodborne illness occur annually resulting in 325,000 hospitalizations and 5,000 deaths (Mead et al., 1999).

Most infectious foodborne illness is characterized by acute symptoms that are limited to the gastrointestinal tract, including vomiting and diarrhea. These illnesses are generally limited in both duration and severity, and most patients without underlying illnesses or malnutrition recover without medical treatment or require only modest supportive care. These illnesses can be especially difficult to quantify because medical treatment is not sought. For example, *B. cereus*—linked to a wide variety of foods such as meat, milk, vegetables, fish, and rice products—may cause diarrhea and abdominal cramps and pain that last approximately one day. *C. perfringens*, which may be present in meat, meat products and gravies, can cause intense abdominal cramps and diarrhea that also generally resolve within a one-day period. Norwalk-like viruses, responsible for an estimated 66.6% of illnesses attributed to known foodborne pathogens (Mead et al., 1999), result in nausea, vomiting, headache, diarrhea, and abdominal pain. The symptoms usually subside within a day or two, and the infections rarely come to the attention of public health workers.

However, some pathogens cause gastrointestinal symptoms that are more severe and take longer to subside, especially in immunocompromised individuals such as young children, the elderly or people with AIDS. For example, *Cryptosporidium parvum* is a parasitic protozoan that causes severe watery diarrhea and sometimes coughing, fever, and intestinal distress. Symptoms may last from four days to three weeks. *Campylobacter jejuni*, usually associated with raw chicken and raw milk, can trigger watery diarrhea, fever, abdominal pain, and nausea that last for days to weeks. Of the estimated illnesses attributed to known foodborne pathogens, *Campylobacter* spp. are responsible for more than 14% (Mead et al., 1999).

Not all foodborne disease is limited to the gastrointestinal tract. Some foodborne pathogens invade deeper tissues or produce toxins that are absorbed and cause systemic symptoms, including fever, headache, kidney failure, anemia, and death. *Salmonella* has been linked to numerous foods, but especially raw meats, poultry, and eggs. The symptoms of salmonellosis include nausea, vomiting, abdominal cramps, fever, and headaches with a duration that ranges from days to weeks. Nontyphoidal *Salmonella* are responsible for an estimated 30.6% of deaths caused by known foodborne pathogens. *E. coli* O157:H7 is most commonly associated with undercooked ground beef and causes severe cramping and bloody diarrhea. After the hemorrhagic colitis caused by *E. coli* O157:H7 and other enterohemorrhagic *E. coli* (EHECs), some children develop a characteristic set of kidney disfunction and anemia called hemolytic uremic syndrome (HUS). In the United States, HUS is the leading cause of acute kidney failure in children.

The major effects of some foodborne pathogens are outside the gastrointestinal tract. *Listeria monocytogenes* causes serious illness in pregnant women, their fetuses, or newborns, often resulting in spontaneous abortion or stillborn babies. An estimated 92% of foodborne cases of listeriosis result in hospitalization, and 20% result in death. *C. botulinum* produces toxins that attack the central nervous system, resulting in weakness, vertigo, double vision and difficulty swallowing and speaking.

In addition to acute illness, some foodborne pathogens cause chronic illness, often within sensitive subgroups of the population. For example, hepatitis A virus (HAV) causes fever, headache, anorexia, malaise, nausea, abdominal discomfort and sometimes jaundice. These symptoms usually take weeks to resolve. Within a genetically predisposed subgroup, prolonged HAV infection may be the precipitating event in the onset of autoimmune chronic active hepatitis (Bogdanos et al., 2000; Naniche and Oldstone, 2000; Rahyaman et al., 1994.

The foodborne parasite *Toxoplasma gondii* causes birth defects. In addition, chronic toxoplasmic encephalitis attributed to *T. gondii* infection may occur when an individual's immune system is impaired. Toxoplasmal encephalitis, characterized by dementia and seizures, has become the most commonly recognized cause of opportunistic infection of the central nervous system in AIDS patients. Activated macrophages, lymphocytes, and cytokines play a major role in control of both the acute infection and maintenance and/or prevention of the chronic stage (Cohen, 1999; Tenter et al., 2000).

Biotoxins also cause foodborne disease, with both acute and chronic clinical manifestations. Because these compounds can be resistant to processing and cooking, biotoxins can be present in a food even in the absence of viable cells of the causative agent. The target organs

for these toxins vary and can include the liver, kidney and gastrointestinal tract as well as the immune, nervous, and reproductive systems. Biotoxins include toxins produced by bacteria (e.g., botulism toxin), fungi (e.g., aflatoxin and fumonisin), marine organisms such as dinoflagellates (ciguatoxin), and plants (phytotoxins, which are not discussed in this report).

The chronic sequelae of foodborne infections in particular often focus on extra-intestinal systems, although foodborne microorganisms also may play a role in chronic enteropathies such as inflammatory bowel disease. Guillain-Barré Syndrome (GBS) is a disorder of the peripheral nervous system that occurs worldwide and is a common cause of neuromuscular paralysis. Victims lose the ability to write or speak and experience motor paralysis with mild sensory disturbances. Cases of severe GBS have been linked to a previous infection with *C. jejuni*, although other enteric pathogens also may trigger the disorder. Evidence indicates GBS is an autoimmune disease, but the immunologic mechanisms that produce GBS after infection with *C. jejuni* are complex. Studies support the hypothesis of molecular mimicry, since peripheral nerves may share epitopes with surface antigens of certain strains of *C. jejuni*. Some data suggest that patients share genetic traits (Smith, 1995). Although it is clear that GBS is an autoimmune phenomenon, evidence indicates that infections with *C. jejuni*, a common foodborne pathogen, frequently start the pathologic process (Allos, 1997; Shoenfeld et al., 1996).

Another example of chronic illness related to a foodborne infection is reactive arthritis. Triggered by infection with *Yersinia enterocolitica, Yersinia pseudotuberculosis, Shigella flexneri, Shigella dysenteriae, Salmonella* spp., *C. jejuni,* and *E. coli,* reactive arthritis is an acute sterile inflammation of the joints. In addition to joint pain, Reiter's syndrome, a subtype of reactive arthritis, affects eyes and the urogenital system. A genetic predisposition to developing post-infectious reactive arthritis has been documented in persons who share certain genetic traits. Other genes acting in concert apparently determine the clinical presentation. Chronic sequelae are thus related to genetically determined host risk factors in combination with an environmental trigger (Kobayashi and Ando, 2000; Parker and Thomas, 2000). It is important to note that as human and microbial genome sequencing projects progress, scientists should gain increasing insights into the bacterial virulence factors and the host factors that interact in the production of these chronic, autoimmune pathologies. Hopefully, these insights will eventually result in rational therapies to prevent or treat these types of diseases.

Although the evidence is not complete, foodborne infections may play a role in the development of inflammatory bowel disease (IBD). IBD is the collective

Pfiesteria piscicida and *Pfiesteria*-like Microbes As Potential Foodborne Pathogens

An association between seafood-borne illness in humans and the occurrence of *Pfiesteria* in the marine environment has not been established, and much remains unknown. *Pfiesteria piscicida* and *Pfiesteria*-like organisms, discovered in 1988, have caught the attention of researchers and funding agencies interested in characterizing the biological functions and effects of previously unrecognized toxic dinoflagellates (Fleming et al., 1999; Glasgow et al., 1995; Smith et al., 1988). Initially presenting as the cause of massive fish kills in North Carolina in the late 1980s, *Pfiesteria*-like organisms also have stimulated public concern over the potential threat these organisms may have on human health and seafood safety. Because of difficulties in identification of the *Pfiesteria* species, these organisms are currently characterized on the basis of morphology and hence referred to as morphologically related organisms (MROs). MROs are believed to occur in waters from Delaware to the Gulf of Mexico (EPA, 1998). Currently, the number of MROs is unknown, and while some are toxic, others are not (Steidinger and Penta, 1999).

It has been proposed that the *Pfiesteria* dinoflagellate, unlike most toxic dinoflagellates, excretes its toxin(s) into the estuary rather than retaining the toxin within its cell (Burkholder *et al.*, 1995; Glasgow et al., 1995). Following exposure to toxic *P. piscicida* and other toxic MROs, fish appear to be narcotized and frequently die (Burkholder et al., 1995; Glasgow et al., 1995; Noga, 2000). However, the toxin (*Pp*tx) is relatively unstable in the marine system, and in spite of continuing research efforts on the life cycle and physiology of *Pfiesteria*, very little is known about the toxin(s) produced by the dinoflagellate. Attempts to obtain purified toxin(s) have been unsuccessful. Mechanisms of action and chemical structure are currently undetermined. Investigators believe that the toxin consists of both water-soluble fractions, which may be responsible for the alleged neurotoxic effects in humans, and highly lipophilic components, which may be responsible for fish morbidity and mortality (Fleming et al., 1999; Noga, 1997). Reported human impacts include respiratory irritation, skin rashes and possible neurocognitive disorders (Glasgow et al., 1995). Although animal models have been developed to investigate neurocognitive effects (Levin et al., 1997), and epidemiological studies to evaluate the potential human health effects (Gratten et al., 1998; Savitz, 1998) are ongoing, results are inconclusive to date.

It is important to note that all of these studies investigate transmission routes other than through the consumption of contaminated seafood. Nonetheless, consumer confidence in seafood is adversely affected by events such as fish kills and health alerts. Extensive media coverage of *Pfiesteria*, closings of recreational and commercial waters, as well as a growing list of scientific unknowns regarding the organism's occurrence, toxin(s), and effects, have generated immediate food safety concerns in seafood consumers, despite the apparent safety of these foods.

term for Crohn's disease (CD) and ulcerative colitis (UC), which are both chronic inflammatory diseases with a prolonged clinical course. Abdominal abscesses are a complication of CD while in UC abdominal perforations may lead to peritonitis. The cause of IBD, and the mechanism(s) for spontaneous exacerbations and remissions remain unresolved. Controversial reports by some investigators about the potential association of *Mycobacterium paratuberculosis*, the etiologic agent of Johne's disease in ruminants, with CD have appeared in the literature for years. A conference convened by the National Institutes of Health Division of Microbiology and Infectious Disease (NIH/DMID, 1998) and the European Commission's Scientific Committee on Animal Health and Animal Welfare (EC, 2000) concluded that there is insufficient evidence to prove or disprove that *M. paratuberculosis* is the cause of CD. Studies to determine how *M. paratuberculosis* could be transferred from animals to humans have focused on milk and results have been conflicting (CAST, 2001). Both the NIH/DMID conference and the EC committee recommended further study of this issue. An association between IBD and the bacterial L-forms of *Pseudomonas, Mycobacterium, Enterococcus faecalis* and *E. coli* also has been suggested (Herman-Taylor et al., 2000; Korzenik and Dieckgraefe, 2000).

The preceding examples are representative, but they do not present a comprehensive discussion of foodborne disease. The differing symptoms, duration and severity make diagnosis and comprehensive tracking of foodborne illness difficult. The large number of foodborne pathogens—each with its own virulence factors—produce an astonishing array of illnesses, and the pathogens continue to evolve. Millions of illnesses and thousands of deaths occur each year as a result of contaminated food in developed countries, and the situation is much worse in the developing world.

Resistance to Microbial Foodborne Disease

Humans are protected from infectious foodborne disease by a variety of nonspecific (innate) and specific immune system mechanisms. When all of these systems are functioning optimally, the chance of foodborne illness is reduced. Many factors cause these systems to function below optimal levels, increasing the likelihood of illness. In addition, some foodborne pathogens have found ways to evade or trick the body's defensive mechanisms.

Immune Response

Foodborne pathogens and their toxins enter human tissue via the gastrointestinal (GI) tract. The GI tract is approximately 30 feet long and includes cells that produce acid, mucus, antibodies, and other substances that protect the host from foodborne pathogens (Burke, 1985). Food entering the mouth is chewed, breaking it into smaller pieces and mixing it with saliva. When swallowed, the food travels via the pharynx and esophagus to the stomach. Upon entering the stomach, the food is broken down by gastric juices that contain pepsin, lipases and acids.

From the stomach, the partially digested food enters the small intestine. The intestine is approximately 20 feet long and has an irregular, folded lining that provides a very large surface area that facilitates digestion and absorption of nutrients. It also facilitates exposure to microorganisms that survived passage through the stomach. The tissues immediately below the epithelial lining cells contain blood capillaries to absorb monosaccharides and amino acids and deliver bloodborne defenses, and lymph capillaries to absorb fatty acids and glycerol. Enzymes and hormones secreted by the liver and pancreas assist digestion and absorption in the small intestine. Because of its length, surface area (equivalent to a singles tennis court), and digestive capacity, more than 80% of absorption occurs in the small intestine. Undigested food material enters the large intestine (colon), through which it travels until it finally exits the anus. Anatomic, physiologic, and pathologic changes in the GI tract influence the level of protection from the effects of pathogens. These GI tract changes also affect the population of nonpathogenic microorganisms living in the GI tract, which influences the likelihood of foodborne infections.

The large surface area, presence of large amounts of nutrients, and absorptive capacity of the alimentary canal make it particularly prone to penetration by microbes and their toxins. Fortunately, the body defends itself with an extraordinary array of nonspecific and specific immune mechanisms.

Nonspecific Immune Mechanisms

Nonspecific or "innate" immunity is the front line of host defense against microorganisms in the gut and other sites (Elwood and Garden, 1999; Pestka, 1993; Takahashi and Kiyono, 1999). The epithelial barriers prevent absorption of more than 99% of the proteins in the intestinal tract (Newby, 1984). These epithelial lining cells are constantly renewed to ensure that damaged villi do not provide a location vulnerable to infection. The continued movement of the gut contents also keeps the microbial populations (microflora) in the small intestine at lower levels compared to the large intestine microflora, which exists in a more static environment.

Digestive secretions are another major form of nonspecific immunity in the GI tract. For example, the high acidity and pepsin content in the stomach work to destroy microbial pathogens and their toxins. Enzymes in bile acids and pancreatic secretions also can protect against microbial pathogens. Gastric and intestinal epithelia are covered by a moving layer of continually replaced mucus, a protein that contains sugar residues to protect against proteolytic attack and microbial attachment. In addition to functioning as a lubricant and protecting the stomach and intestine from acidic pH, the mucus is a vehicle for antibacterial substances (e.g., secretory immunoglobulin A and enzymes) and prevents large molecular weight materials from passing into enterocytes, the epithelial lining cells.

As a stable ecosystem, the normal intestinal microflora diminish opportunities for pathogenic microbial infection. By occupying the available binding sites on the enterocytes, decreasing the pH of the gut lumen, producing volatile fatty acids and selective antibiotics known as bacteriocins, and increasing motility of the gut contents, these nondisease-causing (commensal) microbes provide an important element of the nonspecific defense system.

Microbial agents or antigens that manage to penetrate the epithelial barrier may encounter mononuclear phagocytes (blood monocytes or tissue mac-

rophages) and polymorphonuclear phagocytes (PMNs or granulocytes) that defend the rest of the body from things that get through the superficial defenses. PMNs, a primary defense against infectious agents, can travel via blood vessels. Macrophages travel to an inflamed site where they attempt to kill the intruder. Certain blood proteins also can serve as backup nonspecific defense mechanisms (Pestka and Witt, 1985). Interferon formed by virus-infected cells can inhibit replication of unrelated viruses. Kinins are a group of peptides which, when activated, are involved in inflammation and blood clotting. Finally, the complement system, a series of proteins and enzymatic reactions, can destroy invading cells.

The nonspecific mechanisms described here act together to prevent infection by enteric microorganisms or entry of large microbial toxins. Thus, under normal conditions, relatively large numbers of microorganisms would be required for a few to survive the defenses and initiate infection. A variety of factors may depress nonspecific immunity, such as decreased gastric acidity caused by ingestion of antacids, diminished native microflora following treatment with antibiotics, or damage to the epithelial barriers. When nonspecific immunity is depressed, the likelihood that small numbers of a pathogen will cause an infection is increased. However, even when innate protection fails, specific defense mechanisms can prevent infection and disease.

Specific Immune Mechanisms

In addition to the nonspecific immune defenses described above, ingested microbes face other compounds that circulate in the blood or are secreted into the lumen of the GI tract that are specific to certain microbes or related groups of microbes. This "acquired" immunity recognizes characteristics or components of the microorganism, called antigens, and then inactivates, removes or destroys the microorganisms that possess these antigens. To do this, the immune system must be able to recognize small differences in the chemical structure of an antigen and "remember" these chemical structures for long periods of time. Antigens are typically high molecular weight (>10,000 Daltons) proteins or polysaccharides. Parts of the pathogen—such as the cell wall, flagella, capsule and toxins—serve as excellent antigens, in part because they are multivalent, meaning they have more than one chemical structure that can be recognized by the immune system.

Specific responses can be functionally divided into phases: (1) recognition, (2) activation, and (3) effector (Abbas et al., 1997). In the recognition phase, foreign antigens bind to specific receptors on existing lymphocytes. Lymphocyte recognition of specific antigens triggers the activation phase. Activation events include development of antigen-specific lymphocytes and a shift from recognition to defensive functions. In addition to antigens, activation requires "helper" or "accessory" signals from other cells. Finally, the effector phase implements an active defense based on antigen recognition and lymphocyte activation.

The immune system has numerous possible effector responses to an antigenic stimulus. First, one or more components of the specific immune system can work to remove the antigen. Second, specific and nonspecific immune mechanisms can interact to enhance the host's ability to kill invading microorganisms. Third, an antigenic stimulus can induce "tolerance," which is a "specific" type of unresponsiveness. Thus, a host can recognize and tolerate the host's own proteins, known as self antigens. The ability of the immune system to develop a memory allows the host to both prevent future reinfection by an invading organism and to avoid mounting a self-destructive immune response.

Cells of the Immune System. Many highly specialized cells carry out the critical functions of specific humoral (antibody-mediated) and cell-mediated immune reactions that influence a host's resistance to infection and serious disease. These cells are derived from stem cells in the bone marrow and become the lymphocytes, granulocytes, macrophages, dendritic cells and other specialized protective cells during a process called hematopoiesis. To be responsive to the present needs of the immune system, many aspects of leukocyte development are regulated by cell-to-cell interactions and by cytokines, soluble protein factors that influence cell growth, differentiation and maturation. In most cases, the cell types involved in generalized systemic immunity also play key roles in gastrointestinal immunity.

Lymphocytes carry out critical regulatory and effector activities in specific immunity. B lymphocytes are responsible for humoral immunity (antibody production) and carry immunoglobulins (antibodies) on their surface. T lymphocytes can have both effector and regulatory functions. T cells control the maturation of both effector T and B cells. T cells also are involved in cell-mediated immune responses such as cytotoxicity and delayed-type hypersensitivity. Some B and T cells reside in specific areas in the "secondary" lymphoid organs such as the spleen and gut-associated lymphoid tissue (GALT) to facilitate contact between lymphocytes and circulating antigens.

In addition to B and T cells, accessory cells (macrophages, monocytes, and dendritic cells) can ingest and destroy infectious particles and function in antigen presentation that influences the strength and type of antibody response. Mast cells can respond to various antigens and generate a hypersensitivity response. Mononuclear cells known as "killer" cells can bind to antibodies and facilitate lysis of tumor cells and cells infected with viruses. Other cell types with the ability to spontaneously dissolve or disintegrate neoplastic cells have been called natural killer (NK) cells.

Gut-Associated Lymphoid Tissue (GALT). Differentiating generalized systemic immunity from mucosal immunity is useful. The systemic immune system includes all the tissue involved in protecting the body's interior from invading microorganisms. The mucosal immune system consists of the lymphoid tissue that borders the external environment of the gut lumen or other sites such as the lungs and nose. While this classification is helpful in analyzing diverse functions, many of the specific activities of lymphoid tissue in the systemic and mucosal compartments overlap and can affect the function of each other.

GALT is made up of aggregated and non-aggregated tissue (Elwood and Garden, 1999). The aggregated component includes mesenteric lymph nodes, lymphoid nodules, and groups of nodules called Peyer's patches that occur right under the epithelial cells that line the lumen of the intestine. These sites contain a full complement of the immune cells necessary to launch an immune response. The non-aggregated tissue includes lymphocytes, macrophages, and mast cells in the lamina propria (connective tissue beneath the epithelium) and the intraepithelial lymphocytes in the gut wall.

Antigen Uptake in the Gut. In gener-

al, large molecules in the lumen of the intestines are digested into small component parts before they are absorbed. However, high molecular weight antigens can move from the gut lumen into the blood. Prior to uptake, the antigens must resist proteolytic activity in the lumen and penetrate the mucus layer so they can interact with the various absorptive cell types. Factors that disrupt the mucosal barrier function and facilitate the uptake of antigens include immature gastrointestinal function, malnutrition, inflammation, and immunoglobulin deficiencies (Walker, 1987). At least two different mechanisms may result in uptake of these macromolecules (Stokes, 1984). In the first, the intestinal epithelial cell can incorporate macromolecular aggregates through endocytosis and deliver these to the subepithelial space by exocytosis (Walker, 1987). In the second, antigens can be deliberately "sampled" by the specialized epithelial cells (M cells) that cover Peyer's patches, which bring the intact antigen into the underlying lymphoid tissue to trigger a comprehensive specific immune response.

Specific Humoral Responses in the Gut. Humoral immunity is mediated by highly specific proteins known as antibodies, which are secreted in response to the antigen that originally stimulates the antibody formation. Antibodies are sometimes called immunoglobulins (Igs). There are five major classes or "isotypes" of immunoglobulins, each of which functions slightly differently. Of these, IgA is of predominant importance in local immunity in the gut because much of it is secreted into the gut lumen where it can interact with microorganisms before they invade deeper into the body. In fact, IgA accounts for 60% of total daily antibody production in humans (McGhee et al., 1992). IgA is found both in mucus secretions (secretory IgA or sIgA) of the gut and as a circulating Ig. Antigens in the gut, including those on microbes, are most likely to encounter sIgA before any other Ig. Peyer's patches are usually considered to be the source of most IgA.

Primary roles that have been suggested for sIgA are antigen exclusion, inhibition of adherence of microorganisms, intracellular virus neutralization and excretion of IgA immune complexes. Secretory IgA induces antigen removal by taking advantage of the normal clearing activities of the gut (Newby, 1984). Thus, working with the nonspecific immune system, sIgA is able to inhibit entry of soluble antigens and restrict epithelial colonization by bacteria and viruses.

Specific Cell-Mediated Responses in the Gut. Cytotoxic T cells also can defend a host against living antigens, such as virally infected cells or intracellular pathogens. In such a response, a target host cell bearing an antigen of the pathogen on its surface interacts directly with a cytotoxic T cell resulting ultimately in the lysis of the target cell. The killing is unidirectional and thus cytotoxic T cells can kill numerous target cells.

The Common Mucosal Immune System. It appears that antigenic stimulation in the gut may result in IgA secretion at other mucosal sites such as salivary glands and genitourinary sites, leading to the concept of a "common mucosal immune system" (McDermott and Bienenstock, 1979). While primarily demonstrated in experimental animals, evidence exists for a common mucosal immune system in humans, based on detection of gut antigen-specific IgA at anatomically remote sites. Furthermore, antigen-specific IgA producing cells can be found in blood following oral immunization and preceding their appearance in saliva and tears (Russell et al., 1991). The advantage of a common mucosal response relates to the mobilization of humoral and cellular immune elements to various sentinel sites (e.g., mouth, eye, genitourinary tract) that can prevent infection at all of these sites upon subsequent reexposure to the pathogen.

Stimulation of Specific Gut Immunity. Stimulating the specific immune response within the gut to protect against various microbial illnesses helps prevent disease. However, achieving long-term memory when immunizing orally is difficult because ingested antigens tend to be degraded by acidic pH and proteolysis in the gut (Stokes, 1984). Oral immunization with live organisms is generally more effective than nonreplicating organisms for induction of IgA responses, implying that colonization and/or replication in the GI tract is required (McGhee et al., 1992). Furthermore, particulate antigens function much more effectively than soluble ones. Thus, close contact with key components of the gut is required to induce a GI immune response.

Responses to Infectious Microbes. Many different bacteria, parasites and viruses cause gastroenteritis or penetrate the gut as an entry point to cause systemic infection. The capacity to override the GI defensive barriers depends on several factors, such as the number of microorganisms and virulence factors (see virulence, p. 15). Thorne (1986) outlined five pathogenic mechanisms for bacterial diarrheal diseases: (1) bacteria produce toxin but do not generally adhere and multiply (e.g., *B. cereus, S. aureus, C. perfringens, C. botulinum*); (2) bacteria adhere to the lining of the intestine and produce toxin (e.g., enterotoxigenic *E. coli, Vibrio cholerae*); (3) bacteria adhere and damage the villi that make up the brush border (e.g., enteropathogenic *E. coli*); (4) bacteria invade the mucosal layer and initiate intracellular multiplication (e.g., *Shigella* spp.); and (5) bacteria penetrate the mucosal layer and spread to lamina propria and lymph nodes (e.g., *Yersinia*). With each increasing level of action, the pathogen's focus moves from the mucosal to the systemic compartment, and, hence, the specific immune response must escalate.

The antigen-sampling process itself may become a major portal of entry for pathogens (Owen and Ermak, 1990). Wells et al. (1988) hypothesized that, in some instances, a motile phagocyte may ingest an intestinal bacterium, transport it to an extraintestinal site, fail to accomplish intracellular killing, and then liberate the bacterium at the extraintestinal site. This hypothesis was based on the observation that the intestinal bacteria that most readily translocate out of the intestinal tract are facultative intracellular pathogens. Secondly, intestinal particles without inherent motility (e.g., yeast, ferritin, starch) move out of the intestinal lumen within hours of their ingestion. Thirdly, the rate of translocation of intestinal bacteria can be altered with agents that modulate immune functions such as phagocytosis. Thus, systemic infection by translocating intestinal bacteria could be a result of the antigen-sampling process that evolved to regulate the immune response to intestinal antigens.

Low Molecular Weight Toxins

As discussed above, high molecular weight toxins (proteins, polysaccharides) produced by microbes are cleared by the immune system. However, the immune system does not respond to low molecular weight, nonpolar compounds, such as mycotoxins, which can be rapidly absorbed through the gastrointestinal tract. Higher vertebrates have developed the capacity to metabolize mycotoxins and oth-

er foreign materials (xenobiotics) via a process known as biotransformation (de Bethizy and Hayes, 1994). The liver is the primary organ of xenobiotic biotransformation because of its size and its central location in systemic circulation. However, specific limited biotransformation capacities can be found in other tissues and in the microflora of the intestine.

Biotransformation can be divided into two distinct phases. Phase I reactions add specific functional groups to the toxin that are used for subsequent metabolism by phase II enzymes. Phase II reactions are considered biosynthetic. Biotransformation changes hydrophobic toxins to more polar, readily excreted compounds. Examples of phase I reactions include oxidation, reduction, hydration, and hydrolysis. Examples of phase II reactions include glucuronidation, sulfate conjugation, glutathione addition, methylation, and acetylation.

Although biotransformation is an important mechanism of host defense, in some cases, biotransformation can make xenobiotics more toxic. Notably, aflatoxin B_1 is converted to a reactive epoxide that can react with nuclear DNA and cause mutations that ultimately result in liver cancer, although the original mycotoxin chemical structure is not carcinogenic.

Susceptibility to Microbial Foodborne Disease

The extent to which the human host is susceptible to disease influences the likelihood of foodborne illness. Many factors play a role in the level of susceptibility.

Infectious Disease

Susceptibility to infectious disease is the inability of the host's body to prevent or overcome invasion by pathogenic microorganisms. Susceptibility to infectious disease is increased by conditions that alter the host defenses and suppress the function of the immune system. Altered host defenses and immunosuppression can be caused by an infection, another disease, aging, poor nutrition, or certain medical treatments (see Table 6). These factors have all been implicated in the increased risk of infection or increased severity of illness caused by many foodborne pathogens including *Cryptosporidium, Toxoplasma, Campylobacter, Salmonella, L. monocytogenes,* and *Giardia* (see sidebar, p. 35).

As described above, humans possess a number of general and specific host defenses against foodborne disease. General defenses include normal indigenous microflora, the acidic pH of the stomach, and the antibacterial effect of the various pancreatic enzymes, bile and intestinal secretions. The constant movement of the intestine (peristalsis) helps maintain the balance of normal flora and purge the intestinal tract of harmful microorganisms. Factors that alter these general parameters can increase susceptibility to infection. For example, *Salmonella* infection is more common in patients with decreased stomach acidity from medication or after gastrectomy. Slowing peristalsis with belladonna or opium alkaloids prolongs symptoms of shigellosis. Similarly, treatment of typhoid fever with antibiotics prolongs the carrier state for *Salmonella* Typhi, and some evidence indicates that antibiotic treatment of *E. coli* O157:H7 increases the risk of HUS. Additionally, altering the bowel microflora with broad spectrum antibiotics can lead to overgrowth of pathogenic organisms (e.g., *Salmonella*).

In the United States, end-stage cancer, renal disease, end-stage AIDS, liver disease, and alcoholism are the most common underlying illnesses that diminish cellular immune response. Immunosuppression often accompanies drug or radiation therapy. Corticosteroids, chemotherapeutic agents used in cancer and organ transplantation, and total lymphoid irradiation all suppress the cell-mediated immune (CMI) function. Organ transplant patients receiving combined immunosuppressive therapy (corticosteroids, azothiprine, and cyclosporin) face an increased risk of infection (or reactivation of quiescent infections) with a variety of opportunistic pathogens including *L. monocytogenes, Salmonella, T. gondii, Cryptosporidium,*

Table 6. Factors That Increase Host Susceptibility (adapted from CAST, 1994)

General Factors	Specific Factors	Reasons
Age	Age less than 5 years	Lack of developed immune systems, smaller infective dose-by-weight required
	Age greater than 50 or 60 years (depending on pathogen)	Immune systems failing, weakened by chronic ailments, occurring as early as 50 to 60 years of age
Sensitive populations	Pregnancy	Altered immunity during pregnancy
	Hospitalized people	Immune systems weakened by other diseases or injuries, or at risk of exposure to antibiotic-resistant strains
	Possession of certain human antigenic determinants duplicated or easily mimicked by microorganisms	Predisposition to chronic illnesses (sequelae)
Underlying medical conditions	Concomitant infections	Overloaded or damaged immune systems
	Consumption of antibiotics	Alteration of normal intestinal microflora
	Excessive iron in blood	Iron in blood serving as nutrient for certain organisms
	Reduced liver/kidney function (alcoholism)	Reduced digestion capabilities, altered blood-iron concentrations
	Surgical removal of portions of stomach or intestines	Reduction in normal defensive systems against infection
	Immunocompromised individuals including those on chemotherapy or radiation therapy; recipients of organ transplants taking immunocompromising drugs; people with leukemia, AIDS, or other illnesses	Immune system inadequate to prevent infection

Cryptosporidiosis

Cryptosporidium was first described in the early 1900s but was not considered to be medically or economically important. During the early 1970s, *Cryptosporidium* was linked to diarrhea in calves, and case reports describing its appearance in a variety of animal species began to appear in the literature. In 1976, *Cryptosporidium* was first associated with severe watery diarrhea in a patient who had been receiving immunosuppressive chemotherapy for 5 weeks. The diarrhea resolved 2 weeks after discontinuation of the therapy. Following this report, additional case reports of severe, persistent diarrhea in immunosuppressed or immunodeficient individuals appeared in the literature (Pitlik et al., 1983).

During the early 1980s, two series of observations began to shape the emerging epidemiology of human cryptosporidiosis. First, cases of cryptosporidiosis were reported among persons who had normally functioning immune systems and exposure to infected calves. While these patients typically had self-limited illnesses of mild severity, they demonstrated that calves with diarrhea were a potential source of human infection. The second observation was the occurrence of chronic protracted diarrhea due to *Cryptosporidium* in patients with acquired immune deficiency syndrome (AIDS). In many of these patients with severe cell-mediated immune defects, cryptosporidiosis was unresponsive to therapy and culminated in death (Navin and Juranek, 1984).

During the mid-1980s, the first outbreaks of cryptosporidiosis in child day care centers and the first waterborne outbreaks of cryptosporidiosis were reported. As clinicians and laboratories became more aware of *Cryptosporidium*, its role as a cause of community-acquired diarrhea emerged. This growing awareness of the public health importance of cryptosporidiosis culminated with the occurrence of a massive waterborne outbreak in Milwaukee, Wis., in 1993. More than 400,000 illnesses were attributed to contamination of water distributed by one of two water treatment plants in Milwaukee (MacKenzie et al., 1994).

Cryptosporidiosis is now recognized as an important cause of diarrheal illness, and is estimated to cause 300,000 illnesses each year in the United States (Mead et al., 1999). Genotyping methods have been developed to discriminate between strains of human and bovine origin, although humans are susceptible to infection with bovine strains. Application of these methods to outbreak investigations and surveillance data will improve our understanding of the epidemiology of cryptosporidiosis. Interestingly, although manure runoff from dairy farms and effluent from beef slaughter plants were suspected to be likely sources for the Milwaukee outbreak, *Cryptosporidium* oocysts recovered from outbreak-associated cases were of the human genotype (Sulaiman et al., 1998). Thus, effluent from a plant treating human waste was a more likely source.

and *Trichinella spiralis*. In one study, individuals with chronic heart disease had an increased risk for listeriosis (Schuchat et al., 1992). Cellular immunity declines during pregnancy, which may account for the severity of certain infections.

Evidence indicates that immune deficiency not only increases the number of cases but also the severity of infection from a wide variety of foodborne pathogens. For example, studies conducted in Los Angeles, San Francisco and New York City during the mid-1980s demonstrated that patients with acquired immune deficiency syndrome (AIDS) had rates of *Campylobacter* and *Salmonella* infection that were 19 – 94 times the general population rates in the same cities (Celum et al., 1987; Greunewald et al., 1994; Sorvillo et al., 1994). In addition, 16% of *Campylobacter* infections and 44% of *Salmonella* infections resulted in bacteremia in these compromised patients, much higher rates of severe disease than occurred in the general population. San Francisco residents infected with the human immunodeficiency virus (HIV) also have been shown to have incidence rates of *Shigella* infection 30 times greater than the HIV-free population (Baer et al., 1999), and using an immune suppressive medication was identified as a risk factor for sporadic *E. coli* O157:H7 infections in a FoodNet case-control study (Kassenborg et al., 1998).

It is known that the neonatal, pediatric, adult, and elderly immune systems differ. The fetus and neonates are highly susceptible to infection with a variety of pathogens, presumably as a result of an immature immune system. The development of the immune system begins early in fetal development, but children are not immunologically mature until puberty, putting them at increased risk for foodborne illness.

Improvements in health care and nutrition in this century have increased the life expectancy for most people. One result is that the elderly are the fastest-growing segment of our population. Elderly people experience significantly greater morbidity and mortality from infectious diseases than the general population. This apparent susceptibility to infection in the elderly has been attributed to a decline of immune function with age, termed "immune senescence." The data regarding the effects of aging are confusing and sometimes conflicting. In general, cell-mediated immunity declines, including both functional and quantitative cell counts. Superimposed and interrelated with this generalized impairment are age-related decreases in organ structure and function. Nutritional abnormalities in macro- and micronutrients are common in the elderly and may compound immune senescence. The presence of other illnesses and environmental factors also may contribute to the decline.

Susceptibility to Biotoxins

The variability of human susceptibility to mycotoxins and other biotoxins can be attributed to physiologic and environmental factors, host genetics, and the presence of infection and inflammation.

Physiologic Factors

A number of factors can influence a person's ability to detoxify ingested

biotoxins. For example, biotransformation enzyme activity can vary during perinatal and postnatal development (deBethizy and Hayes, 1994). When the individual is older, environmental forces play a role in host response: the nutritional quality of the diet, the presence of chemicals in the diet, and the intake of prescription or elicit drugs can affect the profile of biotransformation enzymes. Finally, environmental toxins in air (e.g., cigarette smoke) and water can increase or decrease the activity of biotransformation enzymes toward specific mycotoxins or other biotoxins.

Genetic Polymorphisms

Wide differences in the human capacity to biotransform biotoxins appear to relate to genetic background that influences the presence, amount and activation of various enzyme systems that metabolize ingested biotoxins into chemical derivatives that are less or sometimes more toxic than the original mycotoxin (Daly et al., 1994; Kalow, 1993). For example, the level of expression for CYP1A2, a cytochrome P-450 (CYP)-dependent monooxygenase that metabolizes aflatoxin B_1 varies considerably in the human liver (Eaton et al., 1995). The activity of microsomal epoxide hydrolase, which acts coordinately with CYP1A2, can vary up to 40-fold in human tissue (Seidegard and Ekstrom, 1997). It has been suggested that epoxide hydrolase polymorphisms may alter the risk of aflatoxin-associated liver cancer (McGlynn et al., 1995). Specifically, enzymes may vary in both the amount present and their effectiveness/activity, resulting in differing risks of a negative outcome from aflatoxin ingestion.

Infection and Inflammation

The simultaneous presence of an infectious microbe with attendant inflammation can increase the sensitivity of a host to mycotoxic disease. Epidemiologic studies have demonstrated that hepatitis B infection predisposes humans who chronically ingest aflatoxins to develop primary liver cancer (Pitt, 2000). In experimental animals, gram-negative bacterial endotoxin can cause a predisposition to acute liver injury from aflatoxin B_1 (Barton et al., 2000, 2001) and T-2 toxin (Tai and Pestka, 1988) and to depletion of lymphoid tissue by deoxynivalenol due to leukocyte cell death (Zhou et al., 1999; 2000).

Other Factors

Other significant host factors, apart from immune suppression per se, are associated with increased risk of both acute foodborne disease and chronic sequelae. Genetic predisposition and underlying chronic disease have been cited as potential risk factors. For instance, septic *Vibrio vulnificus* infections are most commonly seen in men over 50 years of age with liver and/or blood disorders (Desenclos et al., 1991; Tacket et al., 1984). These underlying conditions frequently result in elevated serum iron levels that play a role in *V. vulnificus* disease pathogenesis, although the exact mechanism is not fully understood (Wright et al., 1981). Similar evidence is available for yersiniosis in iron-overloaded patients treated with deferioxamine, although again the pathogenic mechanisms are not yet clear (Mandell and Bennett, 1995). As previously discussed, genetic predisposition plays a role in the development of chronic reactive arthritis. Recent evidence from a quantitative human challenge study for the Norwalk-like virus indicates a two-phase dose-response relationship that appears to be separately associated with prior exposure (antibody titer) and individual susceptibility, neither of which are associated with any recognized specific host factors (Moe et al., 1999). When taken together, this body of evidence suggests that many factors apart from immune suppression influence host susceptibility to foodborne disease agents.

Individual Choices that Affect Disease Risk

Which foods are consumed and how those foods are prepared affect an individual's risk of foodborne disease. Despite education efforts, consumer behavior continues to play a significant role in exposure to foodborne pathogens (see Table 7).

Behavior Changes

The 1990s saw a tremendous increase in public awareness of food safety issues in the United States. This awareness arose in part because of the continuing interest in personal health and well being, a phenomenon that occurred throughout the world's developed countries. Schools, education programs, media communications, and the Internet have made foodborne and waterborne diseases important concerns to many consumers. Outbreaks of foodborne illness that would have gone unnoticed a decade ago are now the subject of rapid, in-depth news coverage. The increased publicity about infectious foodborne hazards appears to reinforce food safety messages and to increase motivations to handle foods safely.

Proper hygiene and sanitation related to food handling and preparation, appropriate methods of refrigeration and freezing, and thorough cooking of foods comprise a very effective approach to preventing foodborne illness. However, these behaviors are just one aspect of a healthy life-style. Additional behavioral changes—such as consuming probiotics, eating a balanced diet, and exercising regularly to maintain a healthy weight—foster proper functioning of the immune system that may heighten resistance to occasional pathogens in the food supply.

Consumer awareness of food safety issues has placed additional pressure on the food service and food processing industries to improve their efforts to ensure the safety of the products they provide both domestically and internationally. Hazard Analysis and Critical Control Points (HACCP) implementation has become commonplace in the food processing and delivery process. Companies have a strong economic incentive to prevent outbreaks of foodborne illness associated with their product or restaurant.

While many host factors that influence infection, occurrence and severity of illness are associated with human physiology, the factors that influence exposure to foodborne pathogens are often tied to human behavior, specifically consumption, food handling, and preparation behaviors.

Eating outside the home in restaurants and other foodservice venues has been identified as a risk factor for certain foodborne diseases (Friedman et al., 2000), and the number of meals that Americans eat away from home continues to increase. In the 1990s, food eaten outside the home accounted for almost 80% of reported foodborne illness outbreaks in the United States (Bean et al., 1996). Because of the larger number of people involved, these outbreaks are more likely to be recognized and, there-

Table 7. Factors That Increase Risk of Foodborne Disease (adapted from CAST, 1994)

General Factors	Specific Factors	Reasons
Life-style	Stress	Body metabolism changes, allowing easier establishment of pathogens, or lower dose of toxin required for illness
	Poor hygiene	Increased likelihood of ingestion of pathogens
	Geographic location	Likelihood of exposure of endemic virulent strains; limited and/or compromised food and water supply; variable distribution of organisms in water and soil
Diet	Nutritional deficiencies either through poor absorption of food (mostly ill or elderly people) or unavailability of adequate food supply (starving people)	Inadequate strength to build up resistance and/or consumption of poor-quality food ingredients, which may contain pathogens
	Consumption of antacids	Decreased stomach acidity (increased pH)
	Consumption of large volume of liquids including water	Dilution of acids in the stomach and rapid transit through the stomach
	Ingestion of fatty foods (such as chocolate, cheese, hamburger) containing pathogens	Protection of pathogens against stomach

fore, reported to health officials, so the extent of the role of foodservice in foodborne disease may be overstated. Nonetheless, the role of foodservice has become more significant as the percentage of the food budget spent on eating out has increased during recent decades (Manchester and Clauson, 1995). Quick-service restaurants and salad bars were rare 50 years ago but are primary sites for food consumption in today's fast-paced society (Manchester and Clauson, 1995).

Regardless of what has or has not happened to commodities on their way from farm to table, the final common pathway for food involves storage, preparation, and serving prior to the time of consumption. Unfortunately, foods that are free of foodborne pathogens and toxins early in the food chain can become unsafe if not handled properly during the final stages of preparation and service.

Food handling behaviors such as inadequate hand washing, unsafe storage temperatures that permit the growth of low levels of pathogens, incomplete cooking of potentially hazardous foods, and cross-contamination of fresh and cooked foods are a problem whether they take place inside or outside the home. Based on data from a 1993 nationwide survey, an estimated 37% of food handlers did not wash their hands after handling raw meat (Altekruse et al., 1995). As many as 42% of survey respondents did not cook hamburgers at home until well done (Albrecht, 1995). In a 1992 study, 23% of respondents reported eating raw shellfish (Timbo et al., 1995). Fortunately, some high-risk food-handling and consumption behaviors, although still common in 1995 and 1996, did show improvement (CDC, 1998a).

The reasons why some consumers respond to food safety information and admonitions to choose safe food and handle it properly while other consumers do not are poorly understood. Demographic factors such as gender, age and education level are associated with high-risk behaviors. For instance, all behaviors associated with increased risk of foodborne diseases were more prevalent in men than in women. (Albrecht, 1995; Altekruse et al., 1995). The 1993 FDA Health and Diet Survey indicated that men were less likely than women to wash their hands after handling raw meat or poultry (53% versus 75%) (Altekruse et al., 1995). Results of several studies indicate that younger people have a higher prevalence of a number of risky food handling, preparation, and consumption practices (Altekruse et al., 1995; Klontz et al., 1995; Timbo et al., 1995). Education efforts are complicated by decreased opportunities for food safety instruction both in school and at home. Health educators in secondary schools emphasize prevention of other important health concerns (e.g., HIV infection, obesity) over consumer safety issues including food safety education (Collins et al., 1995). In addition, the trend toward two-income families and eating away from home leaves fewer opportunities to pass food safety information from parent to child (Manchester and Clauson, 1995).

Travel, another factor in foodborne illness, has increased dramatically during the 20th century. Five million international tourist arrivals were reported worldwide in 1950, and the number is expected to reach 937 million by 2010 (Paci, 1995). Travelers may become infected with foodborne pathogens uncommon in their nation of residence, thus complicating diagnosis and treatment when their symptoms begin after they return home. In 1992, for example, an outbreak of cholera caused 75 illnesses in international airline passengers; 10 persons were hospitalized, and one died (Eberhart-Phillips et al., 1996). Pathogens also may be carried home and infect family members and other close personal contacts (Finelli et al., 1992).

Changes in Food Consumption Behavior

Potential exposure to pathogens is not only a function of how food is handled, but also what foods individuals choose to eat. Similar to the link between poor sanitation practices and foodborne illness, consumption of certain foods increases the risk of illness.

Changes in food consumption have brought to light previously unrecognized or underestimated microbial hazards. Fresh fruit and vegetable consumption, for example, increased nearly 50% from 1970 to 1994 (BC/USDC, 1996). Produce is susceptible to microbial contamination during growth, harvest, and distribution (see section on microbial ecology, p. 40), which is of special concern for foods eaten fresh and not cooked. Pathogens on the surface of produce (e.g., melons) can contaminate the interior during cutting and multiply if the fruit is held at room temperature (Reis et al., 1990). In the United States from 1990 to 1997, a series of foodborne outbreaks were associated with produce such as sliced cantaloupe (Reis et al., 1990), green onions (Cook et al., 1995), unpasteurized cider (Besser et al., 1993), fresh-squeezed orange juice (Cook et al., 1996), lettuce

(Ackers et al., 1996), raspberries (Herwaldt, 1997), alfalfa sprouts (Mahon et al., 1997), sliced tomatoes (Wood et al., 1991), and frozen strawberries (CDC, 1997a).

In addition to relative changes in quantities, the past few decades have seen dramatic changes in the diversity of foods available to the American public. The growing wealth of Americans and the profitability of fresh produce has led to the introduction and increased availability of a wide variety of produce items—from kiwi, mangoes and papayas, to alfalfa sprouts, specialty lettuces and fresh-cut, packaged produce. Imported produce has played a significant role in increasing diversity. Fresh produce has also become a mainstay of restaurant fare; dinner salads and salad bars have become mainstream. In addition, ethnic cuisines that feature fresh produce ingredients, such as Chinese, Mexican, Thai, and Middle Eastern, have become popular.

Individual Choices

Americans pride themselves on their individual freedoms. In a culture based on these individual rights, we allow people to engage in high-risk behavior and offer products that sometimes cater to these risks. Steps are taken to mitigate risk but ultimately certain behaviors are inherently risky. Sometimes people knowingly engage in high-risk behavior. If people are aware of the risks and continue to engage in risky behavior such as eating raw oysters or eggs, it is appropriate to consider to what lengths our society should go to protect them.

Surveys conducted from 1998-1999 as part of the FoodNet Active Surveillance Program for foodborne diseases have documented certain aspects of consumer behavior. With regard to consumption of fresh produce that is known to be at particular risk for microbial contamination, 19% of respondents reported eating a mesclun lettuce mix in the 7 days before the interview, and 8% reported eating alfalfa sprouts, although these eating habits are highly regional (CDC, 1999a). Among other potentially risky food exposures, 25% of the people who had eaten eggs had chosen to eat eggs that were runny; 11% of persons who consumed hamburgers ate burgers that were still pink inside; 4.4% drank unpasteurized apple juice or apple cider; 3.4% drank unpasteurized milk; and 2.5% ate fresh oysters.

Cultural Differences

Diet selection can create subpopulations at greater risk for certain foodborne illnesses. Some reports of foodborne illnesses involve transmission via foods consumed primarily by immigrant groups. Outbreaks of trichinosis have become relatively rare in the United States because cooking pork thoroughly has become a widespread cultural practice. An exception occurred in 1990, when Laotian immigrants in Iowa prepared and ate undercooked pork, a traditional food, as part of a wedding celebration (Stehr-Green and Schantz, 1986). Other reports involve foods more commonly consumed by ethnic populations. *Y. enterocolitica* outbreaks are also rare, but several outbreaks in African-American communities were associated with preparation and consumption of pig intestines (Lee et al., 1990). The epidemiology of human brucellosis in California has shifted from an occupational disease related to animal husbandry to a foodborne disease most frequently affecting Hispanics who often consume raw milk and cheeses made with raw milk while abroad (Chomel et al., 1994). Consumption of rare hamburgers—a risk factor for *E. coli* O157 infection—is more common in U.S. Caucasians than in any other racial/ethnic group (CDC, 1998a).

Dietary Recommendations

A host of organizations have issued dietary recommendations and provided information to assist in health promotion and chronic disease prevention (U.S. PSTF, 1997). These recommendations may advocate increased or decreased consumption of certain types of foods, outline specific circumstances for consumption, or caution individuals with certain medical conditions. Each type of recommendation has consequences for foodborne illness. In recognition of the importance of food safety, the latest edition of the federal government's dietary guidelines contains a section on food safety.

Counseling the general population to limit dietary intake of fat and emphasize foods containing fiber (i.e., fruits, vegetables, grain products) has increased consumption of foods such as fresh produce and leaner meats such as chicken. Recent increases in the consumption of health-promoting fresh fruits and vegetables have resulted in increased likelihood of exposure to certain diseases like hepatitis A, shigellosis and salmonellosis from contaminated produce (Tauxe et al., 1997). The dietary shift toward increased consumption of chicken may have contributed to the high incidence of *C. jejuni* infection (Friedman et al., 1992), which now exceeds *Salmonella* as the most common bacterial cause of foodborne illness (Mead et al., 1999). A recent study demonstrated that only 17% of Americans ate five or more servings of fresh fruits and vegetables per day (Thompson et al., 1999). Thus, public health marketing campaigns are likely to increase fruit and vegetable consumption in years to come.

Medical and public health advisory bodies also have advised certain subpopulations to use special caution in diet selection and food preparation. For example, the American Academy of Pediatrics has recommended that children should not drink unpasteurized milk or eat unpasteurized cheese, undercooked eggs, raw or undercooked meat or meat products (AAP, 2000). FoodNet population surveys (CDC, 1999a) demonstrate that these types of dietary recommendations do have an impact on consumer behavior, although final analyses are not complete. While 13.5% of adults aged 20-39 who consumed hamburgers ate hamburgers that were pink, only 4.4% of children under 10 years of age did so. Similarly, adults were 2–3 times more likely than children to drink unpasteurized milk, or eat alfalfa sprouts or runny eggs (CDC, 1999a).

Ignoring recommendations about preparation practices such as adequate cooking places consumers at greater risk for foodborne illness. Consumer education is an important part of foodborne illness prevention. As the statistics above demonstrate, the media attention, safe handling labels, public health advisories, and public information and education campaigns to date have left a substantial part of the population unprotected. For a variety of reasons, food safety and other public health messages fail to reach their intended audience, are misunderstood, or are disregarded. For those charged with preventing foodborne disease, there are two inescapable lessons in this data: first, we need to know a great deal more about risk communication, education,

and motivation before consumer and food worker education programs can be considered credible parts of our overall food safety strategy; and second, as long as pathogens are delivered to home and commercial kitchens, some foodborne disease will occur.

Modification of Susceptibility

Because of the significance of the human host in foodborne illness, it is appropriate to look for host-related opportunities for control or mitigation of illness. Certainly, behavioral and demographic issues influence susceptibility and exposure. If the host could be rendered immune to infectious agents, illness would cease to occur despite microbial contamination in the food supply. Although complete protection is currently out of reach for many pathogens, approaches such as immunization and probiotics can decrease human susceptibility. Coupling these approaches with efforts to mitigate exposure would further boost our ability to control foodborne illness of infectious origin.

Immunization

Vaccines use the host's own immune system to combat disease. Knowing how the immune system functions enables scientists to investigate methods to enhance its effectiveness or trigger its protective effects without causing illness. The medical community has had great success with vaccination for some infectious diseases, and potential vaccines to prevent foodborne illness are the subject of considerable research.

Although no vaccines are currently available for most enteric bacterial pathogens, experimental approaches are under investigation. In general, vaccination strategies to control enteric bacterial diseases are complicated by many factors, not the least of which is the complexity of host immunity as well as a general absence of appropriate animal models for oral challenge and subsequent disease presentation for many of these pathogens. For example, an effective broad-spectrum vaccine against enterohemorrhagic *E. coli* (EHEC) will likely need to target systemic immunity against the Shiga toxins as well as local intestinal immunity against intestinal colonization factors (Nataro and Kaper, 1998). Nonetheless, genetic manipulation has enabled researchers to produce bacterial mutants that have an impaired ability to survive in vivo, and some of these have been used as live oral vaccines to immunize against subsequent infection with wild-type virulent strains (Fairweather et al., 1990). An alternative vaccination approach has focused on the use of purified antigens, although this usually results in a comparatively poorer immune response when administered orally (Fairweather et al., 1990).

Scientists have had greater success developing effective vaccines against the human enteric viruses that are commonly spread through food and waterborne routes. For instance, we are in the final stages of worldwide eradication of the poliovirus, an accomplishment made possible by widespread immunization (Jacob, 2000). Vaccines for HAV are another success story. Licensed in the mid-1990s, these vaccines consist of formalin-inactivated organisms and are well tolerated; they produce durable immunity persisting for more than 20 years (Cuthbert, 2001). Recent evidence also indicates that the HAV vaccine effectively prevents secondary HAV infection and may be appropriate for administration to individuals in frequent personal contact with infected persons, replacing the widely used immunoglobulin (Sagliocca et al., 1999). Although there has been interest in mandating routine HAV vaccination for food handlers, there is currently no overwhelming support for this proposal, in part due to the expense of this type of approach. Perhaps more efficacious would be recommendation of routine vaccination of individuals with underlying chronic active hepatitis due to infection with hepatitis B or C viruses, since this subpopulation is particularly susceptible to very serious disease manifestations if concurrently infected with HAV.

In 1998, FDA also licensed a live attenuated rotavirus vaccine (Rotashield, Wyeth-Lederle Vaccines and Pediatrics, Philadelphia, Penn.) for oral administration to infants (AAP, 1998). Unfortunately, in the summer of 1999, CDC reported a clustering of cases of an intestinal complication in the weeks after vaccination, eventually leading to the voluntary withdrawal of the product from the U.S. market and an uncertain future for the vaccine (Weijer, 2000). Some progress has been reported in vaccination against the human gastrointestinal caliciviruses. However, protection against this important group of foodborne infectious agents remains challenging because immunity is poorly understood, the group has tremendous antigenic and genetic diversity, and the viruses cannot be cultivated in model animal or laboratory systems.

Probiotics

Probiotics represent another opportunity to use our understanding of the gut microflora and the immune response to facilitate human health and decrease susceptibility to illness. Although originally used to describe substances produced by one protozoan that stimulated another (Fuller, 1989), the definition for the term "probiotic" now generally implies a viable microbial supplement that beneficially affects the host (human or animal) by improving or maintaining a desirable microbial balance in the gut. The reduction of harmful enteric microorganisms is only one of numerous potential health benefits of maintaining a healthy gut microflora. Probiotic cultures are consumed in foods or capsules or are facilitated by ingesting prebiotics (compounds that enhance the proliferation of beneficial indigenous bacteria). Currently, dairy foods such as yogurt have been the most popular vehicle of choice to deliver viable probiotic cultures. Intestinally-derived lactobacilli and bifidobacteria predominate in this role (Hughes and Hoover, 1991).

The effect of probiotic cultures varies based on numerous conditions. The human gut contains 100 trillion viable bacteria and other microorganisms representing anywhere from 100 to 400 different species; the population dynamics are quite complex. The microflora of the human intestinal tract is affected by genetic or host factors, the composition of microbial populations, and the metabolites produced by these microbes; these factors are in turn influenced by climate, diet, stress, drugs, age, and disease (Mitsuoka, 1990). One can maintain that whenever there is a change in the intestinal microflora from its normal state, the change is detrimental or undesirable. A foodborne intestinal infection that produces diarrhea can be viewed as a period of microbial imbalance or instability in the gastrointestinal tract. However, by establishing themselves in the human GI tract in proportionally high numbers, acidulating bacteria, such as lactobacilli and bifidobacteria, may protect the gut against invasive pathogenic agents.

Thus, regular consumption of foods containing probiotics has a strong potential to help maintain a beneficial and stable intestinal microflora that promotes intestinal health. This is especially true for those subpopulations with compromised or underdeveloped gut flora, such as the elderly, infants, and patients treated with antibiotics or chemotherapy. For example, in the elderly, there is a steady decline in numbers of bifidobacteria and an increase in the numbers of *C. perfringens* with age. With this shift in gut flora, there is a corresponding increase in putrefactive substances in the intestinal tract that are inherently toxic and impose a constant stress upon the liver (Mitsuoka, 1990). In addition to *C. perfringens*, the putrefactive organisms that convert amino acids into amines and other toxic substances include *Salmonella, Shigella,* and *E. coli.* As a means to ameliorate these detrimental conditions, elevated levels of bifidobacteria and lactobacilli from dietary probiotics reduce fecal pH to discourage growth and colonization by acid-sensitive enteric pathogens, whether the pathogens are indigenous to the intestinal population or opportunistic contaminants of food and water (Langhendries et al., 1995).

A similar case for administration of probiotic cultures would be for the newborn (Mitsuoka, 1989). Within a day of birth, bacteria commence colonization and proliferation in the previously sterile intestinal tract. Initially, coliforms, enterococci, staphylococci, and clostridia appear, but in three to four days after birth, lactobacilli and bifidobacteria predominate. Bifidobacteria soon dominate all other bacteria, whether the infant is breast-fed or bottle-fed. However, in bottle-fed infants, populations of coliforms and enterococci are ten times higher than in breast-fed infants, a fact that may encourage the use of infant formula that includes probiotic cultures.

The feeding of probiotic cultures to prevent or treat disease is well established in the scientific literature. Elie Metchnikoff of the Pasteur Institute first promoted the use of probiotics nearly 100 years ago (Metchnikoff, 1908). More recent examples include Saavedra et al. (1994), who showed that supplementing infant formula with *Bifidobacterium bifidum* and *Streptococcus thermophilus* can reduce the incidence of acute diarrhea and rotavirus shedding in infants; Bernet et al. (1994), who found that consumption of a greater number of lactobacilli provided increased protection against cell association by enterotoxigenic and enteropathogenic *E. coli* and *S.* Typhimurium, and against cell invasion by enteropathogenic *E. coli*, *S.* Typhimurium and *Y. pseudotuberculosis;* and Okamura et al. (1986), who used a tissue culture infection assay to demonstrate that administration of *Bifidobacterium infantis* prohibited invasion and intracellular multiplication of *S. flexneri.* In all, probiotic cultures have demonstrated an inhibitive or antagonistic effect against almost all foodborne pathogens, including *Salmonella, Shigella, E. coli, Campylobacter, Clostridium, Yersinia, Vibrio* and *Candida* (Fuller, 1992).

Microbial Ecology and Foodborne Disease

The complexity of the pre-harvest, harvest, and post-harvest environments makes it impossible to control all potential sources of microbial contamination. Efforts at prevention and control are implemented throughout the food production and processing system. Researchers are continually searching for a better understanding of the pathogens and their interaction with the environment, leading to improved control technologies. But at the same time, the pathogens continue to evolve, and human actions sometimes drive that evolution. Even small environmental changes can have unforeseen or even unforeseeable impact on microbial populations.

Improved understanding of these complex factors provides insight into pathogen evolution and opens the door to new and improved prevention and control methods.

PRE-HARVEST ENVIRONMENT

Efforts to minimize microbial contamination of food begin in the pre-harvest environment. Raw ingredients are one way in which pathogens are introduced into the processing environment. Unfortunately, pathogen control in the production agriculture environment is often difficult.

Overarching Issues

In the pre-harvest environment, many of the significant issues in microbiological food safety are broad concerns that apply to many different commodities. Because these issues affect so many commodities, improvements in these areas would have significant food safety impact.

Global Food Trade

Globalization of the world's food supply has contributed to changing patterns of food consumption and foodborne illness. A growing percentage of the U.S. food supply is imported. The sheer volume of these imports adds to the complexity of foodborne illnesses.

Global sourcing provides economic benefits and a wider selection for consumers that improves nutrition worldwide. However, in terms of disease control programs, globalization minimizes traditional geographic barriers to emerging as well as traditional pathogens. Developing economies represent major sources of certain im-

Thus, regular consumption of foods containing probiotics has a strong potential to help maintain a beneficial and stable intestinal microflora that promotes intestinal health. This is especially true for those subpopulations with compromised or underdeveloped gut flora, such as the elderly, infants, and patients treated with antibiotics or chemotherapy. For example, in the elderly, there is a steady decline in numbers of bifidobacteria and an increase in the numbers of *C. perfringens* with age. With this shift in gut flora, there is a corresponding increase in putrefactive substances in the intestinal tract that are inherently toxic and impose a constant stress upon the liver (Mitsuoka, 1990). In addition to *C. perfringens*, the putrefactive organisms that convert amino acids into amines and other toxic substances include *Salmonella, Shigella,* and *E. coli.* As a means to ameliorate these detrimental conditions, elevated levels of bifidobacteria and lactobacilli from dietary probiotics reduce fecal pH to discourage growth and colonization by acid-sensitive enteric pathogens, whether the pathogens are indigenous to the intestinal population or opportunistic contaminants of food and water (Langhendries et al., 1995).

A similar case for administration of probiotic cultures would be for the newborn (Mitsuoka, 1989). Within a day of birth, bacteria commence colonization and proliferation in the previously sterile intestinal tract. Initially, coliforms, enterococci, staphylococci, and clostridia appear, but in three to four days after birth, lactobacilli and bifidobacteria predominate. Bifidobacteria soon dominate all other bacteria, whether the infant is breast-fed or bottle-fed. However, in bottle-fed infants, populations of coliforms and enterococci are ten times higher than in breast-fed infants, a fact that may encourage the use of infant formula that includes probiotic cultures.

The feeding of probiotic cultures to prevent or treat disease is well established in the scientific literature. Elie Metchnikoff of the Pasteur Institute first promoted the use of probiotics nearly 100 years ago (Metchnikoff, 1908). More recent examples include Saavedra et al. (1994), who showed that supplementing infant formula with *Bifidobacterium bifidum* and *Streptococcus thermophilus* can reduce the incidence of acute diarrhea and rotavirus shedding in infants; Bernet et al. (1994), who found that consumption of a greater number of lactobacilli provided increased protection against cell association by enterotoxigenic and enteropathogenic *E. coli* and *S.* Typhimurium, and against cell invasion by enteropathogenic *E. coli*, *S.* Typhimurium and *Y. pseudotuberculosis;* and Okamura et al. (1986), who used a tissue culture infection assay to demonstrate that administration of *Bifidobacterium infantis* prohibited invasion and intracellular multiplication of *S. flexneri.* In all, probiotic cultures have demonstrated an inhibitive or antagonistic effect against almost all foodborne pathogens, including *Salmonella, Shigella, E. coli, Campylobacter, Clostridium, Yersinia, Vibrio* and *Candida* (Fuller, 1992).

Microbial Ecology and Foodborne Disease

The complexity of the pre-harvest, harvest, and post-harvest environments makes it impossible to control all potential sources of microbial contamination. Efforts at prevention and control are implemented throughout the food production and processing system. Researchers are continually searching for a better understanding of the pathogens and their interaction with the environment, leading to improved control technologies. But at the same time, the pathogens continue to evolve, and human actions sometimes drive that evolution. Even small environmental changes can have unforeseen or even unforeseeable impact on microbial populations.

Improved understanding of these complex factors provides insight into pathogen evolution and opens the door to new and improved prevention and control methods.

PRE-HARVEST ENVIRONMENT

Efforts to minimize microbial contamination of food begin in the pre-harvest environment. Raw ingredients are one way in which pathogens are introduced into the processing environment. Unfortunately, pathogen control in the production agriculture environment is often difficult.

Overarching Issues

In the pre-harvest environment, many of the significant issues in microbiological food safety are broad concerns that apply to many different commodities. Because these issues affect so many commodities, improvements in these areas would have significant food safety impact.

Global Food Trade

Globalization of the world's food supply has contributed to changing patterns of food consumption and foodborne illness. A growing percentage of the U.S. food supply is imported. The sheer volume of these imports adds to the complexity of foodborne illnesses.

Global sourcing provides economic benefits and a wider selection for consumers that improves nutrition worldwide. However, in terms of disease control programs, globalization minimizes traditional geographic barriers to emerging as well as traditional pathogens. Developing economies represent major sources of certain im-

Table 8. Sources of Imported Fresh and Frozen Produce (1997 data) (GAO, 1999)

Country	Percent (by $ value)
Mexico	51
Canada	15
Chile	12
Costa Rica	4
Netherlands	3
Guatemala	2
Other	13

ports (see Table 8). For many of these countries, infectious diseases still represent a significant burden of illness. Diarrhea remains among the ten leading causes of death and disease burden in the developing world (Murray and Lopez, 2001).

Imported foods can introduce pathogens previously uncommon in the United States, such as *Cyclospora* and new strains of *Salmonella*. Raspberries from Guatemala, cantaloupes and scallions from Central American countries, coconut milk from Southeast Asia, and a Middle Eastern snack food have all been implicated in recent foodborne disease outbreaks in the United States. As the percentage of imported foods consumed in the United States increases, the importance of ensuring that these foods are safe increases as well. Food safety therefore cannot be achieved by focusing on domestic products exclusively (GAO, 1998).

As shown in Table 9, the import share of some commonly consumed foods is increasing. For example, in 1995, one-third of all fresh fruits consumed in the United States was imported, and this trend is likely to continue.

Manure

The widespread occurrence and use of animal manure as fertilizer is a growing environmental concern, because it contaminates: water for drinking, irrigation, aquaculture and recreation; the hides, coats, and feathers of farm animals; and farm equipment and buildings. In the United States, cattle, hogs, chickens and turkeys produce an estimated 1.36 billion tons of manure annually (EPA, 2000), with greater than 90% attributed to cattle. Each year livestock create an estimated five tons of animal manure per person living in the United States, meaning the amount of animal manure is 130 times greater than the amount of human waste produced (U.S. Senate Agriculture Committee Democratic Staff, 1998).

Many of the most prominent foodborne pathogens in the United States, including *Campylobacter jejuni*, *Salmonella* and *Escherichia coli* O157:H7, are carried by livestock and are principally transmitted to foods by fecal contamination. *C. jejuni* accounts for an estimated 2 million cases of foodborne illness annually, with poultry and unpasteurized milk as its principal vehicles (Mead et al., 1999). *Salmonella* causes an estimated 1.3 million cases of foodborne illnesses annually, with eggs, poultry, beef, pork and produce as primary vehicles (Mead et al., 1999). Both *C. jejuni* and *Salmonella* are carried in the intestinal tract of apparently healthy poultry and livestock. Fecal contamination of hides, feathers and skin occurs during poultry and livestock production and slaughter. This contamination can subsequently carry through to processing. A case-control study of patients with *Campylobacter* infection identified the following risk factors for campylobacteriosis: foreign travel; eating undercooked poultry; eating chicken, turkey or non-poultry meat cooked outside the home; eating raw seafood; drinking raw milk; living on or visiting a farm; and having contact with farm animals or puppies (Friedman et al., 2000). Fecal contamination is a common source of *C. jejuni* contamination for each risk factor. For example, poultry feces frequently contain *C. jejuni* at populations of 10^5 to 10^7 colony forming units of bacteria per gram (CFU/g), resulting in levels of greater than 10^3 CFU *C. jejuni* per gram of carcass in 60 to 90% of retail poultry.

E. coli O157:H7 causes an estimated 73,500 cases of infection in the United States annually. Its principal vehicles of transmission are beef, produce, water (both drinking and recreational), and contact with cattle (Doyle et al., 1997; Griffin, 1998). Because *E. coli* O157:H7 is carried in the intestinal tract of cattle, the pathogen's most frequent origin is direct or indirect contact with cow manure. Manure can contaminate food when used as a soil fertilizer, when it pollutes irrigation water, when cattle defecate near produce or foods of animal origin, and when intestinal contents or manure-laden hides contact carcasses during slaughter and processing. Case-control studies of patients with *E. coli* O157:H7 infections revealed several major risk factors for illness: eating undercooked ground beef, living on or visiting a farm, and having contact with farm animals, especially cattle (Kassenborg et al., 1998). Depending on environmental conditions, *E. coli* O157:H7 can survive in manure for many weeks, and in some instances for more than one year. Similarly, the pathogen can survive well in lake water, with as little as a 10- to 100-fold reduction occurring during 13 weeks at 8 C.

Increased proximity and animal density during production contribute to problems of pathogens in runoff water because the difficulty of manure management is increased with greater volume. Another issue is composting of manure by farmers, including organic farmers. The conditions that effectively destroy pathogens are not well defined. Also, manure handling in other countries may be worse than in the United States, a serious concern for imported foods. Human feces from field workers without access to adequate sanitation facilities remains an issue as well.

Table 9. Percentage of Total U.S. Consumption Provided by Imports (GAO, 1998)

Import Item	1980	1985	1990	1995	% Change (1980-95)
Fish & shellfish	45.3	53.8	56.3	55.3	22.1
Fresh fruits	24.2	28.0	30.7	33.3	37.6
Fresh vegetables	7.6	8.9	8.4	11.7	53.9
Tomatoes for processing	1.4	7.0	5.7	3.5	150.0
Broccoli for processing	9.1	22.2	57.8	84.9	833.0

Water

In the pre-harvest environment, water can be obtained from a variety of

sources, although ground water and well water are perhaps the most widely used. Most farms do not provide specific treatment of water for use in agricultural production, and the water sources routinely used in agriculture can become contaminated by a number of means. Perhaps the most common source is animal manure contamination of runoff water; less common is contamination of water with untreated human sewage, which is largely under control in the United States and other developed countries, but of considerable concern for foods produced in developing countries with inadequate water resources. Water sources also may become contaminated by fecal excrement from wild animals or by general contamination of soil, but the significance of these to food safety is largely unknown.

Associations have been made between the presence of pathogens in watering troughs and their subsequent prevalence in animals. For instance, investigators have reported isolation of *E. coli* O157:H7 in water troughs sampled from cattle farms (Faith et al., 1996; Midgley and Desmarchelier, 2001; Sargeant et al., 2000; Shere et al., 1998), and others have reported that the practice of flushing alleyways with water to remove manure results in as much as 8-fold increases in animal carriage rates (Garber et al., 1999). Furthermore, the organism is able to persist for days at ambient temperature in both soil and water (Maule, 2000; Rice and Johnson, 2000). Likewise, it is recognized that contaminated water is a significant source of *Campylobacter* for infection of commercial poultry flocks (Shane, 2000). Such contamination is usually followed by rapid, intraflock dissemination, which has been exacerbated by intensification of animal agricultural practices (Gibbens et al., 2001; Shane, 2000).

Recent evidence of foodborne disease outbreaks associated with the consumption of fresh produce has prompted some to consider the role of contaminated irrigation and surface runoff waters. Irrigation water containing raw or improperly treated human sewage can be the source of many pathogens, with *Shigella* and the enteric viruses (hepatitis A virus, Norwalk-like viruses, rotaviruses) being perhaps the most significant (Beuchat, 1996; Beuchat and Ryu, 1997). Irrigation water contaminated with animal fecal matter can also be a source of pathogens on fresh produce. Although animal fecal material may contain a wide variety of potential human pathogens, it appears that the heartier survivors, such as parasitic protozoan oocysts (*Cryptosporidium* spp.) are likely to pose the greatest risk (Beuchat, 1996; Jaykus, 1997). Also, the relative importance of contaminated irrigation water as opposed to direct fecal contact is unknown for pathogens such as *Campylobacter*, *Listeria monocytogenes*, *E. coli* O157:H7, and *Cyclospora cayetanensis*.

Typical Pre-Harvest Environment for Foods of Plant Origin

The microbiological status of a food product at the time of consumption is a function of its history. What kinds of microorganisms and how many exist on and in the food are a direct result of the circumstances of its production and handling. During the pre-harvest production period and the harvest process, many opportunities exist for microorganisms to contaminate food materials. During the past 50 years, farming practices have changed considerably. In general, intensive farming practices have improved process control but also have contributed significantly to the rapid spread of human and animal pathogens by creating more concentrated environments for pathogens to multiply and evolve and by generating larger quantities of subsequently contaminated food (Rangarajan et al., 2000). At the same time, distribution networks have become more complex. Complicating the situation further, microorganisms rapidly adapt to new, adverse environmental conditions, allowing them to survive and replicate under extreme conditions involving high and low temperatures, pH, osmotic pressures, and oxygen levels that are inhospitable to most higher forms of life (Jay, 2000; Kushner, 1980).

As discussed in the previous section, American diets contain increasing amounts of fresh fruits and vegetables. Produce is commonly consumed raw (unprocessed), which makes it impossible—with the currently available technologies—to guarantee it is free of contaminating pathogenic microorganisms when consumed.

To control foodborne illness, the entire food supply chain must be considered (Baird-Parker, 2000). In the case of *Salmonella* transmission (Baird-Parker, 1990), direct contributing factors for the contamination of pre-harvest produce include contact with manure, water, humans, livestock, wildlife, pets, environmental pollution and effluent/sewage. The primary source is considered to be contact with human or animal feces. As noted above, water is a major concern because it is used so extensively in farming.

Not all bacterial and fungal food pathogens exist in the pre-harvest environment as a result of human or animal fecal contamination. For example, many sporeforming bacteria of food safety consequence are native to soil and water,

Production Practices and Mycotoxins

A recent example of how changes in ecology and production practices have affected mycotoxin incidence in the United States is the massive increase in *Fusarium* head scab in Midwestern wheat and barley during the last decade (McMullen et al., 1997). Head scab is often accompanied by elevated contamination by deoxynivalenol (vomitoxin) and other trichothecenes. Two factors seem to have driven the head scab epidemic. One is an increased spring rainfall during early wheat head formation. The uncharacteristic increase in rainfall may be a result of the prolonged El Niño during the 1990s, long-term climatic changes, or global warming from human activities. The second causative factor is the increased use of no-till agriculture methods, which have been implemented to reduce soil erosion. Residual stubble left in a field during winter can provide a way for fusaria to contaminate the following year's crop.

As the environment changes, sometimes as a result of human action, the microbial populations adapt. Some environmental changes can increase pathogen levels by providing favorable conditions; other changes can select for traits that result in resistant microorganisms that survive unfavorable conditions.

e.g., *Clostridium botulinum* and *Bacillus cereus*. Mycotoxigenic varieties of fungi include *Fusarium, Claviceps purpurea* and aflatoxin-producing strains of *Aspergillus.* In addition to known pathogens, the environment represents a substantial reservoir for potential emerging pathogens.

Because fresh produce undergoes very little processing, emphasis at the farm level has been directed towards the prevention of microbial contamination rather than relying on corrective actions once contamination has occurred. Although preventing contamination of crops by pathogenic microorganisms is important, it is very difficult to accomplish consistently and reliably, given the large number of possible sources of pathogens prior to harvest. Science-based farming guidelines, known as good agricultural practices, have been developed to control microbial contamination in an effort to improve the safety of produce (FDA/CFSAN, 1998).

Typical Pre-Harvest Environment for Foods of Animal Origin

Meat animal production has increased dramatically since 1975 (USDA/NASS, 2000). The largest increase has been in poultry production, which rose from approximately 10 billion pounds in 1975 to 40 billion pounds in 1999. Cattle production has remained relatively steady since the early 1970s at approximately 41 billion pounds. In addition to cattle and poultry, 24 billion pounds of pork are produced annually. These production quantities, coupled with limited space for livestock on the farm, promote the dissemination of microorganisms such as salmonellae. A higher prevalence of pathogens in food animals increases the chances that meat will become contaminated, providing a route for the pathogens to reach humans. Furthermore, to increase livestock health, feed efficiency, and growth rates in these confined conditions, antibiotics are often added to animal feed, potentially contributing to the development of antibiotic-resistance in microorganisms that live in animals (zoonotic microorganisms) (Angulo et al., 2000; Witte, 1998). Approximately half of the antimicrobials produced today are used in human medicine; most of the remainder is added to animal feed (WHO, 2002). The emergence of antibiotic-resistant human pathogens like *Salmonella* and *Campylobacter* spp. limit therapeutic options available for treating invasive human infections.

Use of Antibiotics

The widespread use of antibiotics in animal production and in the treatment of human illness both facilitate the emergence of antibiotic resistance. Microorganisms can develop resistance to antimicrobials through gene mutations or by acquiring transferable genetic elements, such as plasmids and conjugative transposons, that harbor resistance genes. These mobile genetic elements are important in horizontal transmission of genes from the resident to transient microflora of the intestinal tract (Levy et al., 1976). In addition to the mobility of the genetic elements, the antibiotic-resistant bacteria can be transmitted to different animal hosts. A tetracycline-resistant *E. coli* strain from cattle was traced to humans, mice, pigs, and fowl found at the same location (Levy et al., 1976). The selective pressure caused by antibiotic administration causes the microbial populations that harbor the appropriate resistance determinant(s) to flourish (Levy, 1992). These antibiotic-resistant microbes can make their way to humans through contaminated foods or animal-to-human transmission (Angulo et al., 2000; Holmberg et al., 1984), although the public health impact of the use of veterinary drugs is difficult to measure (Howgate, 1997).

The contribution of sub-therapeutic levels of antibiotics in animal feed to the emergence of antibiotic-resistant pathogens has been debated for years (Feinman, 1998). A growing body of evidence from epidemiological data and trace-back studies indicates that agricultural use of antibiotics plays an important role in the emergence of some antibiotic-resistant bacteria (Angulo et al., 2000). A review of *Salmonella* outbreaks between 1971 and 1983 revealed that antibiotic-resistant strains were more likely to originate from animals than were strains without resistance (Holmberg et al., 1984). Additionally, the emergence of *Salmonella* with decreased susceptibility to fluoroquinolone paralleled approval of the veterinary use of enrofloxacin, a fluoroquinolone antibiotic, even though fluoroquinolones had been used in humans for the preceding six years with little impact on the development of resistant *Salmonella* (Threlfall et al., 1997). Although there is no evidence that the use of antibiotics in feed is responsible for the evolution of the multi-drug resistant strain of *Salmonella* Typhimurium DT104, the rapid dissemination of this strain in animals and humans indicates there is an advantage for strains with the antibiotic-resistance phenotype (see sidebar, p. 44). *S.* Typhimurium DT104 is the second leading cause of human salmonellosis in England and Wales (Anonymous, 1996) and the most common *Salmonella* species isolated from cattle (Hollinger et al., 1998). *S.* Typhimurium DT104 is resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (known as R-type ACSSuT). The resistance genes to the antibiotics are located on the chromosome rather than a plasmid, indicating they are nonmobile and stable (Threlfall et al., 1995). In the United States, the ACSSuT resistant pattern was present in 28% of 976 *S.* Typhimurium isolates collected nationally in 1995, a substantial increase from 7% in 1990 isolates (Hosek et al., 1997). Human infection by *S.* Typhimurium DT104 has greater morbidity and mortality than the other nontyphoid *Salmonella* infections (Wall et al., 1994). Because the accumulating data from molecular subtyping methods and epidemiological investigations suggest that the use of sub-therapeutic levels of antibiotics in animal feeds plays a role in the emergence of antibiotic-resistant foodborne pathogens, the prudent and judicious use of antibiotics in both the agricultural and medical sectors is needed.

Feeding Practices

Another animal production practice with potential ramifications for microbiological food safety is farm animal diet composition. A change of diet, for example, can change the microbial ecology of the ruminant digestive system. Many studies have evaluated the effect of dietary changes on fecal shedding of *E. coli* O157:H7 or acid-tolerant *E. coli* by cattle or sheep, with conflicting results. Some investigators have determined that sheep or cattle fed hay shed *E. coli* O157:H7 in their feces considerably longer than animals fed grain (Hovde et al., 1999; Kudva et al., 1997). In contrast, studies of acid-tolerant *E. coli*, which is a characteristic of *E. coli* O157:H7, revealed that a mostly grain diet promotes shedding of acid-tol-

Development and Dissemination of Resistant Organisms

Microorganisms develop resistance to antibiotics encountered in clinical and environmental settings. This fact has led to calls for the judicious use of antibiotics in human medicine and for restrictions on the use of antibiotics in veterinary medicine and animal production.

There are at least three fundamentally different ways that exposure to antibiotics can promote the development and/or dissemination of resistant microorganisms: (1) mutations and selection of mutants capable of surviving in vivo exposure to the antibiotic (e.g., fluoroquinolone resistance in *C. jejuni*), (2) mobilization and horizontal transfer of genetic elements containing resistance genes among different species of bacteria (e.g., vancomycin resistance among enterococci), and (3) widespread dissemination of strains with previously developed resistance (e.g., *S.* Typhimurium DT 104). The differences between these mechanisms have important implications for the prevention and control of antibiotic resistance among foodborne bacteria.

Resistance of *C. jejuni* to fluoroquinolones is conferred by a point mutation in the *gyr* gene (Engberg et al., 2001). Resistant organisms with the same molecular subtype characteristics as sensitive microorganisms have been isolated from human patients after apparent failure of treatment with ciprofloxicin. During the 1990s, an increased occurrence of resistant *C. jejuni* isolates in Minnesota was associated with treatment with a fluoroquinolone antibiotic and foreign travel. However, a growing proportion of resistant isolates was not attributable to these sources. Surveys of chicken at retail markets in Minnesota demonstrated a 20% prevalence of contamination with resistant *C. jejuni* strains. These strains showed considerable diversity based on polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) (PCR-RFLP) subtyping of the *fla*A gene, suggesting that mutations and selection of mutants was independently occurring among many *C. jejuni* strains. The overlap of molecular subtypes obtained from human and chicken sources suggest that chicken was a primary source of resistant *C. jejuni* for humans that were not treated and did not travel (Smith et al., 1999).

In Europe, a glycopeptide antibiotic, avoparcin, was used as an antimicrobial growth promoter. Following its introduction, resistance of enterococci to vancomycin (a similar glycopeptide antibiotic) was observed in hospitalized patients, exposed animals, and the human population outside of hospitals. Vancomycin-resistance genes were transferred horizontally between species of enterococci (Simonsen et al., 1998; van den Braak et al., 1998). The potential for vancomycin-resistance genes to be acquired by strains of *Staphylococcus aureus* represents an important public health threat (Cetinkaya et al., 2000).

The strains of *S.* Typhimurium DT104 that became a global public health concern during the 1990s appear to be highly clonal (Baggeson et al., 2000). Although DT104 appears to have accumulated multiple resistance genes through horizontal gene transfer, these genes were likely accumulated before the widespread dissemination of the resistant strains. Widespread dissemination of DT104 may have been facilitated by the use of antibiotics on farms, either because individual animals were treated with drugs that DT104 was resistant to, or because use of antibiotics altered the herds' microflora and increased animals' susceptibility to colonization and infection (Besser et al., 2000).

Although fluoroquinolone resistance in *C. jejuni* appears to be a direct response to clinical or environmental exposure to the antibiotics, DT104 represents the epidemic spread of a microorganism that has already developed resistance. The origins and factors contributing to the dissemination of these organisms require public health measures that address the differences. Trying to accomplish comprehensive control of these different situations primarily through restrictions on the veterinary and agriculture use of antibiotics has created an adversarial relationship between public health and animal production communities that has impeded the application of science-based control strategies.

erant *E. coli* in comparison to hay-fed cattle (Diez-Gonzalez et al., 1998). Subsequent studies revealed that changing the diets of cattle from grain to hay or from hay to grain distinctly reduced fecal shedding of Shiga toxin-producing *E. coli* (STEC) within the first week after changing the diet, whereas the *E. coli* cell numbers increased considerably thereafter (Richter et al., 2000). Overall, a major change in the composition (i.e., grain or roughage) of the ruminant diet appears to decrease for a few days the number of STEC shed in feces. Thereafter, cell numbers of the pathogen increase.

Recent evidence indicates that the type of grain fed to cattle can influence fecal shedding of *E. coli* O157:H7 by cattle (Buchko et al. 2000). Feces of cattle fed 85% barley were more frequently *E. coli* O157:H7-positive than those from cattle fed 85% corn, although no major differences were observed in cell numbers of *E. coli* O157:H7 in feces throughout most of the study. Before specific diet and feeding practices can be practically applied to farm production practices for pathogen control, considerably more research is needed to elucidate the influences that different dietary practices contribute to gastrointestinal carriage and fecal shedding of pathogens.

As with humans, "good" bacteria—in some cases nonpathogenic normal gut flora—in the gastrointestinal tract of animals may be able to prevent colonization by pathogens (Nurmi and Rantala, 1973). In chickens for example, the gut of the hatchling chick is sterile until it ingests microorganisms from the environment. If the chicks are exposed to adult bird fecal material, colonization of the gut occurs rapidly. Today's production practices remove this route of colonization, and the hatchling may not acquire a normal gut flora for days or weeks (Spencer and Garcia, 1995). The lack of a fully developed "normal flora" increases the chances of the chick gut becoming a carrier for microorganisms, such as *Salmonella*, that are pathogenic in hu-

mans. The chicken is most susceptible to colonization by human pathogens during the first week of life (Nurmi et al., 1992). The prevention of colonization with pathogens by normal gut flora is called competitive exclusion.

The use of probiotics in animals has expanded to include virtually all food and food-producing animals, as well as companion animals. The concept is the same as for the human use of probiotics, that is, maintaining a healthy gut flora enhances health and may prevent colonization with pathogens. Probiotics are administered to maintain health under the stresses animals experience due to crowding, transportation, overwork, and other external forces, and also to increase feed efficiency. They are touted as possible alternatives to antibiotics in some situations. Most probiotics used in animals currently are single microorganisms or defined mixtures of microorganisms. Current policy prevents unquantified or unidentified (undefined mixtures) of microorganisms to be used as "direct-fed microbial products" that can be regulated as food under Food and Drug Administration (FDA) Compliance Policy Guide (CPG) 689.100 (FDA/CVM, 1997). Many competitive exclusion products, particularly those that claim to exclude *Salmonella*, are regarded as drugs by FDA.

Wild-Caught Shellfish and Fish

Feral shellfish present a unique opportunity to transmit foodborne diseases of bacterial, viral and protozoan origin. Of particular concern are the edible bivalve molluscs of the class *Pelecypoda* that include the species commonly referred to as oysters, mussels, clams, and cockles. Since most of these organisms are filter feeders, they use siphoning organelles and mucous membranes to sieve suspended particles from the aquatic environment as a source of food. If their surrounding water is contaminated by bacteria, viruses, or parasitic protozoa, these mucous membranes may entrap the pathogens, which are then transferred to the digestive tract of the animal. Since these molluscan shellfish may be consumed whole and raw, they can act as passive carriers of human pathogens.

The most recent Centers for Disease Control and Prevention (CDC) statistics (1988-1992) of the overall foodborne disease burden in the United States estimate that 0.7-2.1% of all outbreaks, approximately 1% of all cases, and up to 13.3% of food-related deaths are due to the consumption of contaminated shellfish (Bean et al., 1997), although the total number of cases is likely to be underestimated (Wallace et al., 1999). Two general groups of pathogenic microorganisms may be transmitted by feral shellfish. The first group is termed indigenous pathogens because these organisms are native to the marine environment, consisting predominantly of members of the family *Vibrionaceae*, including the genera *Vibrio*, *Aeromonas* and *Plesiomonas*. The presence of these organisms is unrelated to fecal pollution. The second group, referred to as non-indigenous pathogens, are not natural marine inhabitants, and their presence in shellfish arises from either direct fecal contamination by human or animal reservoirs, or due to poor general sanitation during harvesting, processing, or preparation of the food animals. Within these two major groups, we can further characterize microorganisms contaminating shellfish as bacterial, viral or parasitic protozoan in nature.

The presence of *Salmonella* and *Shigella* species in feral shellfish is well documented, even in the recent literature. These organisms are an excellent example of non-indigenous bacterial pathogens, the presence of which is usually due to fecal contamination of harvesting sites. Fortunately, the National Shellfish Sanitation Program sponsors long-term programs targeting the prevention of shellfish-associated disease caused primarily by enteric bacteria. In the United States, this program has established bacteriological standards for shellfish and their harvesting waters based on the fecal coliform index. Such standards have been quite effective in preventing enteric bacterial contamination of feral shellfish, and with the exception of species imported into the United States from countries with less stringent standards, outbreaks of shellfish-borne disease associated with *Salmonella* and *Shigella* are relatively uncommon in the United States.

This is, however, not the case for the non-indigenous viral and protozoan pathogens that may be transmitted by contaminated shellfish. Enteric viruses, most notably hepatitis A virus and the Norwalk-like viruses (NLVs) are excreted in the fecal matter of infected persons and hence their source in the marine environment is usually the disposal of untreated or inadequately treated human sewage. For a number of reasons, the fecal coliform index is inadequate for monitoring the presence of viral contamination in shellfish or their harvesting waters, and both outbreaks and sporadic cases of enteric viral disease associated with the consumption of contaminated shellfish continue to occur in the United States (Jaykus et al., 2001). Protozoan parasites such as *Giardia* and *Cryptosporidium* species have recently been identified in feral shellfish (Fayer et al., 1998), although the significance of this food with respect to disease transmission has yet to be determined. Shellfish harvesting beds become contaminated with parasitic protozoa as a result of contamination with animal farm runoff or human sewage, both treated or untreated.

The indigenous bacterial pathogens (most notably *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*) are part of the estuarine microflora and have excellent survival capabilities in the marine environment. In addition, *V. cholerae* from human sewage may contaminate the marine environment. While the transmission of *V. cholerae* by seafood is well documented throughout the world, the United States has not had a major outbreak since 1911, although sporadic cases have occurred, usually due to the consumption of imported crustacea such as crabs and shrimp (Bean et al., 1997). Historically, *V. parahaemolyticus* outbreaks are rare in the United States, but two highly publicized recent outbreaks have challenged this trend (CDC, 1998b; CDC, 1999b). Shellfish implicated in these outbreaks were harvested from both Atlantic and Pacific waters during these outbreaks, but in both cases, the mean surface water temperatures were significantly higher (1-5 C) than those reported in previous years. This phenomenon suggests the potential for emergence of this pathogen is associated with natural changes in the temperature of the Gulf Stream (El Niño) or global warming from human activities.

V. vulnificus has been of greater concern in the United States in recent years, since this organism results in a syndrome characterized by gastrointestinal disease followed by primary septicemia, with mortality rates approaching 50%. Individuals with underlying liver dysfunction, circulatory problems particularly related to diabetes, or those who are immunocompromised are especially at risk (Hlady and Klontz, 1996). Evidence exists that other *Vibrio* species also can

The Role of Microbiological Indicators in Assuring Food Safety

The term "microbiological indicator" refers to a microorganism, a group of microorganisms, or a metabolic product of a microorganism, whose presence in a food or the environment at a given level is indicative of a potential quality or safety problem. The selection of an appropriate indicator is highly dependent upon the microbiological criteria for the food product in question. Important considerations in indicator selection include: possible sources of pathogenic microorganisms; their incidence on or in the product; production, harvesting and processing practices; survival and growth of pathogens in the product; and specific analytical methods available for detecting the indicator.

Although frequently used interchangeably, scientists sometimes make a distinction between the terms microbiological indicator and index organism. In general, index organisms are markers whose presence in numbers exceeding pre-established limits indicates the possible occurrence of ecologically similar pathogens (Mossel et al., 1995). Indicator tests are often employed to assess a process control attribute, such as using the extent of mesophilic growth as an indicator of inadequate refrigeration (Ingram, 1977). A given marker can function both as an index and as an indicator organism, even in the same food.

The presence of indicator organisms does not necessarily guarantee the presence of pathogens (Banks and Board, 1983). Ideally, the absence or a low concentration of a specific indicator means the food has not been exposed to conditions that would permit contamination by a specific target pathogen or present the opportunity for its growth. The indicator concept can be used to evaluate raw product directly from the field or farm, or after some decontamination or inactivation process.

Selection of an indicator that is relevant for a given food and a given target pathogen continues to be a challenge. Because no microbiological indicator is ideal, food safety professionals are frequently left with a limited choice of indicators that are relevant to some, but not all, foodborne pathogens. The most widely used indicators are the *Enterobacteriaceae*, coliforms, "fecal" coliforms and *E. coli.* Coliforms are gram-negative asporogenous rod-shaped bacteria that are environmentally ubiquitous and may be associated with the fecal material of animals including humans. Coliforms are frequently used as an indicator of inadequate sanitation and process control in food that receives a pasteurization heat treatment. Among the proposed or accepted uses of *E. coli* are as an indicator of fecal contamination, acceptably conditioned manure, acceptable quality of water for irrigation, shellfish safety and general environmental sampling. Some organizations have used the entire *Enterobacteriaceae* family as an indicator or index of potential pathogen contamination. Other alternative indicators have included the coliphage group and the "fecal streptococci" or enterococci. Each of these groups of microorganisms has shortcomings as indicators of enteric pathogens.

The relationship between the presence of the fecal indicators and the presence of foodborne pathogens of fecal origin, such as *Salmonella* and *Campylobacter*, has been questioned for years. Recently, Kornacki and Johnson (2001) stated that "numerous studies have determined that *E. coli*, coliforms, fecal coliforms and *Enterobacteriacae* are unreliable when used as an index of pathogen contamination of foods." This unreliability applies to fresh produce (Anonymous 2000; DeRoever, 1998; Nguyen-the and Carlin, 2000), fresh meats (Goepfert, 1976; Linton et al., 1976; Roberts, 1976; Tompkin, 1983), and feral shellfish (Jaykus et al., 2001). Indicators for other pathogens such as *L. monocytogenes* have perhaps fared better, and many processors continue to use the absence of generic listeria as an indicator for the absence of *L. monocytogenes*.

Despite the clear need for more reliable indicator systems, all the candidate replacements, such as coliphage, *Bifidobacter* spp., and enterococci, have their own limitations. Beyond the pathogenic bacteria, indicators also are needed that are specific for human enteric viruses such as human caliciviruses (Norwalk-like viruses) and parasitic protozoa such as *Cryptosporidium*, *Cyclospora*, and *Giardia*, all of which tend to be more resistant and persistent than bacterial foodborne pathogens. As in so many other areas of food microbiology, additional work in the area of microbiological indicators remains essential.

result in septic disease, including *V. parahaemolyticus, V. cholerae* non-O1, and *Vibrio hollisae* (Hlady et al., 1993), a finding that may indicate the emergence of non-vulnificus *Vibrio* species as sources of life-threatening shellfish-borne disease. *V. vulnificus* is a leading cause of foodborne disease-related deaths, particularly in the southern states (Bean et al., 1997; Hlady et al., 1993). There is indication that the microorganism is capable of establishing the so-called viable-but-nonculturable (VBNC) state, which means that routine examination by conventional cultural methods may provide negative results although viable and potentially virulent cells may be present in high numbers (Oliver and Wanucha, 1989).

Because regulation of seafood occurs predominantly by pre-harvest monitoring of the fecal coliform index of growing waters, the efficacy of control is highly dependent upon the relationship between this test and the presence of pathogens. A significant relationship does not exist in shellfish between the presence of the fecal coliforms and important human pathogens such as enteric viruses, the pathogenic vibrios, and perhaps the parasitic protozoa. In addition, shellfish harvested from other countries with less stringent standards than those of the United States may be contaminated, and some countries have much higher domestic rates of shellfish-associated disease. Pre-harvest control methods such as relaying (movement of shellfish from one harvest site to an alternative, pristine site to allow the animals to purge them-

selves of pathogens prior to harvest) have been effective for the elimination of many of the non-indigenous bacterial pathogens, but are reasonably ineffective in controlling viral and *Vibrio* contamination. In short, there is a need to further evaluate pre-harvest issues that impact the safety of feral shellfish consumed by the U.S. population.

Specific Production Methods

Sometimes the method used to produce the food commodity has a significant effect on the microbiological safety of foods. Organic foods are of special interest, in part because this production method relies on manure as a major source of fertilizer. In addition, aquaculture methods have some significant food safety differences from traditional fishing.

Organically Grown Foods

Organic foods are the product of a farming system that avoids the use of manmade fertilizers, pesticides, growth regulators, and livestock feed additives. Instead, the system relies on crop rotation, animal and plant manures, some hand weeding, and biological pest control. The organic food industry has been growing at an annual rate of 20 percent during the past decade, and continued growth is projected. Produce grown by about 12,000 organic farmers nationwide grossed approximately $6 billion in 2000. The surging market for organic produce is in part attributed to some consumers' belief that such foods are healthier, better tasting, and produced using environmentally friendly methods. Many consumers who have concerns about pesticides and herbicides cite safety as the main reason for use of organic foods.

In December 2000, the U.S. Department of Agriculture adopted marketing standards for the processing and labeling of organic foods (USDA/AMS, 2000). The new standards do not allow the use of irradiation, genetic engineering, and human sewage sludge fertilizer for any food labeled as organic. In addition, synthetic pesticides and fertilizers cannot be used for growing organic food, and antibiotics and hormones are prohibited in livestock production. Furthermore, livestock agricultural feed products must be 100 percent organic. Products meeting the requirements for labeling as "organic" can carry a USDA Organic Seal. Products with a smaller percentage of organic ingredients may be labeled as "made with organic ingredients" but cannot carry the USDA seal. Although the available data do not indicate that organic foods are safer or more nutritious than conventional food, consumer surveys indicate that foods labeled with the USDA organic seal are perceived as being more healthful.

A significant public health concern that has been largely overlooked by the organic movement is the potential for greater prevalence of contamination by foodborne pathogens that are carried by livestock and poultry and shed in their feces. Manure, a significant vehicle for pathogens, is a major source of fertilizer used for growing organic produce. The available scientific information is insufficient to ensure that foodborne pathogens are killed during composting and soil application.

Furthermore, manure contaminates hides, feathers and skin during livestock production. During slaughter and processing, carcasses occasionally come in contact with manure and become contaminated with pathogens. Because organic production standards prohibit the use of irradiation and chemical treatments during processing, the available methods to reduce pathogen contamination are restricted, resulting in a greater likelihood that organic meat and poultry will have higher levels of pathogen contamination than conventionally processed meat and poultry.

Very few studies have been conducted to address the microbiological safety of organic foods. A relatively small study was done to determine the prevalence of *E. coli* and *Salmonella* on selected organic and conventionally produced vegetables available at Atlanta grocery stores (Doyle, 2000). A total of 216 samples—half organic and half conventional—was tested over a 6-week period. *E. coli* was present on 22 organic samples and 18 conventional vegetables, and *Salmonella* was found in 3 organic samples and 2 conventional vegetables. These data indicate that the organic produce sampled was not safer from a microbiological perspective than the conventional produce. A comprehensive survey of foodborne pathogens in organic foods (including produce, meats and poultry) is needed to more fully evaluate the relative microbiological safety of such foods.

Aquaculture

One area where food production has undergone dramatic change is aquaculture or commercial fish farming. To meet the demand for seafood products, the seafood industry is turning to aquaculture to supplement increasingly limited natural supplies. The Food and Agriculture Organization (FAO) of the United Nations defines aquaculture as the "farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants" (FAO, 1995).

The available data (primarily from temperate zones) indicate a low incidence of enteric pathogens in fish and crustaceans raised in unfertilized systems, and few reports of human illness associated with the consumption of aquacultured finfish and crustaceans (Jensen and Greenless, 1997). Howgate (1997) reported that there was no reason to expect the risk of illness from farmed marine fish to be greater than corresponding wild species. In fact, the most challenging public health risks from aquaculture are those associated with shellfish grown in open surface waters (Jensen and Greenless 1997), where aquaculture faces many of the same issues as wild caught.

Antibiotics are used in aquaculture to control disease in cultured species. Drug use is regulated by FDA, but the number of approved drugs is limited and off-label use has been reported (JSA, 1994). These antibiotic compounds are allowed only for certain species and life stages, with designated withdrawal periods, during which cultured fish and shellfish that have been treated with veterinary drugs cannot be harvested or sold (JSA, 1994). Nevertheless, bacteria resistant to antimicrobials have been isolated in farmed catfish and from their environment (DePaola et al., 1988), in sediments located beneath net pens (Kerry et al., 1994), and from the intestines of cultured fish in net pens (Ervik et al., 1994). A World Health Organization report on food safety issues associated with aquaculture products stated that the risk to public health is probably limited to indirect exposure to antimicrobials (WHO, 1999). However, the use and misuse of antibiotics to control diseases in aquaculture is worldwide and will likely increase as intensive aquaculture systems become more common.

Current shellfish management programs—which include classification and monitoring of growing waters, proper siting of aquaculture areas, enforcement of best management practices and train-

ing of employees and harvesters in sanitation procedures—can help to reduce, but do not eliminate, microbiological food safety issues.

HARVEST ENVIRONMENT

The harvest environment is relatively commodity specific, because the methods used and harvest locations depend on the commodity in question. The harvest environment is especially important for foods such as fruits and vegetables that undergo minimal additional processing prior to consumption.

Produce

Many different approaches are used today to harvest produce depending on a variety of determinants, including the type of produce, site of growing operation, and labor availability. However, from a microbiological safety perspective, there are many common hazards for harvest operations. Water quality, field worker hygiene, field sanitation, truck sanitation, and temperature control are all food safety issues related to the harvest environment (IFT, 2002).

Water as ice or in the liquid form can readily transmit microorganisms to produce if contaminated. Most fruits and vegetables are washed with water at least once, and many types of produce are treated with water several times during processing. In addition to being used for washing, water is used for cooling, conveying produce (flume water), and for applying disinfectants and fungicides. Disinfectants are added to about 50% to 60% of water used in packing facilities. Care must be taken to control the sanitary quality of water.

Field worker hygiene is a major consideration because of the widespread use of human hands in cutting or picking vegetables and fruits in fields or orchards. Approximately 90% of farms that grow fruit or vegetables harvest produce exclusively by hand (USDA/NASS, 2001). The amount of human hand contact that occurs during harvesting varies depending on the type of produce. Melons are handled at most steps of the operation, whereas apples receive considerably less frequent contact. Vegetables such as leaf lettuce may be harvested, trimmed, sorted and bagged by hand. It is very difficult to uniformly enforce proper hand washing and glove use among workers.

An interesting practice in recent years has been the production of fresh-cut produce in the field. Presumably done to maintain product freshness, this practice essentially brings food processing into the harvest environment. For example, many fresh-cut processors are now removing the outer leaves and coring lettuce in the field. Some are even cutting lettuce where it is grown. Unfortunately, it is much more difficult to control contamination in the harvest environment, and this practice may increase the chances for contamination of these products. Certainly contamination of wash and rinse water would be of concern under these circumstances, as would be issues associated with poor hygiene of food handlers and inadequate sanitation of equipment.

Fruits and vegetables frequently come into contact with harvesting equipment (such as knives, machetes, clippers and scissors) and containers (such as bins, boxes, buckets, pans, trailers, and truck beds). Such equipment and containers should be properly washed and disinfected, although studies indicate that washing and sanitizing is done only about 75% and 30% of the time, respectively (USDA/NASS, 2001). Packing equipment such as tables, conveyor belts, flumes, and washing and cooling bins are washed and sanitized about 75% and 50% of the time, respectively.

Equipment and containers used during harvest operations are frequently made of materials, such as wood, that are difficult to clean. Soil from the field, which may contain pathogens, often encrusts equipment and containers. If not removed, soil adhering to equipment used for washing and disinfecting produce will reduce the sanitizing capacity of the disinfectant. Produce operations must prevent accumulation of soil on equipment and containers to enable effective disinfection of food contact surfaces.

Proper temperature control of fruits and vegetables is critical for both safety and quality purposes. Optimal temperatures vary according to the commodity; however, the temperature range for storing fruits and vegetables is usually quite narrow. Most pathogens, but not all, are inhibited by the cool temperatures at which produce is stored. In addition, cool temperatures tend to prolong the survival of viruses and parasites. Temperature control contributes most to the safety of fruits and vegetables that are cut; however, its effectiveness in controlling microbiological hazards is in general less significant than the hazard reduction that occurs by refrigerating raw foods of animal origin (IFT, 2002).

Food Animals

Production livestock are naturally contaminated with a variety of potential human pathogens, both externally and internally. These microorganisms originate from the environment in which the animals are produced, as well as from feedstuffs and the co-mingling of animals from various sources. The animals also can readily transmit these microorganisms among themselves, once they are moved from the production environment to the transportation system. Because of centralized slaughter establishments, production livestock may be transported considerable distances before slaughter.

During transportation, livestock are confined to prevent excessive movement. As the animals are in close physical contact, microorganisms may be transferred from one animal to another either by contact with each other or their excreta. Aerosol transmission of salmonella also has been demonstrated in chickens and mice (Clemmer et al., 1960; Darlow et al., 1961). The upper respiratory tract may be important in transmission, and the tonsils and lungs may be important sites for the invasion and dissemination of *Salmonella* in pigs (Fedorka-Cray et al., 1995; Fedorka-Cray et al., 2000; Gray et al., 1996).

Even under the best of conditions, transportation produces measurable stress upon live animals. Transport stress may have several physiological effects on the live animal that may ultimately impact food safety. Transportation of animals may increase fecal shedding of potential human pathogens, such as salmonella. This increase in shedding can contaminate the trucks or trailers used for transportation, and potentially increase the population of foodborne pathogens in and on many of the animals within the truck or trailer.

Once animals arrive at a slaughter establishment, they may be unloaded into holding pens prior to slaughter, depending on the animal species in question. Animals from several trucks or

trailers may be co-mingled in the same pen, although there is considerable variation between slaughter establishments. Co-mingling increases the opportunity for the spread of potential human pathogens between animals from different sources, with the possible outcome of more animals becoming contaminated either externally or internally with these microorganisms. In addition, these holding pens are difficult to adequately clean and sanitize, and it is not uncommon to detect potential human pathogens in these pens after they have been cleaned and sanitized (Davies and Wray, 1997; Gebreyes et al., 1999). As a result, populations of bacteria may remain in these pens and contaminate animals that are brought in from other sources. The same is true of poultry crates, which are equally difficult to clean and sanitize.

Aquaculture and Wild-Caught Fish and Shellfish

The length of time between harvest and refrigeration is critical to the microbiological safety of fish and shellfish. Time/temperature abuse is of particular concern for the pathogenic *Vibrio* species. Rapid harvesting, cooling and processing influences quality, but it may also improve food safety for certain fishery products by slowing the growth of pathogens and reducing the formation of histamine that causes scombroid poisoning in certain species.

Commercial finfish are harvested by a variety of methods. Fishing vessels may transport their harvests back to shore for processing on a daily basis, or they may remain at sea for several days, weeks or even longer. Fishing vessels use several methods to preserve their catch (e.g., icing, freezing, or refrigerated seawater). Finfish can be minimally processed on board (e.g., whole or eviscerated form). Finfish also can be processed into fillets and steaks and other value-added products, such as surimi (a washed, minced fish product) on commercial factory ships.

Shellfish also may be processed on board, or they may be brought to shore live. Mussels, clams, and oysters are usually quickly transported shore side and shucked or sold as live whole shellstock. Scallops are usually processed on board the fishing vessel and stored iced or frozen for several days or weeks. On the west coast, some crab species are processed on board the fishing vessel, while on the east coast and in the Gulf of Mexico, crabs are usually brought alive to shore for further processing. Lobsters are brought alive to shore, and shrimp are iced or frozen on board the fishing vessel.

All fishery products, regardless of whether they are wild harvested or cultured, can be contaminated with a variety of human pathogens. Although there is no reason to expect cultured species to be any more hazardous than wild caught (Howgate, 1997), there may be differences depending on the harvest area or culture method. For example, FDA recently found a higher percentage of *Salmonella* spp. on farm-raised shrimp (7%) compared with wild-harvested shrimp (<1%) (FDA, 2001). Most of the shrimp consumed in the United States are imported (NMFS, 2000), and a high percentage of these shrimp are aquacultured in tropical countries. Reilly et al. (1992) reported that *Salmonella* spp. is a component of the natural flora of pond cultured shrimp in tropical countries. This finding may be due to the fact that in many tropical countries, chicken manure is often used as a fertilizer in aquaculture ponds. To address food safety issues, FDA is currently developing GAP guidance documents to help reduce pathogens associated with cultured fishery products.

POST-HARVEST ENVIRONMENT

In the post-harvest environment, food safety becomes less commodity oriented, as the food moves through processing into the distribution and retail sectors. Microbiological food safety issues in the processing environment have the potential to affect many different foods. Also, at the processing stage, ingredients from many different commodity sectors may be combined into a single product. As ingredients are combined, the physical characteristics of the food change, and its microbiological profile is altered.

The microbiological controls applied in the post-harvest environment are often designed to intentionally stress the microorganisms present in the food. These stresses may be designed to be lethal on their own or in combination. Environmental conditions also may be modified to limit microbial growth, through such techniques as drying or refrigeration. Each of these stresses has an impact on the microbial population in the food.

Food Animal Slaughter and Meat Processing

Although food animal slaughter could be considered a harvest activity, from a microbial ecology perspective, it occurs in the post-harvest sector of the food chain. Slaughter and meat processing take place in a carefully controlled processing atmosphere that is different from the traditional harvest environment. Because food animals are naturally contaminated with a variety of potential pathogens, meat processors apply many microbiological control methods during the slaughter and processing of meat.

Role of Inspection

An important aspect of meat inspection in the United States and other developed countries is antemortem inspection. All animals presented for slaughter are inspected prior to slaughter. At present, this inspection focuses on observable clinical illness, and not on general hygiene of the animals. However, because of the recognition of the impact of animal hygiene on meat contamination, there have been some attempts to regulate this on the live animal. For example, part of the antemortem inspection in the United Kingdom is an evaluation of the overall hygiene of the animal, and the animals may be rejected for slaughter if they are too "dirty." Efforts to reduce the level of external carcass contamination of live animals have typically focused on either washing the entire animal or trimming the hair from the most heavily contaminated areas of the animal (typically the rump and midline of a steer or cow). These procedures must be balanced with animal welfare issues, which prevent the introduction of excessive, needless or unnecessary stresses on live animals.

Source of Contamination

Contamination of animal carcasses during slaughtering procedures is undesirable but unavoidable in the conversion of live animals to meat for consumption. It is assumed that the muscle tissue of healthy animals entering the

slaughter establishment is free of microorganisms (Ayres, 1955). However, intrinsic bacteria, occurring in the deep muscle tissue of healthy animals, have been reported for many animal species (Ingram, 1964; Ingram and Dainty, 1971). The most frequently characterized intrinsic bacteria are *Clostridium* spp. (Canada and Strong, 1964; Jensen and Hess, 1941; Narayan, 1966; Zagaevskii, 1973). Other potential human pathogens, such as salmonella, have not been reported as intrinsic bacteria in the muscle tissue of healthy animals. The assumption is bacteria that contaminate the muscle tissue are from extrinsic sources (gastrointestinal tract, lymph nodes, external carcass surfaces, and environmental sources). The majority of the microflora transferred to the tissue surfaces, while aesthetically undesirable, is nonpathogenic.

Carcass processing may be divided into processes that impact the external surfaces of the carcass and those that open the body cavity (evisceration). Examples of surface processes include the scalding of poultry and the removal of beef carcass hides. These processes in fact remove significant populations of bacteria from the carcass, but also expose the edible tissue to contamination. While the process of hide removal may be thought of as a primary source of contamination of beef carcasses, it also removes significant contamination from the carcass. In a similar fashion, scalding and de-feathering of chickens removes significant contamination from the chicken carcass, although some still remains on the carcass. The microflora deposited by these processes is primarily from environmental sources, that which is on the carcass from the livestock production environment and transportation. This microflora may include enteric pathogens, such as salmonella and *E. coli* O157:H7, and microorganisms such as *Listeria* and *Clostridium*. From a microbiological perspective, contamination of edible tissue by external surface processing is a relatively common occurrence.

Processes that open the body cavity expose edible tissue to bacteria from the gastrointestinal tract. The primary bacteria of potential public health concern from this source are the enteric pathogens. In the case of red meat processing, this source of contamination is infrequent, and usually confined to leaking bungs or an occasional ruptured gastrointestinal tract.

Impact of Interventions

Over the last twenty years, a number of interventions have evolved specifically to address microbial contamination of animal carcasses. These may be divided into physical methods and chemical methods. In practice, these methods are used in combination, resulting in a series of process interventions or "hurdles" to improve the microbiological quality of meats. Among the physical methods, prevention of contamination has received considerable attention. From the perspective of microbial adaptation, it is better to prevent contamination than to address it through processing methods. However, there are practical limitations to this, given the nature of the process (i.e., converting a live animal to food for human consumption). After prevention of contamination to the extent possible, physical interventions involve either removal of contamination (trimming), heat, or chemical treatments.

Effective physical removal of contamination is dependent on first identifying the area of contamination and then removing the affected area without transferring the contamination to other areas. There are practical limits to the identification of affected areas, as microorganisms cannot be seen. Therefore, identification of an affected area requires that there be sufficient contamination (mud, manure, etc.) to become visible to the operator. Once the area has been identified, the operator must then remove the area in an aseptic manner. The probable outcome of this operation, under controlled conditions, is the complete removal of the contamination with a virtually sterile surface remaining around the affected area. However, while this is easily accomplished under controlled conditions, it becomes much more unlikely in a processing environment. The operator must clean and sanitize the equipment (knife and hook) prior to the operation, remove the affected area from a carcass which is frequently moving on a processing line, and then re-sanitize the equipment prior to trimming the next affected area. In practice, trimming is substantially less effective in processing environments than in laboratory environments (compare Gorman et al. (1995) with Reagan et al. (1996)). Trimming as a process is limited to a specific area of a carcass.

In contrast to trimming, heat may be applied as either a localized treatment or as a whole carcass treatment. The most common form of localized treatment currently in use is the steam vacuum. Steam vacuuming, as the name implies, applies a steam treatment to both loosen contamination and kill bacteria, along with a vacuum process to physically remove contamination. Steam vacuuming may be highly effective in reducing microbial populations under controlled conditions, but as with trimming, becomes less effective under processing conditions (Castillo et al., 1999; Dorsa et al, 1996). As an alternative to a localized treatment, heat may be applied as a whole carcass treatment. Common examples of this are singeing of hog carcasses, hot water washing and steam pasteurization. Heat in singeing processes is commonly applied as open flame from gas jets, and while there is a reduction in microbial populations on the carcass surface, the primary function of this process is to remove residual hair from the carcass. In contrast, hot water washing and steam pasteurization were specifically developed as antimicrobial processes, applied as a final operation before chilling. Hot water washing applies hot water (>80 C) as a whole carcass rinse, while steam pasteurization places the carcasses in a chamber and applies steam to briefly raise the temperature of the carcass surface. Both hot water and steam pasteurization have been demonstrated to be effective in controlling microbial populations on animal carcasses (Barkate et al., 1993; Gill et al., 1995; Phebus et al., 1997). However, surviving pathogens will have undergone stress that may either increase or decrease the likelihood of their survival during the remaining time before consumption.

Chemical interventions involve the application of food grade chemicals to the carcass surfaces to inhibit or kill microorganisms (Dickson and Anderson, (1992; Siragusa, 1996). Typically, the mode of action of these antimicrobials is pH, with organic acids, such as lactic or acetic (low pH) and trisodium phosphate (high pH), the most commonly used. The concerns with the use of any chemical intervention process are both the potential to induce resistance in potential human pathogens and the potential to select for resistant organisms out of the overall microbial population. If re-

sistance becomes widespread in a microbial population, more organisms will survive, making the process less effective.

Perhaps the chemical intervention of greatest interest and concern is the organic acids and their potential to induce acid tolerance. In controlled experiments, acid-tolerant bacteria were no more tolerant to organic acid rinse processes and were in general more sensitive to heat than their homologous non-acid-tolerant strains (Dickson and Kunduru, 1995). This suggests that the development of acid tolerance does not present a unique hazard with organic acid rinses on animal carcasses.

Acid adaptation has been shown to enhance the ability of salmonella to survive in acidic food systems (Leyer and Johnson, 1992). These authors reported that when salmonella were briefly exposed to mildly acidic conditions (pH 5.8), the survival of these bacteria was dramatically enhanced in cheese. Another study reported that *S.* Typhimurium briefly exposed to mildly acidic conditions (pH 5.8) was significantly more resistant to strong acid conditions (pH 3.3) than the non-adapted parent strain (Foster and Hall, 1990). Acid shock at pH 4 has also been reported to enhance the thermotolerance of *L. monocytogenes* (Farber and Pagotto, 1992). Foster and Hall (1990) reported that the adaptive acid tolerance response of *S.* Typhimurium did not appear to induce cross protection with hydrogen peroxide or heat shock. Rowbury (1995) discussed the impact of a variety of environmental factors on acid tolerance, and noted that organisms attached to surfaces were more tolerant to acid than non-attached cells. Buchanan et al. (1999) reported that prior growth at acidic conditions increased the resistance of *E. coli* O157:H7 to ionizing radiation at acidic pHs.

Acid-adapted microorganisms may have a competitive or ecological advantage in the human stomach, which could potentially impact pathogenicity. The theory is that an acid resistant microorganism would be more capable of surviving the acid pH of the stomach, and therefore more organisms would enter the small intestine. This could then result in a lower infectious dose for the microorganism.

Chilling

Rapid chilling of hot (body temperature) animal carcasses is essential to reduce the outgrowth of contaminating microbes. The common methods of carcass chilling involve either forced air, forced air and water, or water chilling. While the primary intention of chilling is to limit microbial growth, some chilling methods do contribute to a reduction in microflora. For example, forced air chilling dries the carcass surface and may injure or kill some microorganisms by dehydration. The initial combination chilling process using forced air and water was the Chlor-Chil process (Swift and Company, 1973), which used chilled chlorinated water to simultaneously reduce microbial populations and chill the carcasses more rapidly. Although the use of chlorine in the water is no longer widely practiced, the process now commonly known as spray chilling is almost universally used in the beef industry. Although it does not have the benefit of drying the carcass, spray chilling reduces the surface temperatures of carcasses more rapidly than air chilling, and, since virtually all of the bacteria are on the carcass surfaces, it effectively reduces microbial growth to a greater extent than air chilling. Water chilling, widely used in the poultry industry, has evolved from a significant source of contamination between poultry carcasses to a potential microbial intervention process, with the use of counter-flow chillers and the addition of processing aids.

Fabrication

The disassembly of chilled carcasses is referred to as fabrication. The outputs of fabrication are fresh meat to go to the consumer, and meat intended for further processing. During fabrication, microorganisms may be transferred from carcass surfaces to other parts of the carcass, and may be transferred from one carcass to another by common contact surfaces. Because of the latter phenomenon, one carcass has the potential to contaminate several other carcasses. As the fabrication process proceeds, the carcass identity is lost, and the potential for trace back is seriously diminished. Other than the physical intervention of preventing or reducing contamination, there are essentially no commercially viable interventions in fabrication at this time. This certainly provides opportunities for research and development, but it must be kept in mind that the objective of the slaughter operation is to place the least contaminated carcass into the chilling and fabrication processes. Assuming that the microbiological objectives have been achieved in the slaughter process, and that the fabrication process is maintained at a reasonable level of hygiene, further interventions may be unnecessary.

Ground Meats

Ground meats are a special category of fresh meats. Ground meats are made from the less desirable cuts of fresh meat and from the trimmings of the more desirable cuts of meats. Unlike a steak or a chicken breast, which originate from a single carcass, ground meat may contain meat from many different carcasses. The nature of the process is to grind and mix the meat, which increases the likelihood that a single contaminated carcass may contaminate a larger quantity of meat. An additional factor is that the trimmings from the intact cuts of meat are often from the surface of the cut. Because the most consistent source of contamination on animal carcasses are the processes which affect the external surfaces of the carcass, the raw materials used for grinding have a higher probability of being contaminated with bacteria of potential human health significance. A consequence of these factors is that the microbial populations in ground meat are consistently higher than those on intact meat. Other than irradiation, there are essentially no commercially viable interventions for raw ground meats at this time.

A process that has also been used to recover edible tissue from bones is mechanical de-boning. This process was first developed in the poultry industry, but is also used in the red meat industry. Meat produced from mechanical deboning processes resembles ground meat, but is used almost exclusively as a processing ingredient for cooked meats. This meat does not generally reach the consumer as fresh meat. Because of the nature of the process, mechanically deboned meat commonly has a higher microbial population than other meats, including ground meats.

Processing

Fresh meats are frequently further processed for specific flavor characteristics or for increased shelf life. The processes involved with meats are the processes commonly used with other foods, including thermal processing (cooking, canning), dehydration, and fermentation. These processes do not result in any

unusual microbiological hazards unique to meat products, but some of the common microbiological hazards are worth restating.

Fermented meats, such as pepperoni, select for microorganisms that can tolerate higher osmotic pressure and acidic pHs. Microorganisms that can survive and cause human health concerns include *S. aureus* and some enteric bacteria, including *E. coli* O157:H7. Although it is less likely that enteric bacteria will survive, cases of foodborne disease outbreaks from these bacteria have been documented.

Cooked, ready-to-eat meats are a special category of processed meats. These products undergo a thermal process that renders them fully cooked with a very low population of microorganisms, and they are typically vacuum packaged and refrigerated. The refrigerated shelf life of these products can reach 120 days, which provides an extended opportunity for psychrotrophic bacteria to grow. The specific human pathogen of concern is *L. monocytogenes*, which accounts for approximately 30% of the foodborne deaths in the United States (Mead et al., 1999). Because *L. monocytogenes* in this case is a post-processing contaminant, the intervention (heat) has already been applied, and there are few intervention strategies that are viable after packaging.

A discussion of microbiological controls in meat processing would not be complete without considering the use of irradiation (for detail, see irradiation, p. 56). Irradiation has the advantage of being able to penetrate packaging materials. A product that is packaged and then irradiated is protected from recontamination while the packaging material remains intact. Irradiation may be the only effective microbial intervention process for fresh products that, by definition, cannot be processed by conventional processes. In a similar manner, irradiation may ultimately serve as an intervention for packaged, processed meats.

Future Challenges

The net result of processing changes has been improved microbiological control and reduced levels of enteric pathogens. Since the adoption of these more comprehensive systems for pathogen control, the prevalence of salmonella has decreased as determined by the USDA/FSIS monitoring program. But newly recognized food safety issues, such as *E. coli* O157:H7 in cattle and *C. jejuni* in broilers, challenge the industry's efforts. Continued improvements during slaughter may occur, but the better long-term strategy would be to minimize the presence of human pathogens on the incoming live animals. This approach would require changes in farm management practices that are based on scientific research.

Post-Harvest Processing of Other Commodities

Further processing of other commodities occurs as well. For example, fresh fruits and vegetables are frequently sent to packinghouses where they may be washed, trimmed, or otherwise changed prior to packaging. The environment of the packinghouse, and anything that comes into contact with the fresh produce (e.g. water, conveyor lines, packaging material) can thus contribute to the microbial ecology of the produce, and can contaminate the produce with pathogens. Since many fresh produce items are ready-to-eat, the cleanliness of the packinghouse environment is very important.

Produce may be more extensively processed as "pre-cut" fresh fruits or vegetables. Pre-cut or shredded lettuce has become a large industry serving the chain restaurant industry, as have other pre-cut vegetables for salad bars. Pre-packaged salads are also a popular item in grocery stores, and consumers frequently believe no further washing is required when the items are brought into the home for serving. Because the cutting exposes more surface area and disrupts natural barriers of the fruit or vegetable, these items are more permissive of microbial growth.

The seafood processing industry is complex, and to maximize food safety, processors and importers of seafood are subject to HACCP requirements as mandated by FDA (FDA, 1995). The processing of wild and aquacultured fishery products can be as simple as washing molluscan and crustacean shellstock or finfish with potable water. It also may include shucking, filleting, beheading and peeling followed by chilling and/or freezing, for sale to wholesalers, distributors and retailers. In some instances, finfish, shellfish and crustaceans are sold live at the retail market, or processed by hand or machine at commercial facilities into fillets and other value-added products. Fishery products may be consumed raw, or they may be processed into ready-to-eat products. Salmon, trout and other fish species are often processed into cold smoked and hot smoked fishery products. Fishery products also are used in a variety of salads and spreads, and they are cured, fermented, pickled, dried, and canned. Rapid harvesting, cooling and processing can reduce or prevent the occurrence of certain biological and chemical food safety hazards associated with some wild caught and cultured fishery species (e.g., molluscan shellfish and scombrotoxin susceptible species) (NAS, 1991). In other instances, the use of food additives or other post processing interventions (e.g., high hydrostatic pressure, irradiation, or thermal pasteurization) may be required to control food safety hazards.

Regardless of the commodity, processing hazards can include pathogens that may be naturally present on the food, that may be introduced during processing and handling, or that may increase to hazardous levels during distribution and storage. Strict attention to good manufacturing practices, sanitation control procedures and hygienic practices of plant employees are effective in controlling many of these hazards.

Water

Water is used extensively in the post-harvest processing environment, making water quality a significant concern. Advances in water treatment over the last 100 years have resulted in dramatic improvements to the microbiological safety of the public water supplies of developed countries (Dawson and Sartory, 2000). Perhaps of greatest present-day concern in these countries are large community-wide waterborne outbreaks of parasitic protozoa that are associated with either unfiltered or inadequately flocculated or filtered water, such as the *Cryptosporidium* outbreak that occurred in Milwaukee in the early 1990s (MacKenzie et al., 1994; Moe, 1996). It is important to note that, although drinking water is quite safe in the United States, it remains a significant cause of morbidity and mortality in developing countries, where water remains a common source of bacteria, viruses, and parasitic protozoa that impact human health. If this water is used in food processing, waterborne diseases of developing countries can be passed on

to the consuming public in more developed regions of the world through importation of contaminated foods.

From the perspective of post-harvest issues for produce, wash water, rinse water, and ice can serve as a potential source for contamination of fresh produce (Beuchat and Ryu, 1997). Perhaps predictably, recent evidence suggests that puncture wounds on fruit usually harbor greater numbers of pathogens to greater depths than seen at other locations of the intact fruit (Burnett et al., 2000). More significant, Burnett et al. (2000) found that a negative temperature differential (application of cold rinse water to warm fruit) may increase the relative attachment and infiltration of bacterial pathogens in intact fruit. This observation has significance for the use of hydrocooling technology, which has been applied to produce items such as strawberries, cherries, and field crops. Although hydrocooling water is frequently decontaminated by chlorination, inadequate control of pathogens in water recirculated through hydrocoolers could provide a source of pathogens that then infiltrate produce items because of the temperature differentials.

Historically, water for food processing in the United States has originated from municipal systems. However, with increased volume, as well as stricter regulations, water usage in the processing environment has increased dramatically over the last decade. Water reclamation and reuse has received considerable attention, with guidelines provided by the Environmental Protection Agency (EPA) (EPA/AID, 1992), although actual standards remain the responsibility of state agencies. These EPA guidelines address water reclamation for nonpotable urban, industrial and agricultural reuse; direct potable reuse is not practiced in the United States. Food processors have expressed some interest in water reuse as well, particularly in animal slaughter facilities and the USDA/FSIS has issued guidance for this application. In general, the reuse of water from any one location in the slaughter process is restricted to certain other point locations in the slaughter process, as provided by specific recommendations of the agency. In almost every instance, decontamination steps such as addition of chlorine, as well as microbiological monitoring, must be done on the reconditioned water, with very specific standards recommended. Reuse water from advanced wastewater treatment facilities can be used on edible product only if the facility meets EPA requirements. Unfortunately, these facilities are extremely expensive and most U.S. slaughter operations practice little if any routine water reuse at the current time.

Alternative Processing Technologies

Preservation techniques currently act in one of three ways: (1) preventing pathogen access to foods, (2) inactivating them should they gain access, or (3) preventing or slowing their growth should the previous two methods fail (Gould, 2000a). Traditional food processing has relied on thermal treatments to kill/inactivate microbiological contaminants. Unfortunately, thermal processing induces physical and chemical changes in the food. Canned green beans do not have the same taste and texture as fresh, despite having similar nutritional profiles. Chemical preservatives and naturally occurring antimicrobial compounds also have been used extensively for food preservation (Davidson, 1997). Again, a "pickled" or acidified food such as cauliflower does not have the same role on the menu as a fresh stalk of cauliflower, despite their identical origin. Beyond the use of singular food preservation techniques, many strategies employ a combination of preservation techniques, e.g., refrigerated storage under modified atmosphere, reduced heat treatment with some acidification, and mild heat with reduced water activity (Gould, 2000b). Leistner (2000) states that the important preservation approaches often use combinations of several factors to assure microbiological safety. These factors, often called "hurdles," include heat, acidity, water activity, redox potential, preservatives, competitive flora, low temperatures, and more than 40 other possible factors. Increasingly, the American public has sought "fresh" products, fueling efforts to develop many alternative processing technologies that result in products that have minimal process-induced changes in sensory and nutritional characteristics. It is expected that these technologies will play an increasing role in food processing in the future.

Any discussion of emerging food safety issues must consider the impact of these alternative food processing technologies. This analysis must consider both the immediate impact and the potential ramifications further down the farm-to-table chain. Altering any parameter along the entire food chain can have consequences beyond the immediate change, be they intended or unintended. For example, modifying the feed of a food animal may alter the microbial contamination of the final product that may in turn contaminate the kitchen of the unaware consumer. A new method of heating food (e.g., microwave) may speed up the heating and change the types of microorganisms that survive the heating process, whether the heating takes place in a food processing plant or the consumer's kitchen.

Overview

A recent report generated by IFT for FDA (IFT, 2000b) defined alternative technologies, identified the pathogens of public health concern that are the most resistant to various technologies, described the mechanisms of pathogen inactivation including the inactivation kinetics, identified ways to validate the effectiveness of microbial inactivation, identified critical process factors, and described process deviations and ways to handle them. The report also described synergistic effects among technologies, when data were available, and articulated future research needs for each technology.

Kinetic parameters and models are frequently used to develop food preservation processes that ensure safety. They also allow scientists to compare the ability of different process technologies to reduce microbial populations. Kinetic parameters, with their recognized limitations, use empirical coefficients experimentally determined from microbial reduction measurements to document the relationship between microbial population decreases and different process conditions. Kinetic parameters for microbial populations exposed to thermal treatments have been assembled over a significant period of time. Published literature has included kinetic parameters needed to control most process, product and microbial situations (Pflug and Gould, 2000).

IFT (2000b) used the models and kinetic parameters to present and compare microbial inactivation data from thermal, pressure and electromagnetic processes. Thermal parameters apply to microwave energy, electrical resistance

(ohmic), and other temperature-based processes. Researchers have spent substantial time studying how various microbial populations respond to thermal treatments. The scientific literature contains kinetic parameters for most process, product and microbial situations. These thermal parameters provide a sound basis for development of microwave energy and ohmic technologies. The parameters currently used for pressure or pulsed electric field (PEF) treatments should be applicable to other processes where pressure or electricity is the primary critical factor in reducing microbial populations. Given the scarcity of data, parameters for pressure or pulsed electric field treatments must be estimated, highlighting the urgent need for additional research. For several other technologies, the quantity of data describing the treatment's reduction of microbial populations is insufficient for a comparison.

Scientists face limitations in interpreting these parameters. When the parameters are used to develop a process,

Table 10. Limitations to Alternative Processing Technologies Currently Under Development (IFT, 2000b)

Limitations applicable to all or most of the technologies:

Linear first-order survivor curve model may be inadequate. Appropriate model(s) would be beneficial to all preservation technologies.

Standardized experimental protocol(s) for obtaining statistically reliable kinetic parameters to describe survivor curves for microbial populations exposed to various alternative technologies.

Different inactivation action/mechanism(s) among alternative technologies unidentified and undefined to date.

Synergism or antagonism of one alternative process used with another and their combined effect on microbial inactivation efficiency undetermined to date.

Potential formation of unpalatable and toxic by-products due to processing.

No reliable methods for measuring and monitoring temperatures or other treatment actions within individual, large, solid particulates.

Possible new or changing critical process factors and their effect on microbial inactivation.

Pulsed Electric Field:

Few published reports

Kinetics based on 2-point survivor curves

Few reports state threshold field strength for inactivation

Treatment vessel design is major variable among studies

Mechanisms of action need confirmation

Most resistant pathogens and appropriate surrogates need to be identified

Development of resistance after sublethal treatment needs to be tested

Kinetic models and critical process factors affecting kinetics are needed

Effective monitoring systems, uniform treatment chamber design, electrode construction, and other hardware components are needed to assure consistent delivery of specified treatment

Electrothermal:

Difficult to monitor temperature distributions and heating patterns in solids and particles

No well-developed models for process deviations and use of alternative or variable frequencies of processing energy

Some food factors influence process effectiveness

Most resistant pathogens and appropriate surrogates need to be identified

Development of resistance after sublethal treatment needs to be tested

Kinetic models and critical process factors affecting kinetics are needed

High Pressure Processing:

Influence of pressure on reduction of microbial populations using the proper experimental design (statistically valid, collection of data at different pressures and control of temperature and product), so that z(P) (increase in MPa to reduce D value by factor of 10) and/or activation volumes (V) are quantified. Synergistic effects among pressure, temperature and other variables unknown.

High capital costs of equipment

Questionable reliability of equipment

Solid food must be batch processed and pumpable foods only semi-continuous

Minimal inactivation of bacterial spores in low-acid foods unless mild heat is applied

Survival curves often nonlinear complicating kinetics and calculation of process parameters

Food enzymes respond differently from each other

Excessive pressure denatures proteins and changes food

Most resistant pathogens and appropriate surrogates need to be identified

Development of resistance after sublethal treatment needs to be tested

Kinetic models and critical process factors affecting kinetics are needed

Others:

Some of the technologies present greater limitations than others or are at a development stage that requires extensive further scientific research before they can be commercially used. For these technologies, data are insufficient to calculate kinetic parameters. For example, **high voltage arc discharge** (application of discharge voltages through an electrode gap below an aqueous medium) causes electrolysis and highly reactive chemicals. Although microorganisms are inactivated, more recent designs need to be developed before consideration for use in food preservation. Likewise, **oscillating magnetic fields** have been explored for their potential to inactivate microorganisms. However, the results are inconsistent; different studies have shown the level of microorganisms may increase, decrease or not be affected. Data on inactivation of food microorganisms by **ultrasound** (energy generated by sound waves of 20,000 or more vibrations per second) are scarce and limitations include the inclusion of particulates and other interfering substances. **Ultraviolet light** (UV) is a promising technique especially in treating water and fruit juices. A 4-log bacterial reduction was obtained for a variety of microorganisms when 400 J/m^2 was applied. Apple cider inoculated with *E. coli* O157:H7 treated in that manner achieved a 5-log reduction. To achieve bacterial inactivation, the UV radiant exposure must be at least 400 J/m^2 in all parts of the product. Critical factors include the transmissivity, the geometric configuration of the reactor, the power, wavelength and physical arrangement of the UV source, the product flow profile, and radiation path length.

care should be taken to compare the resistance of different microbial populations or to identify the appropriate target microorganisms. When a new alternative processing technology is under review, it is essential to determine the pathogens of greatest public health concern.

When exploring the new preservation technologies, their preservation level should be compared to that of classical thermal pasteurization or commercial sterilization technologies. Thermal pasteurization focuses on inactivating vegetative cells of pathogenic microorganisms, i.e., microbial cells that are not dormant, highly resistant spores. However, to have a commercially sterile product, the process must control or inactivate any microbial life (usually targeting spores of *C. botulinum*) capable of germinating and growing in the food under normal storage conditions. Commercially sterile products generally require more extensive treatment than those that are pasteurized. This goal must be considered when evaluating alternative processing technologies as well.

Development of a number of alternative processing technologies is underway, although many of these technologies require additional research before they are ready for commercial application (see Table 10). Validation of effectiveness is an important step in the development of any new technology (see sidebar below).

High Pressure Processing

To preserve food using high hydrostatic pressure, the food (normally packaged) is submerged in a liquid (usually water) contained in a vessel that generates pressure by pumping more liquid into the pressure vessel or reducing the volume of the pressure chamber.

In the 19th century, scientists realized that applying pressure in this manner inactivated microorganisms and could be used to preserve foods (Hite, 1899). Larsen and coworkers (1914) confirmed that high pressure processing (HPP) can kill microbial cells. Vegetative bacteria were inactivated after 14 hours at 607 MPa; bacterial spores were extremely resistant to pressure but could be inactivated at 1,214 MPa. Today, HPP of foods uses pressures within the range of 300 to 700 MPa.

Over the last fifteen years, use of HPP as a food preservation method has been pursued in an effort to produce safe foods with a reasonable shelf life with the use of minimal heat. Reducing or eliminating high temperatures in food processing can deliver a food product with flavor, texture, appearance, and nutrient content very similar or identical to fresh or raw food. The first commercial products treated by HPP—fruit products

Validation of Treatment Effectiveness Using Microbiological Surrogates

The function of surrogate organisms is different from that of microbiological indicators. Surrogates are used to evaluate the effects and microbial responses to processing treatments. The main difference between surrogates and indicators is that the latter is naturally occurring and the former is introduced as an inoculum. In the case of fresh and fresh-cut produce where no traditional processing inactivation steps are used (e.g., heat pasteurization), surrogates could be used to assess and validate decontamination procedures. In the case of alternative processing technologies, surrogates could be used to validate specific processing efficacy and treatment delivery. Surrogates may be selected cultures prepared in a laboratory and inoculated onto or into the product, or they may be an inoculum of naturally occurring microorganisms that conforms to the requirements of a surrogate and has been confirmed to exist at adequate concentrations in the specific product. Generally, surrogates are selected from the population of well-known microorganisms that have well-defined characteristics and a long history of being nonpathogenic. It can be especially difficult to identify surrogates that are not pathogenic for highly susceptible subpopulations and that are unlikely to undergo transformation into a pathogenic phenotype in the production environment. In selecting surrogates, the following microbial characteristics are desirable:

- Nonpathogenic
- Inactivation characteristics and kinetics that can be used to predict those of the target organism
- Behavior similar to target microorganisms when exposed to processing parameters (for example, pH stability, temperature sensitivity, and oxygen tolerance)
- Stable and consistent growth characteristics
- Easily prepared to yield high-density populations
- Once prepared, population is constant until utilized
- Easily enumerated using rapid, sensitive, inexpensive detection systems
- Easily differentiated from other microflora.

The validity of an established or new preservation or decontamination process is frequently confirmed using an inoculated test pack consisting of the food product inoculated and tested under actual plant conditions, which includes processing and control equipment, product handling and packaging. Because pathogens should not be introduced into the production area, surrogate microorganisms should be used in inoculated pack studies, and their survival or growth can be measured to validate the process. For instance, surrogates have been used for many years in the low-acid canning industry to establish and validate the destruction of *C. botulinum* spores. The use of nonpathogenic spores of the putrefactive anaerobe *Clostridium sporogenes*, or spores of the flat-sour thermophilic organism *Bacillus stearothermophilus* as surrogates for *C. botulinum*, have helped the industry develop thermal processes that ensure products are safe and commercially sterile. *Listeria innocua* M1 has thermal resistance profiles similar to *L. monocytogenes* but is designed for easy detection as a surrogate and is not a pathogen (Fairchild and Foegeding, 1993). In addition, nonpathogenic strains of *E. coli* have served as surrogates for *E. coli* O157:H7. In all cases, the surrogate organism is added to the food product and used to obtain quantitative information to determine and validate the efficacy of food processing or decontamination methods.

such as jams and jellies—reached the marketplace in Japan in 1991. An acidic pH is an important element in the safety of HPP preserved foods because the pressures used in commercial application of the technology have limited effectiveness against bacterial spores.

Certain principles apply to HPP inactivation of pathogenic bacteria: (1) increasing the pressure magnitude or time of pressurization will usually increase the number of bacteria destroyed (with the exception of bacterial spores); (2) an acidic pH or temperatures above ambient enhance pressure inactivation rates; (3) gram-positive bacteria tend to be more resistant to HPP than gram-negative bacteria; (4) cells in exponential phase are generally more pressure-sensitive than in stationary phase; and, (5) incomplete inactivation of bacteria using HPP can result in injured cells that are capable of recovery under optimal growth conditions, a common phenomenon known as sublethal injury.

Although increasing the pressure kills more bacteria in less time, higher pressures can cause far greater levels of protein denaturation and other detrimental changes in sensory quality that affect the food's appearance and texture as compared to the unprocessed product.

The major factors affecting the effectiveness of HPP are: the type of bacteria present in the food; its growth conditions; the composition, pH and water activity of the food; and the temperature, magnitude, and time of pressurization (Hoover, 1993).

As described by LeChatelier's Principle, pressure enhances reactions that result in a volume decrease and inhibits those reactions leading to an increase in volume (Johnson and Campbell, 1945). For this reason, pressure alters the equilibrium of the interactions that stabilize the folded three-dimensional shape of proteins (Masson, 1992). The extent of denaturation by pressure depends upon the structure of the protein, the pressure range, and other external parameters such as temperature, pH and solvent composition. Pressure will primarily affect the hydrophobic interactions of proteins; covalent bonds are not affected. Consequently, the extent of hydrophobicity of a protein can significantly determine the degree of protein denaturation at any given pressure (Jaenicke, 1981). Pressurized membranes normally show altered permeabilities and it is believed that denaturation of membrane proteins is one of the primary reasons for loss of membrane integrity leading to injury and death of the bacterial cell (Paul and Morita, 1971). Pressure-induced malfunctions of the membrane inhibit amino acid uptake that is probably due to membrane protein denaturation, but it has been shown that bacteria with a relatively high content of diphosphatidylglycerol (shown to cause rigidity in membranes in the presence of calcium) are more susceptible to inactivation by HPP (Smelt et al., 1994), and those compounds that enhance membrane fluidity usually impart pressure resistance to an organism (Russell et al., 1995). It is well established that a loss of intracellular components occurs when microorganisms are exposed to high levels of hydrostatic pressure.

Some foodborne pathogens are more resistant to inactivation by HPP than others. In work by Patterson et al. (1995), *Yersinia enterocolitica* was reduced 5 $\log_{10}$ cycles when exposed to 275 MPa for 15 minutes in a phosphate-buffered saline solution. For comparable 5-$\log_{10}$ reductions using 15-minute treatments, *S.* Typhimurium required 350 MPa, *L. monocytogenes* required 375 MPa, *S.* Enteritidis required 450 MPa, and *E. coli* O157:H7 and *S. aureus* required 700 MPa. The bacteria showed more pressure resistance in ultra high temperature processed milk than meat or buffer. Thus, the variability of pressure response is related to bacterial differences and different food substrates. By using treatment temperatures of 50 C instead of ambient, similar reductions could be accomplished for *E. coli* and *S. aureus* at 500 MPa instead of 700 MPa (Patterson and Kilpatrick, 1998).

For most foods, 10-minute exposures to pressures in the range of 250-300 MPa (37,500-45,000 psi) result in what can be called "cold pasteurization." Here, levels of inactivation represent a reduction of microorganisms (primarily vegetative bacteria) of approximately 4 to 6 $\log_{10}$ CFU/mL or g. Cold pasteurization is a currently popular term used widely for any "nonthermal" food process or processes (such as HPP or irradiation) that do not depend on extensive heat as the major mechanism of microbial inactivation. Foods that are cold-pasteurized are not cooked or heated in the conventional sense and thus do not significantly lose sensory quality (e.g., appearance, texture, and flavor) and nutrient content; however, there is a significant reduction in microbial population.

Pressures greater than 500 MPa are usually required for greater microbial reduction or consistent product sterilization, but use of low pH and mild heat treatment (45 – 70 C) is often necessary to attain commercial sterility. For example, in the case of green infusion tea, Kinugasa et al. (1992) produced a commercially sterile product using 700 MPa at 70 C for 10 minutes. These treatment parameters were successful even when the tea was inoculated with 10^6 spores of *B. cereus, Bacillus coagulans* and *Bacillus licheniformis.* The tea's flavor was unchanged as were the catechins, vitamin C and amino acids in the tea. In another example of using HPP in a combined preservative approach, Shearer et al. (2000) measured the reduction of spores of *Bacillus, Clostridium* and *Alicyclobacillus* in test foods using HPP and 45 C in combination with such preservatives as sucrose laurates, sucrose palmitate, sucrose stearates, and monolaurin. Other preservative combinations that include HPP also used carbon dioxide (Haas et al., 1989) and acidification plus addition of nisin (Roberts and Hoover, 1996).

HPP continues to be developed as a nonthermal food processing method; however, scientists still need to develop a reliable method to predict the HPP process endpoint, the point at which all pathogenic bacteria are inactivated. The heat resistance of a pathogen does not directly correlate to its pressure resistance, and the potential emergence of pathogens with unusual pressure resistance is an issue to address. As a relatively new commercial food process, concern still exists for the safety of some foods processed using HPP, especially given the ever-evolving nature of microorganisms.

Irradiation

Food irradiation, first commercially introduced in the early 1960s, uses ionizing radiation to decontaminate and disinfect food and inhibit sprouting and ripening. The ionizing radiation is usually in the form of gamma rays produced by radionuclides such as ^{60}Co (cobalt) or ^{137}Cs (cesium). Newer irradiation technologies include e-beam, where ordinary electricity is used to produce a stream of electrons, or x-ray irradiation, where the electron beam is bounced off metal to create x-rays (Farkas, 1998). To get away from using the term "irradiation," food

irradiation is more frequently referred to as cold pasteurization, because the much shorter wavelengths of gamma and x-rays and e-beams penetrate the food very rapidly and little or no heat is produced. The purpose of food irradiation depends on specific applications; in general, food irradiation is used to reduce the levels of foodborne pathogens on the food, inactivate the food spoilage microorganisms, and prolong the shelf life of fresh foods by decreasing the normal biological changes associated with growth and maturation processes, such as ripening or sprouting. Both gamma irradiation and x-rays can be used for thick foods as they penetrate several feet, whereas e-beam irradiation can only penetrate several inches. As well, both e-beam and x-ray irradiation are considered environmentally friendly, due to the absence of a radioactive power source and the ability to switch the system off and on at will.

FDA has expanded the use of x-ray and e-beam irradiation for the treatment of prepackaged foods, and companies in the United States have recently begun using e-beam technology to pasteurize ground beef. In addition, companies in the United States will soon begin using x-ray systems for irradiation of packaged foods. For example, a new x-ray test center has opened, allowing food producers to fine-tune x-ray irradiation protocols for a variety of foods.

Like other physical processes such as cooking and freezing, irradiation can cause some alteration of the chemical and sensory profiles of a food. Treatment with ionizing radiation results in chemical modification of extremely small amounts of the major constituents of food (carbohydrates, proteins and lipids), and can also affect minor components of food, specifically vitamins and DNA. However, these changes are considered insignificant with respect to nutritional adequacy. In general, most food nutrients are unaffected by irradiation, with the exception of some vitamins for which minor decreases may occur. It is unlikely, however, that any vitamin deficiency would result from the consumption of irradiated foods (Diehl, 1995; Josephson et al., 1978; Kilcast, 1994).

U.S. commercial production of irradiated foods for food safety purposes is relatively recent, although the technology has a longer history as a treatment for medical devices and for control of insect infestation and sprouting in fresh produce. Applications of ionizing radiation accepted by FDA for food safety purposes include the more recent addition of microbial control in fresh and frozen meat and poultry.

To establish the safety of a proposed food irradiation application, FDA requires data on the radiological, toxicological, and microbiological safety, as well as the nutritional wholesomeness of the irradiated product (Pauli and Tarantino, 1995). From the perspective of radiological safety, the energy produced by approved radiation sources is too low to induce radioactivity in foods. The issue of toxicological safety is more complex and has been thoroughly studied in the past. Recent petitions in the meat and poultry area have indicated that FDA's principal current interest lies in specification of the conditions for food irradiation (such as temperature and packaging atmosphere) and their impact on microbiological safety and nutritional adequacy (Olson, 1998). The two most important concerns related to the microbiological safety of irradiated foods are (1) the potential to create highly virulent mutant pathogens, and (2) the potential that reducing the harmless background microflora could eliminate competitive microbial forces and allow uncontrolled pathogen growth.

The relative radiation resistance of microorganisms can be summarized as follows (in order of most resistant to least resistant): viruses>spores>gram-positive bacteria>gram-negative bacteria>yeasts and molds>parasites (Monk et al., 1995). However, there are some nonsporeforming bacteria, such as *Deinococcus radiodurans* and *Acinetobacter radioresistens*, that are extremely radiation resistant by virtue of their genetic make-up. Although the exact cellular mechanism(s) responsible for such resistance is unknown, scientists believe that these radiation-resistant bacteria possess particularly effective nucleic acid repair mechanisms. Although these organisms are not known to be pathogens, they may provide a genetic pool from which pathogens could theoretically pick up resistance genes through mechanisms of genetic exchange. Furthermore, some researchers have proposed concerns that irradiation may produce mutant strains and/or radiation-resistant pathogens by means of natural selection. To date, various studies and independent reviews by researchers and international organizations have found no indication of specific bacteriological hazards associated with food irradiation. However, as irradiation becomes more widely used, the potential for the production of resistant pathogenic mutants could be magnified, so continued surveillance is warranted.

Treatment with low to medium doses (i.e., non-sterilizing doses) of ionizing radiation greatly reduces, but does not necessarily eliminate, bacteria and other organisms that may be present in the food (Monk et al., 1995). Complete sterilization of foods is not the purpose nor, in most cases, even desirable for irradiation. Because non-sterilizing doses of radiation do not kill all bacteria, certain pathogenic bacteria (e.g., *C. botulinum*, *Salmonella*) may survive and, in the absence of competition from harmless bacteria, may multiply to potentially hazardous levels. Before using irradiation as a food safety measure, experimental evidence is required to demonstrate that the proposed treatment achieves the intended microbiological control without allowing *C. botulinum* growth and toxin production.

Most of the recent interest in food irradiation has focused on its efficacy in controlling bacterial pathogens such as *Salmonella, E. coli* O157:H7 and *L. monocytogenes* in muscle foods. As with heat, higher irradiation doses kill greater numbers of bacteria (Olson, 1998). While different bacterial species and strains demonstrate differences in relative radiation resistance, recommended irradiation doses for fresh meat and poultry result in destruction of greater than 99.9% of *Salmonella* and *L. monocytogenes*, and more than adequate control of *E. coli* O157:H7 (Farkas, 1998; Olson, 1998). The marine *Vibrio* pathogens, of concern in bivalve molluscan shellfish, are also extremely radiation-sensitive (Rashid et al., 1992). The parasite *T. gondii* is readily inactivated by gamma irradiation at doses of 0.25 kGy (Dubey et al., 1986), whereas *T. spiralis* is more resistant to gamma irradiation and requires doses of 7-9.3 kGy to kill the parasite in situ and 0.18 kGy to stop development of larvae to the adult stage (Monk et al., 1995). Unfortunately, foodborne viruses such as hepatitis A virus and the Norwalk-like viruses are resistant to radiation inactivation, and this is not a promising control technology for these foodborne pathogens (Bidawid et al., 2000; Mallett et al., 1991).

Besides having an effect on pathogen load, an added benefit of irradiation is a reduction in the numbers of spoilage mi-

croorganisms. For instance, the gram-negative psychrotrophs, which are the predominant spoilage organisms for fresh meat and poultry, are very susceptible to irradiation. In general, irradiation of raw meats results in a significant extension of shelf life, as much as twice that of non-irradiated, refrigerated products (Olson, 1998). However, regardless of the impact of food irradiation on reducing spoilage and bacterial pathogens, proper storage and handling, including temperature control, after processing is necessary to ensure that the food will be safe. It also should be noted that the effect of irradiation treatments on the sensory qualities of foods depends largely on the type of food product undergoing treatment, as well as the dose of radiation used. In some instances, the dose of radiation necessary to destroy pathogens produces undesirable organoleptic changes in the food product. For example, oxidation of lipids in the food can cause discoloration and rancidity. In short, irradiation is not an effective treatment for pathogen control in all food products.

Irradiation represents one tool among several (e.g., fumigants, carcass rinses, steam pasteurization, chemical sprout inhibitors, food preservatives) for enhancing food safety. It is likely that in the future, food irradiation would be used to complement rather than replace many of the other techniques already in use. Alternatively, combination treatments in line with the hurdle concept (e.g., irradiation plus MAP) may become more commonplace and offer an additional level of control. In some cases, irradiation may be a safer alternative. For example, when used to reduce the microbial load on spices, irradiation can serve as an alternative to fumigants such as ethylene oxide or methyl bromide, which are particularly toxic to occupationally exposed individuals.

A key advantage of food irradiation for controlling pathogens is that it reduces the microbial load at the point at which the product has been packaged, which increases the likelihood that the product the consumer receives will be safe. However, like other processes, irradiation only protects against pathogens that contaminate the product at the time of processing; it does not protect against future contamination that may occur during handling, storage and preparation of the food.

A number of additional issues must be addressed as food irradiation facilities are established. For example, the occupational health and safety of workers in irradiation facilities and the transport of radioactive materials for gamma irradiation facilities merit consideration. If gamma irradiation of food becomes a significant industry in the future, it will likely entail the construction of more facilities and the increased transport of radioactive materials. These concerns may increase advancement of the more environmentally friendly irradiation technologies, namely x-ray and e-beam irradiation.

There is also a need to do more work on indicators that one can use to determine if foods have been irradiated and if so, the level of irradiation given. At the recommended doses for specific applications, there are no major chemical, physical, or sensory changes in irradiated foods. Therefore, detection methods must focus on minute changes such as minor chemical, physical, histological, morphological, and biological changes in the food. Some promising methods for measuring these changes in food include hydrocarbon and cyclobutone for lipid-containing foods, electron spin resonance for bone-containing food, thermoluminescence for foods containing silicate minerals (Olson, 1998) and the DNA comet assay for analysis of foods with low fatty acid content (Cerda et al., 1997).

Active and Intelligent Packaging

The primary purpose of food packaging is to protect the food from physical, microbial and chemical contamination. Therefore, the type of packaging used plays an important role in determining the shelf life of a food. Today's consumers increasingly demand mildly preserved convenience foods with fresh-like qualities. In addition, advances in retail and distribution practices (e.g., centralization of activities, Internet shopping, global procurement) lead to greater distribution distances and longer storage times for a variety of products with different temperature requirements, thereby creating immense demands for innovation from the food packaging industry. Active and intelligent packaging technologies are used to extend shelf life, improve safety and improve the sensory properties of packaged foods. This is achieved by providing the best microenvironment within the package through optimal gas composition and humidity level. The challenge for food manufacturers is to maintain product safety while providing these storage and preservation conditions.

Ideally, active packaging would sense the microenvironment within the package and modify conditions accordingly to extend shelf life, improve safety or enhance sensory properties. This active approach is different but complementary to intelligent packaging, which provides information about critical parameters such as temperature, time, gas content, or microbial contamination. New packaging advances are starting to combine these two concepts in the next generation of food packaging.

Active packaging is not one technology, but a collection suited to specific problems. Active packaging concepts can be divided into three major categories: modified atmosphere packaging, active scavenging (oxygen, ethylene, carbon dioxide) and releasing concepts [emitters (carbon dioxide, ethanol, flavors, fragrances), and microbial control systems (chlorine dioxide, sulphur dioxide)].

Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) involves the creation of a modified atmosphere by altering the normal composition of air (78% nitrogen, 21% oxygen, 0.03% carbon dioxide and traces of noble gases) to provide an optimum atmosphere for increasing the storage length and quality of food (Moleyar and Narasimham, 1994; Phillips, 1996). The atmospheric modification can be achieved by using controlled atmosphere storage (CAS) and/or active or passive MAP. Active modification creates a slight vacuum inside the package that is then replaced by a desired mixture of gases. Passive modification occurs when the product is packaged using a selected film type, and a desired atmosphere develops naturally as a consequence of the product's respiration and the diffusion of gases through the film (Lee et al., 1996; Moleyar and Narasimham, 1994; Zagory, 1995). The choice of the film is an integral part of this system, because gas diffusion rates vary greatly among films, and therefore films differ in their ability to maintain the desired modified atmosphere. Also taken into consideration is the storage temperature, which will also affect gas diffusion rates.

The increased product shelf life re-

sults from the effect of the modified environment on microbial growth and, for respiring food products such as fruits and vegetables, on the product (Molin, 2000). Low oxygen levels negatively affect aerobic microorganisms. Carbon dioxide inhibits the growth of some microorganisms, however, it is not inhibitory for all microorganisms, and it is important to understand that under modified atmospheres, the growth rates of some microorganisms will be reduced, while others may increase or stay the same.

MAP is not without safety concerns. Beneficial microorganisms may be inhibited, potentially allowing certain pathogens to proliferate and cause foodborne illness. *C. botulinum, L. monocytogenes* and potentially pathogenic psychrotrophs are of primary concern. Psychrotrophs grow well at or below 7 C and have their optimum growth temperature between 20 and 30 C, meaning they can multiply under refrigeration conditions. If the oxygen level of the modified atmosphere is too low, the anaerobic environment could facilitate *C. botulinum* growth. Although studies indicate that many foods become inedible before any toxin is produced (Molin, 2000), foods that remain organoleptically acceptable after toxin production are a significant public health concern. For example, recent studies have shown that *C. botulinum* can grow and produce toxin in products such as MAP pizza and English-style crumpets, while the products remain organoleptically acceptable (Daifas et al., 1999a,b). With respect to meat and fish products, Molin (2000) concluded that most products are judged inedible well before any toxin is produced. However, there have been studies where toxin production in MAP meats preceded or coincided with the development of unacceptable sensory characteristics (Lawlor et al., 2000), as well as reports of botulism due to consumption of vacuum-packaged smoked fish products (Korkeala et al., 1998). *L. monocytogenes* is a problem with products, such as ready-to-eat products, fruits and vegetables, that are not heated adequately before consumption. Low concentrations of CO_2 (less than 10%) used in MAP of some products may inhibit the natural microflora and increase the growth rate of *L. monocytogenes*. Combined with storage at refrigeration temperatures, which can select for *L. monocytogenes*, a low-CO_2 MAP environment may pose a food safety concern. A low partial pressure of carbon dioxide, combined with refrigerated storage, can also favor the growth of *Aeromonas* and the *Enterobacteriaceae*. MAP containing elevated levels of CO_2 (70-100%) inhibits the growth of *L. monocytogenes* in a variety of products (meat products, cottage cheese, turkey roll slices), whereas 100% N_2 allows multiplication of the pathogen (Phillips, 1996). Fresh-cut and whole fruits and vegetables, in particular, do not usually tolerate CO_2 concentrations above 15%, well below the inhibitory level for *L. monocytogenes*. The CO_2 rich atmosphere, however, can select for lactic acid bacteria, which have been shown to be inhibitory towards *L. monocytogenes* (Francis and O'Beirne, 1998).

Active Scavenging Concepts

Active scavenging concepts include oxygen, ethylene and taint scavengers as well as moisture absorbers or humidity controllers.

The presence of oxygen in food packages accelerates the spoilage of many foods. Although vacuum packaging and MAP have been somewhat successful in extending the shelf life and quality of food, aerobic spoilage can still occur because of residual oxygen in the headspace. Oxygen residual remains due to oxygen permeability of the packaging material, small leaks due to improper sealing, air enclosed in the food or inadequate evacuation and/or gas flushing. Oxygen can cause off-flavors, color change, nutrient loss, and growth of microorganisms. Oxygen scavengers are useful for removing residual oxygen and can be applied in different ways: sachets and labels containing oxygen-scavenging components, closures (mainly used for plastic beer bottles), and oxygen-scavenging flexibles. One of the largest applications of oxygen-scavenging systems is mold control in packaged baked goods and cheese food packages.

The majority of current commercially available oxygen scavengers are based on the principle of iron oxidation. Inserting a sachet into the food package is effective, but can meet with resistance among food manufacturers, because of fear of ingestion of the sachet, notwithstanding the labelling, and the potential for the sachet to leak the contents into food, resulting in an adulterated product.

The main advantage of using oxygen scavengers is that they can reduce oxygen levels to less than 0.01%, which is much lower than the typical 0.3-3.0% residual levels achievable with modified atmosphere packaging. As well, oxygen scavengers are sometimes used in combination with carbon dioxide scavenger systems.

In terms of public health, the main issue surrounding the use of oxygen scavengers is their potential to create an environment that may promote the growth of potentially harmful anaerobic bacteria. Similar concerns have been discussed with respect to MAP conditions that result in a low oxygen atmosphere within the package. The main organisms of concern in this situation are *Clostridium* species, mainly *C. botulinum*, but also *C. perfringens*, due to their growth and toxin production in anaerobic environments.

Active Releasing Concepts

Considerable research has focused on the release of antimicrobials from packaging materials to limit microbial spoilage, however the choice of antimicrobial compound is often limited by its compatibility with the packaging material and by the ability to withstand the heat during extrusion (film formation). Examples of such compounds are chlorine dioxide, sulphur dioxide, ethanol, carbon dioxide emitters, antimicrobials (zeolite, triclosan and bacteriocins) and antioxidants (BHT/BHA or vitamin E).

Intelligent Packaging Systems

Intelligent packaging can sense the environment and/or convey information to the user about its contents. Although research in this area has been extensive, only a few technologies have made it past the research and development phase. Two main strategies are employed in the development of a new intelligent packaging system: the use of novel materials and processes, and the application of electronics and MEMS (microelectro-mechanical systems). The first strategy is usually the least costly, but the development and specification process can be time-consuming. Electronic or MEMS technology is costly, but far more versatile and flexible. The technology can be used to micro-fabricate sensors that will detect pressure, acceleration, humidity, temperature, physical damage and exposure to radiation.

Key applications for intelligent technology are in the areas of tamper evidence, quality monitoring (i.e., temperature abuse), counterfeiting (i.e., holograms), theft protection and supply chain management and traceability (i.e., automatic data capture coupled to the Internet). Traceability in the food chain is a high profile issue, because of its importance in determining which foods are part of a potentially contaminated lot. Tracing technology is already being used in many parts of the food chain, however, a number of gaps exist.

An example of quality management packaging is a newly developed method of preparing packaging material for food and other products that contain diagnostic properties. The technology involves immobilizing and protecting antibodies (or other ligands) on the polymer film surface. These substances react with targets—such as food pathogens and toxins, pesticide residues or proteins in the food—and create a visual sign on the film surface, alerting the consumer or retailer that the food may be contaminated. However, this technology would likely only be applicable for surface contamination. Other examples of quality management technologies are temperature indicators, time indicators (aging strip), time/temperature indicators, and microwave cook indicators.

Future Directions and Concerns

From a human health and safety standpoint, one must consider the effects of active packaging on the microbial ecology and safety of foods. As previously discussed, removing oxygen from within packs of high water activity, chilled, perishable food products may stimulate the growth of anaerobic pathogenic bacteria. In addition, the modified atmospheres created with MAP and scavenging technologies may sufficiently change the competitive microbial environment, allowing for pathogen growth. To control undesirable microorganisms on food, antimicrobial substances can be incorporated into the surface of food packaging materials. The major potential food applications for antimicrobial films include meat, fish, poultry, bread, cheese, fruits and vegetables. However, antimicrobial films that only inhibit spoilage microorganisms without affecting the growth of pathogenic bacteria, will raise food safety concerns that must be addressed. A better understanding of the effects that new technologies have on the interrelationship among food, microorganisms and package is required. Questions remain about the by-products of oxygen-scavenging systems, the effectiveness of antimicrobial films on products with irregular surfaces, and the spectrum of activity of the antimicrobial additives, to name a few examples. In addition to improving packaging technology, attention must be given to fully understanding the effects on food quality and safety.

Active and intelligent packaging offer great benefits to both consumers and manufacturers and are undoubtedly one of the areas of future innovation in the food industry. However, there are still concerns regarding the regulatory approval of these technologies. Some of the packaging concepts, such as the active release of antioxidants or antimicrobials, have the potential for these additives to migrate into the food product. Food-contact approval must be established before any form of active packaging is used, and labeling may be needed in cases where active packaging gives rise to consumer confusion. As well, it is important to consider environmental regulations covering disposal of active packaging materials. Public perception of antimicrobial or antioxidant use in food packaging must also be considered. Consumers are seeking more natural foods that are free of contaminants and additives, and their acceptance of packaging that incorporates these substances may be questionable. The use of natural antimicrobials, such as those from plants, and natural antioxidants, such as vitamin E, in food coatings or packaging may be more accepted by consumers.

In the future, we will most likely see different combinations of active and intelligent packaging, e.g., combining antimicrobial films with MAP. Research is needed to see how these new packaging technologies will impact on the spoilage microflora and survival/growth of foodborne pathogens.

With additional research, the combination of active and intelligent packaging technology may emerge as perhaps the most important preservation technology of the 21st century.

Transportation and Storage

Following the harvest of nearly all commodity types, raw foodstuffs are normally transported to holding, shipping, or processing facilities. Even processed foods that have received microbicidal treatment are frequently transported to other locations for bottling, packaging, and/or shipping. Transport conveyances are thus a part of the food chain where contamination can occur.

In 1994, an estimated 224,000 persons developed salmonellosis from a nationally distributed brand of ice cream (Hennessy et al., 1996). *S.* Enteritidis was the cause, and the most likely scenario was that pasteurized ice cream premix was transported by a tanker trailer that had carried non-pasteurized eggs just before being loaded with the premix. Eggs are a known source of *S.* Enteritidis. The authors concluded that to prevent more such occurrences, food products not destined to be re-pasteurized before use should be transported in dedicated containers.

As more and more food commodities are grown and harvested in the United States and abroad, transportation will continue to be a factor in foodborne illness and, in fact, may grow in importance. This is not an easy issue to deal with, as the types of conveyances are as varied as the types of commodities they transport.

The storage of foodstuffs also can be an entry point for pathogenic microorganisms or permit the growth of pathogens if present. It is generally accepted that most foods need to be maintained at cold temperatures from harvest to consumption. This "cold chain" is subject to abuse at several steps, and temperature abuse can contribute to the growth of pathogens that can increase the likelihood of foodborne illness.

Storage of foodstuffs is carried out in warehouses and specialized storage facilities, as well as in virtually all institutions that serve food, including hospitals, nursing homes, schools, restaurants, retail stores and the home. FDA has determined that improper cold holding of food is the most frequent temperature violation for nearly all facility types. For example, in a survey of fast food restaurants, 31% were out of compliance in that potentially hazardous foods were being stored at temperatures above 41 F (FDA, 2000).

The situation is no better, and is probably worse, in the home setting. In a recent survey of homes, 16% were holding refrigerated ingredients at too high a temperature, and 55% of the participants

that were improperly holding cold ingredients did not know at what temperature a refrigerator should hold product. The remaining 45% of participants were unaware that their refrigerators were not holding products at the proper temperatures (Daniels et al., 2000). A previous survey showed that 23% of the 106 households had refrigerators that held food at too high a temperature (Daniels, 1998).

While improper transportation and storage are hardly new or emerging food safety issues, they appear to be problems that do not go away. As such, they will continue to contribute to the burden of foodborne illness. As the food chain becomes even more complex, perhaps their effect will become even greater.

Retail and Food Service

The retail and food service environments are part of the post-harvest environment. As more meals are eaten away from home, this environment becomes increasingly significant. No matter how well or how poorly food safety measures are applied prior to consumption, avoiding illness often depends on how well food is handled immediately prior to consumption.

While the role of human handling in foodborne disease has been recognized for years, recent food safety initiatives have increased our awareness of particular risks. For instance, strong epidemiological evidence supports the transmission of *Salmonella* and *Campylobacter* to ready-to-eat (RTE) food products via cross-contamination with uncooked poultry (D'Aoust, 1989; Deming et al., 1987; Harris et al., 1986; Hopkins et al., 1984). Equally strong evidence exists for the transmission of viral foodborne disease by poor personal hygiene of infected food handlers, with recent data suggesting that 50-95% of confirmed viral foodborne disease outbreaks are attributable to human

Outbreaks of *Shigella sonnei* Infection Associated with Fresh Parsley

In August 1998, the Minnesota Department of Health (MDH) received multiple independent complaints of illness and reports of confirmed *Shigella* infections among persons who had eaten at two restaurants (CDC, 1999c). The restaurants were in different cities, had separate water supplies, and had no employees in common. Preliminary results of interviews with patrons and food handlers at both restaurants suggested that ill food handlers likely played a role in contaminating ice and fresh produce items.

S. sonnei isolates from ill patrons and food handlers at the two restaurants were submitted to MDH for molecular subtyping by pulsed-field gel electrophoresis (PFGE). Results of PFGE demonstrated that both restaurant outbreaks were caused by the same strain of *S. sonnei* and that it was a strain that had not previously been isolated in Minnesota. Strains with similar PFGE patterns had been isolated from travelers returning from Mexico.

Because the outbreaks at the restaurants appeared to have a common source, food histories of restaurant patrons were re-evaluated by food ingredients rather than by menu item. In one restaurant, uncooked chopped parsley was associated with illness (odds ratio 4.3, 95% confidence interval 2.4, 8.0). In the other restaurant, parsley was not associated with illness, but a high proportion of cases ate dishes that were served with chopped parsley. These results suggested that chopped parsley was the likely common source for the two restaurant outbreaks.

In collaboration with CDC, other public health agencies and public health laboratories in the United States and Canada were notified of the outbreaks in Minnesota. Six similar outbreaks that occurred during July-August were identified, two in California, and one each in the states of Massachusetts and Florida and the provinces of Alberta and Ontario. *S. sonnei* isolates were available from five of these six outbreaks. All had the same PFGE patterns seen in the Minnesota outbreaks. In each, chopped parsley was sprinkled on foods that were either implicated by the results of a formal investigation, or eaten by a high proportion of cases. Thus, in simultaneous outbreaks linked by PFGE subtype, a common food item was implicated.

Tracebacks to determine the sources of parsley in the outbreaks linked by PFGE were conducted by state and local health officials, FDA, and the Canadian Food Inspection Agency. One farm, in Baja California, Mexico was identified as the likely source of parsley served in six of the seven outbreaks. Field investigations at the farm found that municipal water used for chilling the freshly picked parsley was unchlorinated and vulnerable to contamination. This water also was used to make the ice with which the parsley was packed for shipping.

The widespread distribution of the outbreaks linked to this parsley source suggested that the parsley was contaminated in the field or during packing. However, results of these investigations also suggested that handling of the parsley at the restaurants contributed to the occurrence of the outbreaks. Food handlers at six of the eight implicated restaurants reported washing the parsley before chopping it. The parsley was usually chopped in the morning and left at room temperature during the day before being served to customers. Studies at the University of Georgia Center for Food Safety demonstrated that *S. sonnei* decreased by 1-log CFU/g per week on refrigerated parsley, but increased by 3-log CFU/g in 24 hours on chopped parsley at room temperature. In addition, in at least two of the restaurants, food handlers became infected with the outbreak strain of *S. sonnei* and appeared to have contributed to ongoing transmission in those outbreaks.

These outbreaks demonstrate the complexity of foodborne disease transmission and outbreak investigations. A contaminated food ingredient was widely distributed, contamination was amplified by handling practices in some restaurants, and infected food handlers further amplified the outbreak by contaminating ice and other ready-to-eat foods. Only by linking this series of apparently unrelated outbreaks by PFGE subtyping of the agent, were public health officials able to identify the common source and the other contributing factors.

handling (Bean et al., 1997). Another particularly hazardous behavior is the consumption of high-risk foods (predominantly raw or undercooked foods of animal origin) (Beletshachew et al., 2000; Klontz et al., 1995).

While all of these behaviors can occur at home, institutions and retail establishments are more significant venues with respect to large, recognized foodborne disease outbreaks. That is, single illnesses due to unsafe food handling at home are unlikely to be attributed to food and to be reported, even though home food handling is an important cause of foodborne disease. Poor handling practices are influenced by a large number of demographic factors including age, gender, race, education and income (Beletshachew et al., 2000; Klontz et al., 1995). While targeted food safety education programs have reported some success, they are only one component of a larger initiative to inform and motivate food handlers about food safety (Meer and Misner, 2000; Yang et al., 2000).

Recent outbreaks of viral gastroenteritis illustrate some new discoveries regarding the significance of the food handler in the initiation and propagation of outbreaks. Parashar et al. (1998) reported on the role of an asymptomatic food handler in a viral gastroenteritis outbreak associated with the consumption of contaminated sandwiches. Researchers discovered that NLVs could be shed in the feces for up to 10 days after diarrhea ended in the food worker or by asymptomatic food handlers, and, through poor personal hygiene, subsequently contaminate food. Green et al. (1999), in describing a prolonged viral gastroenteritis outbreak at a large hotel, noted that toilet rims (72%) and carpets (70%) had a high incidence of contamination. These environmental surfaces remained important reservoirs for the propagation of the outbreak. In perhaps the most interesting study, Becker et al. (2000) reported a primary foodborne NLV outbreak associated with the consumption of boxed lunches that were served to a North Carolina college football team. During a subsequent game in Florida the next day, many members of the North Carolina team developed diarrhea and vomiting. Twenty-four hours later, similar symptoms developed in some of the opposing team members, illustrating the role of direct person-to-person transmission in the propagation of a primary foodborne viral disease outbreak.

Microbial Stress Responses to Processing

Human efforts to control microorganisms in the food production, processing and distribution systems have changed the environment for foodborne pathogens. As the scientific understanding of foodborne pathogens has become more sophisticated, so too have the control methods. These control efforts are one of many driving forces in pathogen evolution, and as such, their impact on the virulence and survival of foodborne pathogens must be fully considered (Archer, 1996). Scientists study the effect of processing technologies and other changes to the microbial environment to evaluate the effectiveness of control technologies and also the potential that control efforts will drive pathogen evolution.

The many varied processes used to prepare and preserve the wide range of foods are frequently designed to inactivate, inhibit or prevent the growth of pathogenic or spoilage microorganisms in the specific product. Any of these microorganisms that survive the process may be damaged. Consequently, process evaluations and the microbial inactivation kinetics on which they are based must consider sublethal injury of cells and spores, as well as resuscitation of cells and alternative germination pathways of spores. These considerations must be built into hazard analysis and risk assessment to adequately assess and control emerging food safety situations.

Resistance to Controls

The efficacy of a preservation technology is influenced by a number of microorganism-related factors that are generally independent of the technology itself. These factors include the type and form of the target microorganism; the genus, species and strain of microorganism; its growth stage; selection by environmental stresses; and sublethal injury.

Among the foodborne microbiological hazards, bacteria are generally the primary targets for most preservation processes, and bacterial susceptibility to sublethal cellular injury is of special concern. Processes designed to inactivate pathogens also must address the resistance properties in foods of other microorganisms—such as viruses, yeasts, molds, and parasites—that may persist or grow in foods even if they do not experience sublethal injury. The activity of the entire microbial ecosystem influences the survival and subsequent pathogenicity of the target cells whether or not the target microorganism has been sublethally damaged.

A few genera of foodborne bacteria (for example, *Clostridium* spp. and *Bacillus* spp.) are capable of existing in two forms: active vegetative cells and dormant spores. These two forms often differ in their resistance properties to heat, chemicals, irradiation and other environmental stresses. Similarly, spores are typically more resistant than vegetative cells to the alternative processing technologies. Pasteurization inactivates vegetative cells of disease-producing microorganisms. To have a commercially sterile product, however, the process must inactivate all microbial spores (usually targeting spores of *C. botulinum*) that are capable of germinating and growing in the food under normal storage conditions.

Differences in microbial resistance to control methods may be found not only between genera and species but also between strains of the same species. For instance, at the genus level, some bacterial strains with unique resistance to thermal inactivation, irradiation, and high pressure processing have been identified, such as *D. radiodurans* and the thermoplasmals, making it possible that, in the future, a pathogenic "super bug" could emerge. Within species, some strains of common enteric pathogens such as *E. coli* and *Salmonella* are more resistant to the effects of low pH and high temperature than other strains of the same organism. If a bacterial strain with resistance to multiple control technologies emerged, it could be a potential food safety hazard that would be uncontrollable with technologies that have produced safe products for generations. If the microorganisms proved to be pathogenic, the control process would have to be redesigned to specifically inactivate it. Alternatively, if the "super bug" were not a pathogen or spoilage microorganism, it might be very useful as a possible surrogate during process development and validation.

Another factor that can affect bacterial resistance to preservation processes is the stage of growth. Cells in exponential or log phase of growth are generally less resistant than cells in stationary

phase. The development of stress resistance proteins in stationary phase is a contributing factor to this phenomenon.

Selection by Environmental Stresses

Extreme environments usually kill most bacterial cells and can result in the selection of mutant cells that are resistant to the severe conditions (see selection, p. 21). Studies have suggested that bacterial stress may induce hypermutability, which would in turn lead to a microbial population of greater resistance (Buchanan, 1997a). Therefore, the exposure of cells to some form of stress may induce and allow the survival of microorganisms with unusually high durability to a given inactivation process.

The responses to stresses in the food system may play a major role in the emergence of pathogens (Sheridan and McDowell, 1998). Bacteria are capable of adapting to an immediate environmental stress, but the response is temporary, and the genes involved are switched off when the stress is removed (see stress, p. 22). The stress response may be triggered by a single parameter or by several simultaneously, causing variations in response.

Some stress responses have particular relevance to the food processing environment. For example, stress responses to temperature shifts can affect *L. monocytogenes* attachment to food contact surfaces (Smoot and Pierson, 1998).

The cross protective effect in which exposure to one stress triggers resistance to other stresses is a special concern in food processing environments. Mazotta (1999) found that the heat resistance of acid- or salt-adapted, heat-shocked, or starved *E. coli* O157:H7 cells was higher than that of cells grown to exponential or stationary phase under optimum conditions. To add an extra safety factor, Mazotta suggested using stress-inducing culture conditions when studying the thermal resistance of vegetative pathogens in specific products. The cross protective effects of the bacterial stress response vary. Lou and Yousef (1997) determined that sublethal stresses to ethanol, acid, hydrogen peroxide, heat, or salt had variable effects on subsequent exposure of *L. monocytogenes* to normally lethal levels of the same stressors. For example, heat shocking increased the resistance of the microorganism to ethanol, hydrogen peroxide and salt, but not to acid. Similarly, resistance to food antimicrobials can be acquired through previous exposure or through cross protection triggered by environmental or processing factors including stresses such as heat or acid (Davidson, 1999). Some researchers have attempted to differentiate various multiple stresses, e.g., lethal pH and water activity. Shadbolt et al. (2001) hypothesized that pH-induced stress causes an energy drain that sensitizes the cell to other environmental constraints. Similarly, the effect of habitual exposure to reduced water activity increased the heat tolerance of *Salmonella* spp. (Mattick et al., 2000). Another example of cross protection is the increased radiation resistance caused by the induction of acid resistance in enterohemorrhagic *E. coli* (Buchanan et al., 1999).

Because environmental stress can increase a pathogen's resistance to control technologies, the potential for increased resistance must be factored into the process. The analysis should consider: (1) if the food environments are likely to induce stress conditions in the microorganisms; (2) whether stress-induced resistance could possibly occur at any point in the food processing operation; and (3) if it did, whether it would significantly impact the inactivation process and lead to possible underprocessing. Considering that most food processing systems are designed to expose microorganisms only once to any given stress-inducing factor (for example, heat, acid, or antimicrobials), a resistant population is unlikely to develop. However, it is possible that sublethal injury to *E. coli* O157:H7 as it passes through the low pH of a cow's gastrointestinal tract and then through an acid rinse at slaughter may change its resistance to heat inactivation during preparation. Likewise, *L. monocytogenes* cells in the production environment that are sublethally injured by repeated exposure to sanitizers may have altered survival or virulence characteristics. An additional exception is previously processed material that is reintroduced into the process stream. In this case, in-depth studies of the impact of processing-induced stress are needed.

Sublethal Injury

The microbial ecology of food is influenced dramatically by food processing and preservation technologies. Whether or not the actions are directly or indirectly aimed at microorganisms, many actions within the food processing system—decontamination, sanitation, and product formulation and preservation—can injure cells that survive the event (see Table 11).

Some of these processes are classic and well established as effective against pathogens that have existed in the past, but new product formulations, new equipment, or modified regimens can create opportunities for some microorganisms to survive, frequently in a sublethally injured state. New processes also have the potential to create sublethally injured pathogens.

It would be a rare genus of bacteria of concern in the food industry that has not in some situation demonstrated the capacity to be sublethally stressed or injured by a food-related physical or chemical insult. Many yeasts and molds associated with food also have shown susceptibility to sublethal damage. Early in the study of cell injury, lactic acid bacteria used as starter cultures for dairy fermentations were shown to be cold-damaged, requiring special nutrients for normal rapid growth.

Sublethal injury may be demonstrated or exhibited as more exacting requirements for growth, greater sensitivities to antagonistic agents (e.g., selective agents in media or preservatives in a food), increased resistance to subsequent inactivation treatments by the same or different agents, increased lag time before exponential growth ensues, or changed virulence as a pathogenic cell. The injured cell may return to its initial native state by repairing the cellular damage under suitable conditions. This resuscitation in the absence of antagonistic agents or in the presence of appropriate substrates will result in a cell with its original capabilities, resistances, and virulence. In other words, the damage of sublethal injury is reversible—it is not a permanent genetic change. This phenomenon is different from selection of a resistant mutant with a permanent genetic change.

The effectiveness of a microbial inactivation process is often measured by enumerating any surviving organisms in a selective medium. Because viability in microorganisms is generally based on the ability to increase in numbers to some measurable level, death may be defined as an irreversible loss of the ability to generate progeny (Mackey, 2000). Ideally, a microorganism exposed to processing conditions would be either viable or dead; in actual practice, the control technology of-

Table 11. Conditions That Can Produce Sublethally Injured Cells (Ray, 1989)

Environmental Stress	Processing Parameter
Moderate heat	Pasteurization Concentration
Low temperature	Refrigeration/chilling Freezing
Low water activity	Dehydration High solutes (salt, sugar)
Radiation	X-rays Gamma rays Ultraviolet rays
Low pH	Organic or inorganic acids
Preservatives	Sorbate Benzoate
Sanitizers	Chlorine Quaternary ammonium compounds Short-chain fatty acids Peroxyacetic acid
Pressure	High hydrostatic pressure
Electric fields	Pulsed electric fields
Nutrient deficiencies	Very clean surfaces

ten produces a continuum of effects including some degree of cellular injury.

Injured cells can be easily underestimated, resulting in misleading conclusions about the efficiency of the inactivation method. If the cell damage is not recognized, the loss of specific identifiable characteristics as a result of sublethal injury leads to faulty data. Injury occurs in vegetative cells and bacterial endospores of pathogenic and nonpathogenic microorganisms. A cell that appears to be "dead" because it is unable to multiply and demonstrate its viability may be able to repair itself under some special circumstances. So a food, process, or other microbial environment could appear to be pathogen-free only to become dangerous when the injured cells recover. Mackey (2000) has described the nature of sublethal injury, emphasizing the many conditions that influence injury, resuscitation, and recovery, and highlighting the role that sublethal injury may play in the design of preservation processes.

Another irregular or unnatural state or condition that microorganisms may enter is the viable (or metabolically active) but nonculturable (VBNC) condition in response to stress. These microorganisms lose their ability to grow in even nonselective environments that normally support their growth but are considered still viable because the cells remain physically intact and demonstrate metabolic activity. The VBNC state of microbial cells has been reported to occur in a number of foodborne pathogens as an outcome of environmental stress (McKay, 1992; Xu et al., 1982). The possibility exists that these VBNC cells, which are non-detectable by traditional cultural methods, may cause disease if consumed (Colwell et al., 1985). If they are developed in a food production environment, a food safety risk may be presented. The very existence of the VBNC state has been questioned, based on studying the concept from alternative perspectives (Bloomfield et al., 1998; McDougald et al., 1998). New approaches to studying the VBNC phenomenon need to be taken, to arrive at an agreement regarding its occurrence, and then to determine if these microorganisms have any importance in the food production environment and to food safety and the public health.

Future Implications

The responses to stresses in the food system may play a major role in the emergence of pathogens (Sheridan and McDowell, 1998). Stress responses may increase the pathogen's resistance to inactivation methods, improve its ability to survive in the food processing environment, and enhance its ability to cause illness when consumed by humans.

Scientists are studying the response of cells and spores to multiple stress treatments in an attempt to develop new and better control systems for specific situations. Combined treatment methods take advantage of stress-induced microbial weaknesses. For example, pressure-damaged *E. coli* are more sensitive to acid than native cells (Pagan et al., 2001). Addition of 5% ethanol enhances inactivation by organic acids and osmotic stress (Barker and Park, 2001). On the other hand, cross protection from microbial stress responses must be considered when evaluating treatment effectiveness. Growth under low a_w conditions increases the heat resistance of *Salmonella* spp. (Mattick et al., 2000). Combined approaches are not new, nor are they limited to vegetative cells. Apparent inactivation and control of heat-damaged *C. botulinum* spores was considerably less in food that contained lysozyme (Peck and Fernandez, 1995).

As scientists continue to improve their understanding of microbial stress responses, it is increasingly possible to try to anticipate potential stress-related problems in the food processing environment. The food industry is adopting or considering a variety of methods for sanitizing beef, pork, lamb, and poultry carcasses and reducing or eliminating pathogens in meat products (Mermelstein, 2001). Whether it is steam pasteurization, rinsing with various antimicrobials, e-beam or x-ray treatment, high pressure processing, or some yet to be identified procedure, it is essential that the stress-induced responses be considered.

The same considerations are essential for the decontamination procedures used with fresh and fresh-cut fruits and vegetables. High pressure processing of orange juice (Zook et al., 1999), pulsed electric field treatment of orange-carrot juice (Rodrigo et al., 2001), UVB treatment of surface waters (Obiri-Danso et al., 2001), sanitizer treatment of *Salmonella* attached to apples (Liao and Sapers, 2000), and disinfectants killing *Alicyclobacillus acidoterrestris* spores prior to pasteurizing fruit juices (Orr and Beuchat, 2000) are only a few examples of possibilities for the future. If sublethal injury were to be ignored in these very promising food safety developments, new food safety problems may occur. These concerns also apply to multiple or combination processes that are intended to benefit from additive or synergistic effects. For example, pulsed high pressure processing with modest elevated temperatures appears to inactivate spores with high heat resistance (Meyer et al., 2000).

New Tools for Pathogen Research

New tools are needed to more easily and specifically monitor populations of

pathogens in the post-harvest stages of food production. Although much of the emphasis in microbiological methods development traditionally has been on pathogen detection in the food, it is critical to develop better ways of monitoring the food processing environment, so that the factors that influence food contamination may be understood. This understanding is a necessary step in the development of rational control strategies.

Traditional identification methods based on microbiological culture are time-consuming, prohibiting scientists from accumulating the large amounts of data needed to develop an understanding of the ecology of pathogens in the food production environment. The commercial market has provided no shortage of rapid method "kits," which employ either immunochemical or nucleic acid-based detection technology, but they are expensive and must be validated for each particular application.

Research on biofilms, a persistent problem in the food plant environment (Wong, 1998), is slow because the unique methods and instrumentation needed for their study are not widely available (Zottola, 1997), and because food microbiologists generally have not been trained in these techniques. Biofilms are a growing colony or mass of bacterial cells, attached to each other and a surface, that entrap debris, nutrients, and other microorganisms (Zottola, 1994). To research such issues, food microbiologists must adopt more tools that are traditionally the province of the microbial ecologist.

Although many traditional culture methods are available and many rapid methods have been commercialized for screening/detection of foodborne pathogens (FDA, 1998), the options are much more limited if a quantitative determination is desired. Sensitive quantitative methods are necessary for assessing pathogen growth, survival and inactivation, as well as for accurate risk assessment. Specific quantitative determinations are traditionally obtained by the most probable number (MPN) technique, a sample dilution technique for statistically estimating the number of microorganisms, or if background populations are not too intrusive, by direct plating (FDA, 1998). The MPN technique is the most sensitive, because it provides enrichment conditions that allow for the recovery of many of the injured cells (see sublethal injury, p. 63). In direct plating, the sample is placed directly on or in selective media, which may inhibit the growth of the injured cells. The MPN technique, however, is extremely labor intensive. Molecular techniques, such as the coupling of immunochemical or nucleic acid-based assays to the MPN, or nucleic acid probe hybridizations of colony blots (de Blackburn and McCarthy, 2000; FDA, 1998; Miwa et al., 1998), are increasingly being applied to traditional methods for obtaining results more quickly. PCR, originally developed for screening/detection applications, has been modified for use as a quantitative assay (Nogva et al., 2000). Recombinant bioluminescent strains of food pathogens have been used to quantitatively assess survival in foods and the food processing environment by measurement of light emission (Ramsaran, et al., 1998; Siragusa et al., 1999). These techniques and others need to be further developed, refined and validated as our need for quantitative information on pathogens increases.

The development of genetic fingerprinting techniques has provided the ability to finely discriminate strains within a species of pathogen. Several variations of fingerprinting techniques are available (Farber et al., 2001), and new ones continue to be developed. Genetic fingerprinting has been extremely useful in epidemiological work and identification of foodborne illness outbreaks (e.g., PulseNet) (CDC, 1999d). The technology has begun to be applied to the study of pathogens in the food plant environment (Norton et al., 2001). This novel approach allows questions about pathogen evolution, persistence and routes of contamination in the food plant to be examined.

New tools also need to be developed and applied for assessing stress and injury of pathogens in the food production environment. Sublethally injured cells may have different survival characteristics during food storage or after consumption, and may show greater resistance to control measures and heightened virulence than the original population (Abee and Wouters, 1999; Bower and Daeschel, 1999; Gahan and Hill, 1999). If the injured cells are not detectable by the method chosen, the safety of a food process may be assumed, when, in fact, a food safety risk may be present. Conventional methods for determining microbiological injury generally involve plating on two types of media, i.e., selective and non-selective; the rationale being that injured cells cannot survive selective culture and can grow only on non-selective media, whereas healthy cells can grow on both types of media (Ray, 1979). This strategy is somewhat imprecise; with each additional selective agent incorporated into the medium, greater percentages of injury in the population can be revealed, indicating the presence of different subpopulations of cells having varying degrees or different types of injury. Molecular methods of analysis, in addition to those based on culture, can provide useful insights for assessment of injury and viability of pathogen cells. Molecular probes of cellular functions, e.g. membrane permeability, respiratory electron transport, membrane esterase activity, etc., may be useful for broadly categorizing types of cellular injury (Breeuwer and Abee, 2000; McFeters et al., 1995; Porter et al., 1995). Expression assays that build on technologies such as the reverse transcription (RT)-PCR (Sheridan et al., 1998), the nucleic acid sequence-based amplification (Blais et al., 1997; Simpkins et al., 2000), and use of reporter gene constructs (Cha et al., 1999; Stewart et al., 1997) may be useful for studying the expression of genes known to be associated with stress. However, because we do not know what all of these genes may be, the development of new tools, such as genomic microarrays (Graves, 1999) for food pathogens, is needed. A genomic microarray or "gene chip" could be, for example, an ordered set of all of the known genes of a particular microorganism, deposited in precise locations on a small solid surface. A promising use for genomic microarrays is in expression analysis, in which changes in the pattern of expression of thousands of genes can be studied simultaneously, known as "functional genomics" (Oh and Liao, 2000; Tao et al., 1999). Studying the effect of a particular stress on a pathogen, then, no longer needs be limited to expression of one or a few stress response genes. Functional genomics will become an invaluable tool for understanding and monitoring stress responses in the food production and processing environment.

Ability of Pathogens To Survive in the Environment

The Vaudois University Hospital Medical Center in Lausanne, Switzerland, generally diagnoses a mean of three listeriosis cases per year. However, a cluster of 25 listeriosis cases (14 adults and 11 maternal/fetal) was observed at the same medical facility between January 1983 and March 1984, with 15 additional cases diagnosed at surrounding hospitals (Bille, 1988; Malinverni et al., 1986).

Epidemiology

This epidemic appeared atypical because of the high number of healthy, immunocompetent individuals affected, the high rate of brain-stem encephalitis, and a mortality rate of 45%. The organisms isolated from clinical specimens from thirty-eight of the 40 patients involved in the outbreak were serotype 4b. A high number (92%) of the *L. monocytogenes* serotype 4b cultures were of two unique phage types, compared with only 44% of the serotype 4b cultures obtained during the previous 6 years. This evidence indicated the outbreak might be traced to a single source. A thorough case study did not identify the source or mode of *Listeria* transmission, however, public health officials initiated a prospective case-control study, assuming that a similar listeriosis outbreak was likely the following winter. Overall, 16 additional cases were identified between November 1984 and April 1985.

After a 1985 listeriosis outbreak in California was linked to consumption of Mexican-style cheese, Swiss officials initiated a baseline study to detect *Listeria* spp. in a variety of dairy products. While surveying soft, semi-hard, and hard cheeses, *L. monocytogenes* was isolated from five of 25 surface samples of Vacherin Mont d'Or, a soft smear-ripened cheese manufactured from October to March and consumed primarily in the outbreak region. Furthermore, all five isolates belonged to serotype 4b, and two of the phage types isolated from the cheese were identical to most clinical strains isolated during the 1983-1986 epidemic period.

In 1987, a third case-control study demonstrated that 31 of 37 individuals who became ill had consumed Vacherin Mont d'Or cheese, as compared with only 20 of 51 people in the control group. Investigators isolated the epidemic strain of *L. monocytogenes* from a piece of Vacherin Mont d'Or cheese that had been partially consumed by one of the victims. Therefore, Swiss officials halted production of the cheese and recalled the product throughout Switzerland. Between 1983 and 1987, a total of 122 cases of listeriosis resulting in 34 deaths were recorded in the western part of Switzerland.

Several years following the outbreak, the isolates were further typed using a variety of new methods, and the clinical and cheese isolates were identical. The epidemic strain had the same phage type, enzyme type, ribotype and PFGE type as strains isolated during the 1985 listeriosis outbreak in California.

Management

Immediately following the recall, Swiss officials began investigating how the cheese could have become contaminated. The cheese implicated in this outbreak was produced at 40 different factories located in western Switzerland, and all contaminated cheese was reportedly prepared from *Listeria*-free bovine milk. After coagulating the milk, the resulting curd was dipped into wooden hoops and allowed to drain for 1-2 days. When drained, the cheese was transported to one of 12 cellars located throughout the area and ripened for 3 weeks on wooden shelves, during which time the cheeses were turned daily and brushed with salt water. Once ripened, they were packaged and returned to the cheese factory. To validate suspicions that the contamination occurred during ripening, investigators took samples from the wooden shelves, brushes and the surface rind of the cheese. Analysis of these samples revealed fairly high levels of *L. monocytogenes* (10,000 to 1,000,000 bacteria). Further investigation showed that almost half of the 12 ripening cellars were contaminated with one or both epidemic strains of *L. monocytogenes*, suggesting cellar-to-cellar spread of the pathogen, which would explain why cheeses in all 40 plants were contaminated. Although first detected in 1983, investigators speculated that the outbreak began several years earlier, based on evidence that the epidemic strain had been isolated from a listeriosis victim in 1977. Thus, this particular strain of *L. monocytogenes* had established itself and survived in the factories and/or cellars of various plants for up to 10 years.

All 40 factories and 12 cellars were thoroughly cleaned and sanitized. The wood from the ripening cellars was removed and burned, and the cellars were refitted with metal shelves. Examination of experimental batches of the cheese produced over a 2-month period indicated the cleanup effort was successful.

It is evident that *L. monocytogenes* can adapt to different plant conditions and persist in the manufacturing environment for months and even years. From these environmental niches/biofilms, the organism can find its way into finished product, and cause sporadic cases or outbreaks of foodborne listeriosis. As a part of an overall control strategy, aggressive environmental and product monitoring is needed.

Application of Science to Food Safety Management

Effective application of our current scientific knowledge is a vital part of continuing efforts to improve food safety. Our food safety policies are based on the best scientific information available at the time they were created, but our knowledge continues to improve. Flexible, science-based policies should incorporate new information as it becomes available. Everyone benefits from science-based policies, although factors such as economic impact and other trade-offs are part of the policy-making environment as well. Ideally, food policy will draw on a variety of science-based tools and be structured to provide the flexibility to apply these tools as science dictates.

The final decades of the twentieth century saw tremendous change in our knowledge of pathogenic microorganisms, their toxins and their metabolites. Scientists identified a wide array of microbial hazards, and foods previously thought to be safe were found to be important vehicles of foodborne disease. This new knowledge resulted in new policies to protect consumers. This scenario will continue into the new century.

Unfortunately, current systems cannot deliver a risk-free food supply. The scientific knowledge, technology and equipment are not available to eliminate all microbial hazards from all foods. This vulnerability will continue to influence regulatory policy as regulatory agencies and industry seek solutions to these hazards. The primary weakness in today's control system is the presence of enteric pathogens on raw agricultural commodities (e.g., meat, poultry, milk, eggs, fruits, vegetables, and seafood).

As a result, an essential part of science-based policies for food safety and public health protection is risk analysis. Risk analysis is generally considered to have three components: risk assessment, risk management, and risk communication. New scientific tools can provide ways to assess risk, enabling decisions to be based on data and fact. These decisions are not easy, but risk assessment gives us the basis for managing these risks in an informed, intelligent way. An effective food safety system integrates science and risk analysis at all levels of the system, including food safety research, information and technology transfer, and consumer education.

Risk Assessment

Broadly defined, risk assessment is the use of scientific data to identify, characterize, and measure hazards; assess exposure; and characterize the risks involved with a food. However, risk assessments do not specifically determine whether a product is "safe" or "unsafe."

In terms of foodborne illness, risk is a function of the probability of an adverse health effect and the severity of that effect. In other words, risk is a measure of the likelihood that illness will occur within a population as a result of a hazard in food and the severity of that illness (Buchanan, 1997b).

When used in food safety regulation or policy development, risk assessment reflects the expected impact of a particular food safety problem, the expected impact of protective mitigation measures, and the levels of urgency and controversy surrounding an issue. Risk assessment has long been applied to assessing risks associated with chemical exposure but only recently has it been formally applied to foodborne pathogens. Therefore, a specific format for these risk assessments is still in the definition process; currently used formats vary depending upon the scope and objective of the assessment.

Risk assessments also play an important role in international trade by ensuring that countries establish food safety requirements that are scientifically sound and by providing a means for determining equivalent levels of public health protection between countries. Without systematic risk assessment, countries could set requirements unrelated to food safety, creating artificial barriers to trade. Recognizing the importance of this science-based approach to fair trade, the World Trade Organization requires each country's food safety measures to be based on risk assessment. The Codex Alimentarius Commission (Codex), which establishes international food safety standards, has developed principles and guidelines for conducting risk assessments (CAC, 1999).

Regardless of the specific format, a risk assessment has four main components:

- Hazard identification involves identifying the hazard (e.g., pathogen), the nature of the hazard, known or potential health effects associated with the hazard, and the individuals at risk from the hazard.
- Exposure assessment describes the exposure pathways and considers the likely frequency and level of intake of food contaminated with the hazard.
- Hazard characterization explores the relationship between the exposure level and the nature of the adverse effects, considering both frequency and severity.
- Risk characterization identifies the likelihood that a population of individuals would experience an adverse health outcome from exposure to the food that might contain the pathogen. The risk characterization also describes the variability and uncertainty of the risk and identifies data gaps in the assessment.

These same four components are considered in both quantitative and qualitative risk assessments. Qualitative risk assessments may be chosen to identify, describe, and rank hazards associated with a food. Quantitative risk assessments may be chosen when substantive scientific data are available for analysis,

and these risk assessments almost always yield a numerical expression of risk.

Qualitative Risk Assessment

Qualitative risk assessment is generally considered a valuable method to determine which hazards are associated with a particular food. This process is often used by an expert panel. Also, qualitative assessments are useful when many gaps in the available data limit the precision necessary for a quantitative risk assessment. For instance, scientists often have little exact information about the relationship between the quantity of pathogen ingested and resulting frequency and severity of adverse health effects, especially for susceptible subpopulations. Information about exposure—the probability of contamination, the extent of pathogen growth in the food, and the amount of the food consumed by various populations—is sometimes limited. Qualitative risk assessments can be useful in identifying these data gaps and in targeting or prioritizing research that would have the greatest public health impact.

Qualitative risk assessments have numerous applications in food safety analysis. These assessments can help companies develop more effective Hazard Analysis Critical Control Points (HACCP) plans based on scientific data. For instance, a qualitative risk assessment can help identify likely hazards, although the result might only be to rank a hazard as high, medium, or low in terms of prevalence or potential contamination level. The assessment will have an increased power to inform decision-makers when the risk of an adverse health effect can also be described, at least qualitatively as high, medium or low.

A significant disadvantage of qualitative risk assessments is the inability to compare the extent to which particular mitigating factors or risk management options can successfully reduce risk. A qualitative risk assessment can compare two options that both address the exposure to a hazard—e.g., two different disinfecting agents—or it can compare two options that address the effect—e.g., two methods to prevent susceptible individuals from consuming the food. However, qualitative risk assessments present difficulties when trying to compare one option that addresses only exposure and another option that addresses only effect.

Notwithstanding the disadvantages of qualitative risk assessments, they are frequently used and serve a purpose in science-based food safety management. Qualitative risk assessment will continue to play a role in the future when time and money constraints prohibit a full quantitative risk assessment. However, scientists must develop a better understanding of the exact role that qualitative risk assessments can play and a better overall defined structure for these risk assessments.

Quantitative Risk Assessment

Quantitative risk assessment is formally defined as the technical assessment of the nature and magnitude of a risk caused by a hazard. The technique, first developed in the 1950s to evaluate nuclear proliferation risks, has since been used to evaluate the toxicological risks to plants, animals, and public health posed by chemical exposure and more recently in microbiological food safety evaluation.

As the name implies, quantitative risk assessment ultimately provides numerical estimates of risk that can be used in regulatory decision-making and risk management. Quantitative microbial risk assessment as a discipline has been under scrutiny recently, purportedly because of a lack of rigor and precision. Because the process inherently depends upon the input of many scientists from frequently diverse disciplines, as well as the incorporation of assumptions when definitive data are lacking, some individuals believe that the process is little more than an academic exercise. Of all the limitations in the current risk assessment methodology (Jaykus, 1996), the need for more and better data is the most pressing.

After the hazard identification phase is complete, the exposure assessment estimates or directly measures the quantities or concentrations of risk agents received by individuals or populations. As such, exposure assessment is designed to characterize the circumstances, source, magnitude, and duration of exposure, the final goal of which is to produce a mathematical expression for exposure, generally in the form of the probability of ingestion of the infectious agent through the food vehicle of interest. Common data sources include survey information on the prevalence and levels of contamination for a particular pathogen in a particular food commodity. Scenario analysis using various computer software packages is frequently done to model the many circumstances that may surround exposure. And since foodborne pathogen growth, inactivation and survival are highly dependent upon intrinsic (food-related) and extrinsic (environmental) factors such as relative humidity and storage temperature, mathematical models such as USDA's Pathogen Modeling Program can be used to estimate the effect of these factors on microbial persistence and levels in foods.

The relationship between the ingestion of pathogenic microorganisms and possible health effects may be described as the quantitative relationship between the intensity of exposure (dose) and the frequency of the occurrence of illness within the exposed population of hosts (response). For pathogenic microorganisms, this is dependent upon the number of units of infectious agent ingested in the food, the infectivity and pathogenicity of the infectious agent, and the vulnerability of the host. The purpose of the hazard characterization step is to quantify or statistically describe the relationship between the risk agent and the magnitude of the adverse effect. Included in this step is a full description of the severity and duration of adverse effects that may result from the ingestion of a microorganism or toxin in a food. The source of data used for hazard characterization is usually human challenge studies, whereby a defined population of consenting adults is given various doses of the infectious agent, and their response is measured as infection or disease. For pathogens causing mild disease, this is feasible; for those associated with more severe diseases, or affecting particularly susceptible populations, risk assessors must rely on animal models of disease or other data sources. The raw input data from these types of studies is used in conjunction with statistical methods that describe the dose-response relationship in mathematical terms. Some frequently used dose-response models include the Beta-Poisson, Exponential, and Gamma-Weibull models.

Risk characterization is the final step of risk assessment and represents the integration of the exposure assessment and hazard characterization to obtain a risk estimate of the likelihood and severity of the adverse effects that would occur in a given population. The final risk estimate should incorporate information about the variability, uncertainty and assumptions

identified in all previous steps of the risk assessment. Statistical methods are used to characterize variability associated with a well-characterized phenomenon, while Monte Carlo and Bayesian approaches can be applied in an effort to better characterize uncertainty associated with a poorly characterized phenomenon. Sensitivity analysis is frequently done to better understand the contribution of the individual factors that influence the overall risk estimate. Because the risk assessment process usually involves extensive data input, the use of various mathematical modeling approaches, and some degree of assumption on the part of the assessors, risk assessment teams now seek to provide "transparent" documents that give the reader a full and detailed description of the process.

At present, the first reports of microbial risk assessment in food safety have appeared in the scientific literature, and several regulatory agency-driven risk assessments have been completed in the United States during the last 5 years: the USDA/FDA *Salmonella* Enteritidis risk assessment for shell eggs and egg products (USDA/FSIS, 1998a); the USDA/FSIS risk assessment for *Escherichia coli* O157:H7 in beef and ground beef (USDA/FSIS, 1998b); the FDA draft assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat (RTE) foods (FDA/CFSAN, USDA/FSIS, and CDC, 2001); and the FDA draft assessment on the public health impact of *Vibrio parahaemolyticus* in raw molluscan shellfish (FDA/CFSAN, 2001). Other risk assessments have been completed by the Canadian Food Inspection Agency. Despite these very significant efforts, the application of quantitative risk assessment techniques to microbiological foodborne hazards is still in its relative infancy.

Quantitative risk assessment provides a formal, conceptual framework for the evaluation of foodborne disease risks, one that effectively uses all available information and expertise. Prior to the advent of this discipline, decisions about food safety regulation and management were much less systematic. Furthermore, recent risk assessments provide a clear picture of the role of uncertainty in overall risk modeling, using some of the more robust methods, such as Monte Carlo simulation, to characterize uncertainty. Finally, risk assessment is, by nature, an iterative process; if the models are properly constructed, they can be readily updated as additional information becomes available.

Role of Expert Panels

The process used by expert panels is very similar to that for qualitative risk assessment. The major difference is that expert panels, in general, collect and review available information and develop guidance or recommendations for use by government and/or industry. Thus, specific risk management options are an expected outcome from the expert panel process. In addition, the time frame for an expert panel would normally be in days or perhaps several months, the extended time being used to prepare the report of the panel's deliberations and recommendations.

Expert panels can effectively gather information, evaluate available data, and develop recommendations in a relatively short period of time. Expert panels can be used to address a variety of circumstances: when a rapid decision is needed to address a newly recognized concern, when resources and/or data for a quantitative risk assessment are limited, or when few management options exist. When epidemiological evidence indicates a hazard is not under control, panels may identify ways to increase consumer protection. Also, panels may address concerns raised by changes in food processing technologies, food packaging, or distribution systems. Expert panels can address such situations and evaluate the risk, based on scientific evidence.

Government and international bodies have made extensive use of expert panels (e.g., Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) consultations) to address concerns about the safety of a particular hazard-food combination. In addition, industry expert panels have considered the factors leading to foodborne disease and developed recommendations for their control (e.g., the Blue Ribbon Task Force established by the National Livestock and Meat Board to address *E. coli* O157:H7) (NLSMB, 1994).

Expert panels rely on epidemiologists, public health specialists, food microbiologists, food technologists and others with knowledge about the foodborne disease or the conditions of food production, processing, distribution and use. Often, the panel uses a process that is very similar to a HACCP hazard analysis. In some instances, a rough estimation of the risks associated with different likely scenarios is sufficient. One approach is to assign relative probability and impact rankings—such as negligible, low, medium, or high—to the likelihood of exposure and adverse outcome. The panel must clearly define and justify the rankings to enable people to use the final result without misinterpretation.

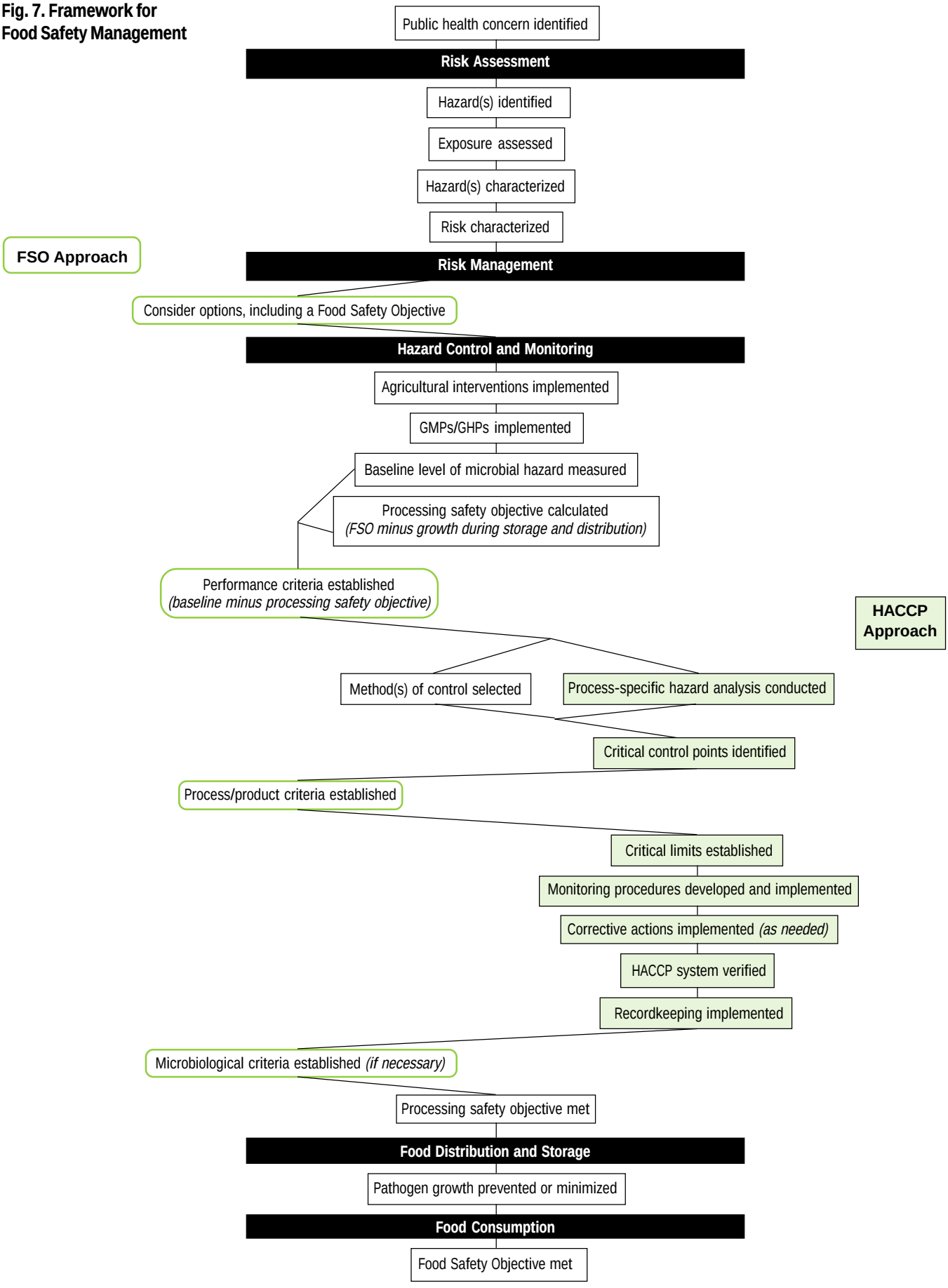
An expert panel considers various aspects of the issue and provides its best judgment based on information available at that time. Because the process is simpler, it is also faster than a quantitative risk assessment. Although the complexity of the risk evaluation may vary, the panel follows the standard four-step format for a risk assessment and should provide information on the conditions that lead to hazardous food. In the end, the panel may recommend one or more measures to control a hazard or, if necessary, the expert panel may recommend banning the product or process. An expert panel also may recommend establishing a Food Safety Objective when it would be an effective means to enhance the safety of the food under consideration.

Risk Management Using Food Safety Objectives

A recently proposed risk management approach revolves around the concept of Food Safety Objectives (FSOs). As defined by the International Commission on Microbiological Specifications for Foods (ICMSF, 1997, 2002), a food safety objective (FSO) is a statement of the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides the appropriate level of protection. The FSO approach can be used to integrate risk assessment and current hazard management practices into a framework that can be used to achieve public health goals in a science-based, flexible manner. Fig. 7 demonstrates how the FSO concept (boxes with rounded corners) can integrate with HACCP (shaded boxes).

Although the FSO concept is relatively new and is still evolving, its acceptance is growing because it offers a practical means to convert public health goals into values or targets that can be used by regulatory agencies and industry. For exam-

Fig. 7. Framework for Food Safety Management



ple, a public health goal may be to reduce the incidence of foodborne illness attributed to pathogen A by 50% from 20 to 10 cases per 100,000 people per year. A regulatory agency or manufacturer cannot design control systems that would be certain to meet such a goal. However, if this goal were translated into a numerical measure of the microbiological hazard's frequency or concentration (e.g., less than 100 CFU/g of *L. monocytogenes* or less than 15 mg/kg of aflatoxin), then the regulatory agency could establish inspection procedures and industry could design control processes based on the FSO.

The FSO concept can be a useful tool for creating policies that are consistent with current science. The limits imposed in an FSO should reflect not only the best available scientific information but also nonscientific input from a variety of sources. FSOs should reflect societal values with regard to levels of consumer protection. The FSO development process should be transparent and facilitate input, both scientific and societal, from all affected parties.

The FSO approach integrates scientific data from risk assessment to set quantifiable standards that address specific public health outcomes. Because the FSO must be met at the time of consumption, it is necessary to consider the potential for pathogen growth during storage and distribution. The processing safety objective is the FSO minus any projected pathogen growth. For example, if the FSO is less than 100 CFU/g of *L. monocytogenes* and 1 log cycle of growth is projected, the processing safety objective is calculated as no more than 10 CFU/g of *L. monocytogenes*. If no pathogen growth is projected, the processing safety objective is the same as the FSO.

The processing safety objective is then used to develop the performance and process/product criteria and to establish verification and acceptance procedures. Good hygienic practices (GHPs) and good manufacturing practices (GMPs) are important to minimize the hazard and prevent recontamination after processing. HACCP manages the application of control methods, ensuring that the process is effective. Table 12 provides three examples of how the FSO approach might be used to address specific issues of microbiological food safety. Regulatory agencies can use FSOs and processing safety objectives to communicate the level of control expected in food processes and then to evaluate the adequacy of a facility's control system.

FSOs differ from the microbiological criteria that have been traditionally used to determine the acceptance of food products. Microbiological criteria specify details such as a sampling plan and the method of sample preparation and analysis, but the criteria cannot be readily used to evaluate a process. Microbiological testing of finished product from a plant provides a snapshot for the time the food was produced. Review of the same plant's food safety management system using an FSO approach would provide a more meaningful assessment of long-term control.

FSOs can be used to communicate food safety requirements for food processes; whereas microbiological criteria are used to determine the acceptability of specific lots of food with respect to quality and/or safety. The principles for the establishment of microbiological criteria for food have been described by Codex (CAC, 1997a) and have been recently further elaborated upon by the ICMSF (ICMSF, 2002). For example, the following components are recommended when microbiological criteria are to be established:

- a statement of the microorganisms of concern and/or their toxins/metabolites and the reason for that concern;
- microbiological limits considered appropriate to the food at the specified point(s) of the food chain,
- the number of analytical units that should conform to these limits;
- a sampling plan defining the number of field samples to be taken, the method of sampling and handling, and the size of the analytical unit; and
- the analytical methods for their detection and/or quantification.

In addition, a microbiological criterion should also state:

- the food to which the criterion applies;
- the point(s) in the food chain where the criterion applies; and
- any actions to be taken when the criterion is not met.

When used for food safety, microbiological criteria should reflect the severity of the disease and whether risk is likely to decrease, remain the same, or increase between when a food is sampled and when it is consumed. As criteria are established to address newly emerging food safety concerns, there may be little information available. It is the responsibility of regulatory agencies to establish criteria that can be used to assess the safety of foods. Preferably, the criteria should be considered interim standards that will be adjusted to be more or less stringent as more information becomes available.

The FSO approach assumes that a food is distributed, stored, and prepared as intended and expected when the food safety system was designed. Deviations in handling and storage after the food meets the processing safety objective at the factory could trigger a failure to meet the FSO. Proper food handling and preparation practices are essential under the FSO approach.

Hazard Control and Monitoring

Once an FSO has translated public health goals into quantifiable limits, hazard control and monitoring practices must be developed. Although hazard controls were developed long before the more recent formal risk assessment and risk management approaches, the application of hazard controls can be directed and informed by the broader perspective that results from an integrated food safety framework. Mandatory hazard control processes have traditionally been the focus of regulatory agencies, but recent initiatives to develop risk-based approaches offer the opportunity for flexible, science-based hazard control.

Often, many different approaches are combined to achieve the desired result. In the farm-to-table approach to food safety, good agricultural practices (GAPs) can provide ingredients with improved microbiological safety. GMPs, also known as GHPs in the international arena, set basic standards for facility sanitation and hazard control. Performance criteria quantify the hazard control results necessary to meet the processing safety objective, and process/product criteria define the process variables and product characteristics that will achieve the performance criteria. HACCP establishes the critical control points at which the process/product criteria are applied, further defining the conditions that must be met into a specific set of critical limits for the process. HACCP also monitors and documents successful implementation of the control process. Finally, microbiological criteria and testing may be used, if nec-

Table 12. FSOs in the Food Safety Management Framework (Derived from ICMSF, 2002)

Managing Microbiological Food Safety	***Salmonella*** **in Dried Milk**
Public health concern *(often based on epidemiological data)*	Consumed as reconstituted milk, particularly by children
Risk Assessment	
Hazard identification	*Salmonella* are heat-sensitive bacteria that are a leading cause of diarrheal disease worldwide, especially among the very young and elderly
Exposure assessment	Post-processing contamination in the factory rarely occurs, and the concentration of *Salmonella* in dried milk is low
Hazard characterization	Considering reconstituted dried milk is often consumed by children, assume worst case scenario: one *Salmonella* cell per 10 g serving of milk may cause illness
Risk characterization	Based on limited data, probability of illness is <1 in 10^8 servings
Risk Management	
Food safety objective	To maintain the current estimated level of risk, FSO may be established as <1 *Salmonella* per 10^8 gram at the time the dried milk is reconstituted for consumption
Hazard Control and Monitoring	
GMPs	GMP to control recontamination after processing
Performance criteria	Processing to achieve at least 8-log reduction for salmonella
Hazard analysis, method(s) of control, and CCPs	Pasteurization
Process/product criteria	Time and temperature specifications for pasteurization
HACCP implementation and verification	Monitor environment after pasteurizer by testing for indicator organisms and *Salmonella* to document effectiveness of GMPs
Microbiological criteria *(if necessary)*	Testing product is not recommended

essary, to further verify that the processing safety objective has been met.

Good Agricultural Practices

It is impossible to guarantee that crops will be free of all harmful microbiological contamination because disease-causing organisms have too many opportunities to enter the food system through the production sector. Nonetheless, it is possible to minimize the food safety risks and take preventive steps to protect produce and other agricultural commodities at the farm level. The Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables, published by FDA's Center for Food Safety and Applied Nutrition (FDA/CFSAN, 1998), lists basic principles to guide farmers and farm workers on pre-harvest safety.

Management and control of manure has become a critical issue in GAPs. Properly treated manure can be an effective and safe fertilizer, but untreated, improperly treated, or recontaminated manure can contain pathogens that can reach fresh produce in the field or nearby water supplies. Evidence indicates that some pathogens can survive for extended periods of time in the soil and on produce (Rangarajan et al., 2000). Manure should be composted to effectively eliminate pathogens and applied appropriately to minimize the possibility of pathogen survival and subsequent crop contamination.

Irrigation water also is a key factor. The source of irrigation water should be

L. monocytogenes in Ready-To-Eat Meats	*Escherichia coli* O157:H7 in Ground Beef Patties
Listeriosis has been associated with certain ready-to-eat (RTE) meats, including hot dogs	Outbreaks of illness associated with undercooked ground beef
Listeriosis is a rare but serious disease affecting immunocompromised individuals and the developing fetus	*E. coli* O157:H7 infections can result in moderate to severe disease or death; children under 5 years and the elderly are the most sensitive populations
L. monocytogenes is a frequent contaminant in certain RTE foods (e.g., up to 5%)	*E. coli* O157:H7 is frequently present on the hide and in the intestines of cattle. The occurrence of *E. coli* O157:H7 in ground beef is estimated as <1% in the U.S.
The majority of cases appear to be associated with dose levels in excess of 10^4 CFU/serving of RTE meats (FAO/WHO, 2001)	Fewer than 100 cells can cause disease, especially among young children
Estimated median number of cases per serving: 3×10^{-6} for perinatal populations; 5×10^{-8} for elderly populations; and 5.9×10^{-9} for intermediate populations (FDA/CFSAN, USDA/FSIS, CDC, 2001)	One estimate found 26×10^4 patties per year nationwide may contain viable *E. coli* O157:H7 after cooking
Based on epidemiologic data. FSO set at no more than 100 CFU/g of *L. monocytogenes* in RTE meats when consumed	To achieve a 25% reduction in the number of illnesses, the FSO may be a concentration of *E. coli* O157:H7 in ground beef of no more than 1/250g (equivalent to 1 cell per two 125g patties)
GMP to control recontamination after processing	GMP to minimize contamination during slaughter and processing
Processing must result in 6-log reduction of *L. monocytogenes*	Performance criteria cannot be specified at this time
Thermal processing	Moist heat and/or acid sprays
Time and temperature for cooking	Parameters for sanitation and control measures (prevention of contamination during slaughter and decontamination) may be defined
Emphasis on environmental testing to verify sanitation. Consider options to control pathogen growth if food should become contaminated.	Sanitation and control parameters are monitored
Product testing is of little value in controlled environments but should be considered when control is uncertain and pathogen growth can occur in the product	Testing of raw materials may enable plants to select suppliers with desired microbial quality. Lot testing may be conducted to identify high prevalence lots, but lots that test negative cannot be considered free of the pathogen or "safe"

known, and periodic testing may be appropriate.

For pathogen control, GAPs include recommended practices prior to planting (Rangarajan et al., 2000). Where possible, the field should be upstream of the farm's animal housings, and plans should be put in place to prevent any runoff or drift from animal operations from entering the field. Grazing livestock should be located away from produce fields, and traffic of wild and domestic animals in produce fields should be minimized wherever possible.

The hygiene of field workers should be maintained, monitored and enforced. Employees should have clean restrooms with access to soap, clean water and single-use towels. All employees should be properly trained to follow good hygienic practices (FDA/CFSAN, 1998).

Good Manufacturing Practices

Current GMPs—the conditions necessary for each segment of the food industry to protect food while under its control—are well defined and established in post-harvest food processing. These conditions and practices provide the basic environmental and operating conditions that are necessary for the production of safe, wholesome food. These conditions and practices, many of which are specified in federal, state, and local regulations and guidelines, are now considered to be prerequisite to the development and implementation of effective HACCP plans (NACMCF, 1998). GMPs

cover sanitation issues, such as equipment design and cleaning, and pest control.

Performance Criteria

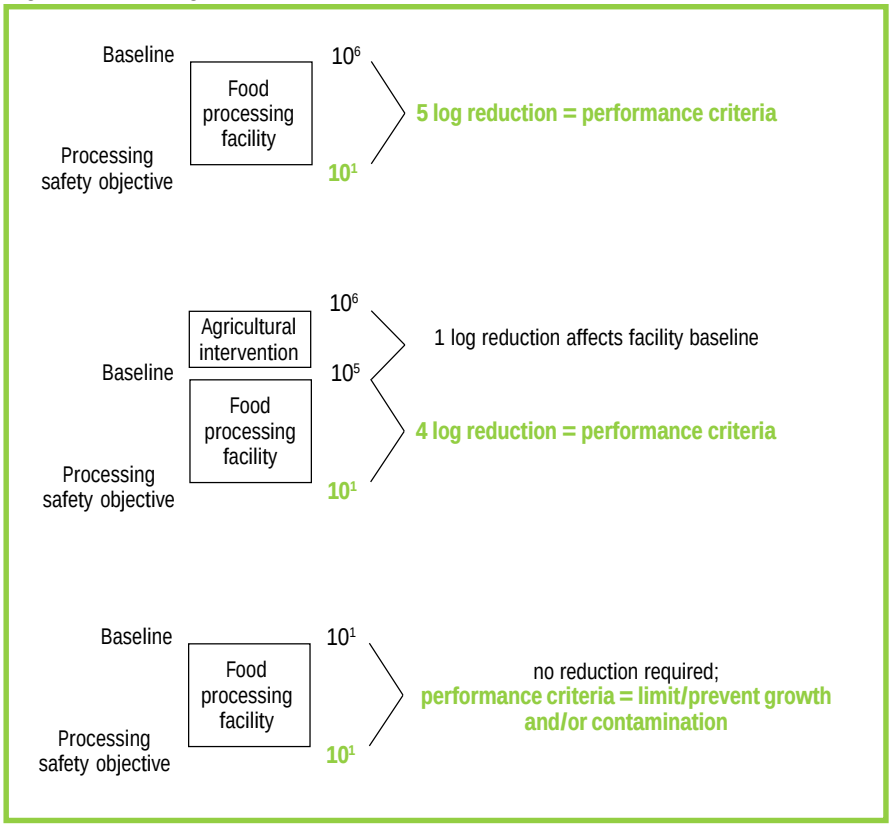
FSOs are met using performance criteria, which are the required outcome of a control step or a combination of steps. At certain points in food processing, control measures can be applied to either prevent an unacceptable increase in a microbiological hazard or reduce the hazard to an acceptable level. Chilling cooked meats and stews prevents the growth of *Clostridium perfringens* (i.e., increase of a hazard), and pasteurization of milk or fruit juices eliminates enteric pathogens (i.e., decrease of a hazard). The performance criteria are the reduction necessary (e.g., a 5-log reduction) to achieve the processing safety objective; they are calculated from the baseline level of the microbial hazard (see Fig. 8). Performance criteria also may address the prevention of pathogen growth (e.g., less than 1-log growth).

Under the FSO approach, it is important not to confuse performance criteria and performance standards. Some current food safety regulations (i.e., performance standards) mandate specific pathogen reductions as a result of processing, but this approach would not ensure compliance with an FSO. For example, under the current system, a performance standard may require a 5-log reduction in pathogen levels for a raw agricultural commodity (e.g., fresh juice) (see Fig. 9). Although a food processor could design a system to achieve the required reduction, a higher baseline level of pathogens could result in higher pathogen levels after processing. Under the FSO approach, the processor would know the level of hazard that is allowed in the final product and would calculate the performance criteria based on the initial number of pathogens.

Process and Product Criteria

Performance criteria are implemented through application of process and/or product criteria, which are the variables in the control process or the characteristics of the product that achieve the necessary reduction or limit pathogen growth. A process criterion could be the time and temperature of a thermal process; a product criterion could be a pH value. Process and product criteria may be used alone or in combination. Control of spores of *Clostridium botulinum* could be accomplished with a process criterion of heating low acid foods for a specified time at a specified temperature, or with a product criterion of reducing the pH below a specified level for acidic foods. Often, more than one combination of criteria will meet the processing safety objective. The specific values for a particular process are established as critical control limits through the HACCP process (see below).

Fig. 8. Establishing Performance Criteria



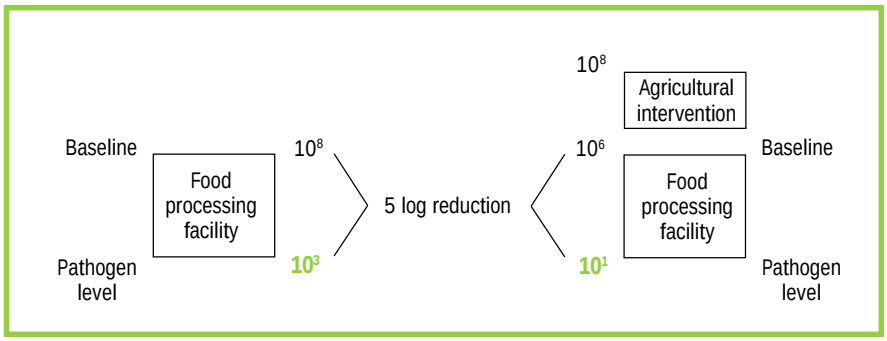
HACCP

HACCP is a management tool used by the food industry to enhance food safety by implementing preventive measures at certain steps of a process. When HACCP principles are properly implemented, microbiological hazards that have the potential to cause foodborne illness are controlled, i.e., prevented, eliminated or reduced to an acceptable level.

The pathway to HACCP began in 1959 because existing quality control techniques could not provide the desired level of safety for food produced for the space foods program (Bauman, 1992). Traditional microbiological testing of finished product was impractical and ineffective because of the small quantities of food produced, and the high product cost limited the amount available for sampling. In addition, the food industry had no uniform approach to managing food safety. HACCP was developed to meet this need.

Over the years, the fundamental concepts that comprise a HACCP program have been refined, and their application has become more practical. The U.S. National Advisory Committee on Microbiological Criteria for Foods (NACMCF)—a federal advisory committee assembled to provide impartial, scientific advice to federal food safety agencies for use in policy development—has revised and expanded the original principles based on industry experience (NACMCF, 1992; 1998). The committee consists of experts in microbiology, risk assessment, epidemiology, public health, food science, and other relevant disciplines. At the same time as the NACMCF activities, Codex adopted a similar HACCP document (CAC, 1997c). NACMCF adopted seven

Fig. 9. Unequal Levels of Food Safety



HACCP principles:

- Principle 1: Conduct a hazard analysis.
- Principle 2: Determine the critical control points.
- Principle 3: Establish critical limits.
- Principle 4: Establish monitoring procedures.
- Principle 5: Establish corrective actions.
- Principle 6: Establish verification procedures.
- Principle 7: Establish record-keeping and documentation procedures.

The fundamental HACCP principles, associated definitions, and principles of application were intended to help the food industry implement food safety management systems. First, companies identify hazards that have the potential to cause illness or injury to the consumer and determine whether or not these hazards are significant. For significant hazards, the company identifies critical control points (CCPs) in the food system, where possible, and establishes critical limits for their control. Critical limits are based on evidence that the control is effective in practice. Next, CCPs must be monitored to assure critical limits are not violated. This monitoring must be a systematic procedure that verifies that the HACCP system is functioning as intended and that it is effective. Finally, the company must maintain comprehensive records associated with the HACCP system.

HACCP is an essential part of implementing an FSO. Through the identification of CCPs, the process and/or product criteria can be translated into process-specific critical limits. Monitoring of these critical limits ensures that the performance criteria are met.

Using the principles identified in the NACMCF and Codex documents as a standard, HACCP was widely adopted by the food industry in the United States and throughout the world beginning in the 1990s and continuing to the present. While many companies voluntarily adopted HACCP as part of their responsibility to provide safe food, it also became mandatory for some U.S. companies under new regulations. In 1995, the Food and Drug Administration (FDA) published a rule that required all seafood processors and, in effect, those companies exporting to the United States to develop and implement HACCP systems by Dec. 18, 1997. In July 1996, USDA published a final rule that required all meat and poultry processors to implement a HACCP system. On Jan. 19, 2001, FDA published a rule that requires large juice processors to implement HACCP by 2002, with later compliance dates for smaller companies. These regulations are playing a major role in HACCP adoption in the food industry.

Successful HACCP implementation has not been limited to food processing. HACCP has been used worldwide to improve food safety in food service and retail, and food distribution.

The application of HACCP to production agriculture, however, is limited. Applying the HACCP principles reveals few, if any, CCPs in the production of raw agriculture commodities. A CCP is a point in the process where control can be applied and where the control is essential to prevent or eliminate a food safety hazard or to reduce it to an acceptable level (NACMCF, 1998). The difficulty in production agriculture is two-fold: identifying the source of a hazard and finding an effective control. Additional research is needed to develop methods that effectively control hazards in the production agriculture environment. Currently, hazards in this part of the food system are most effectively controlled by GAPs, such as those recommended by FDA for fresh fruits and vegetables (see GAPs, p. 72).

For HACCP to be successful, it is critically important that regulatory policy be based on the best science currently available. In an effort to ensure diligent HACCP implementation, regulatory agencies may constrain the process and ultimately undermine HACCP's scientific basis. Although it is an extremely useful hazard management tool, HACCP is not appropriate for all situations. Regulatory policies must allow the flexibility to apply science in a product- and process-specific manner that best achieves the FSO. Some current policies mandate the development of a HACCP plan, even when a scientific analysis fails to identify a point in the process that meets the CCP criteria; it is impossible to have a valid HACCP plan without a critical control point. In addition, certain policies generally prescribe the CCPs, without regard to the particular circumstances at a given food manufacturing facility. As a result, the food industry may pursue mere regulatory compliance, following the form of HACCP without its proper substance. HACCP is a science-based hazard management tool, not purely an administrative system. When critics claim that HACCP has failed, the failure is often in applying science and the HACCP principles to managing food safety.

Microbiological Criteria and Testing

Routine microbiological testing can be useful in certain applications. It can be helpful for surveillance purposes and process verification, and it is sometimes helpful for lot acceptance. However, microbiological testing of finished product can be misleading, and negative test results do not ensure safety. Statistical limitations of microbiological testing are of significant concern, especially when the rate of contamination is very low. As the defect rate in the product becomes low, emphasis should shift to improving the implementation of food safety management strategies, such as HACCP, rather than relying on microbiological testing.

Statistical Limitations to Testing

To analyze food for microbiological agents, a sample of the product must be taken. Ideally, the sample or samples taken from a production lot of food will in some way reflect the whole of the lot, the underlying concept in statistically-based

Table 13. Probability of Acceptance (P_a) of Defective Product Using a 2-class Sampling Plan with n=10 to n=300 and c=0 (ICMSF, 2002)

Composition of lot	Number of Samples						
Percent defective	10	20	30	50	100	200	300
1	0.90	0.82	0.74	0.61	0.37	0.13	0.05
2	0.82	0.67	0.55	0.39	0.13	0.02	<
3	0.74	0.54	0.40	0.22	0.05	<	
4	0.66	0.44	0.29	0.13	0.02		
5	0.60	0.36	0.21	0.08	0.01		
6	0.54	0.29	0.16	0.05	<		
7	0.48	0.23	0.11	0.03			
8	0.43	0.19	0.08	0.02			
9	0.39	0.15	0.06	0.01			
10	0.35	0.12	0.04	0.01			

sampling plans. So-called "lot acceptance sampling plans" are in widespread use around the world to determine whether or not a food product meets a certain set of specifications. These specifications can target maximum numbers of bacteria per unit size that are set by a purchaser of the food or by governments. When applied to determining food quality, lot acceptance sampling plans in conjunction with 2- or 3-class sampling plans (ICMSF, 1986; 2002) are of some value.

The acceptance of a "quality-only defect" that will occur occasionally no matter how rigid the sampling scheme is quite different from a situation in which consumer health is at risk. When sampling food for pathogens, sampling has sufficient inherent limitations to be rendered misleading. The following are some examples of the possible consequences of rigorous sampling plans when applied to making accept/reject decisions for safety reasons.

Most microbiological sampling plans involve anywhere from a single sample to as many as 60 samples per lot. Table 13 indicates that:

- If 10 samples are collected from across a lot of food that has a defect rate of 1%, there is a 90% probability that the defect will not be detected and the lot will be accepted.
- If 300 samples are collected from across a lot of food that has a defect rate of 1%, there is a 5% probability that the defect will be not be detected and the lot will be accepted.
- If 10 samples are collected from across a lot of food that has a defect rate of 10%, there is a 35% probability that the defect will not be detected and the lot will be accepted.

The implications of the table become apparent when the prevalence of contamination for various foods is considered. For example, during the years 1998-2000, the U.S. Department of Agriculture (USDA) monitoring program for salmonella in raw meat and poultry detected a range of prevalence in various commodities (see Table 14). Contamination in some foods is much more likely to be detected by sampling when the prevalence of the pathogen is high, as compared to foods with a lower defect rate. However, the prevalence of contamination for many foods is more likely to be at the lower end of the scale, particularly in the case of ready-to-eat (RTE) foods. For example, the prevalence rate for *L. monocytogenes* in RTE foods during 1994-1998 was reported to be from 1.08% to 4.91% (FDA/CFSAN, USDA/FSIS, CDC, 2001), and the prevalence rate for *L. monocytogenes* in most categories of ready-to-eat meat and poultry products was below 5% (Levine et al., 2001).

The above examples demonstrate that microbiological testing can have utility for detecting pathogens in certain types of food; however, as the prevalence rates decrease, testing becomes less reliable for detecting contaminated lots, even with large numbers of samples. For many foods there is a favorable history of safety, and testing for pathogens is not routinely done. Thus, as more effective control measures are adopted by industry and the prevalence of contamination decreases, a point is reached where product testing is no longer practical or justifiable. At that stage, greater benefit can be achieved by shifting verification procedures to comprehensive analysis of control systems that have been validated to control the pathogens of concern.

The effectiveness of a sampling plan is influenced by a number of factors such as whether random samples can be collected from a lot of food, how samples are prepared to obtain analytical units, and the sensitivity and reliability of the analytical method. Sensitive analytical methods do not exist for many of the pathogens responsible for foodborne illness. This includes the viruses that have been estimated to be responsible for more than 50% of all U.S. foodborne illness caused by known pathogens (Mead et al., 1999).

Lot acceptance sampling plans assume the microbial population is randomly distributed throughout each lot of food that is to be sampled. In reality, this is often not the case, particularly for foods that are not liquids. Nonrandom distribution of pathogens is a major contributing factor to the unreliability of product testing to prevent contaminated food from entering the food supply. This is a particular problem for detecting *E. coli* O157:H7 in ground beef where the prevalence rate is less than 1%. In this case, the current USDA Food Safety and Inspection Service (FSIS) sampling plan

Table 14. USDA Monitoring Program for *Salmonella* (1998-2000) (USDA/FSIS, 2000)

Product	Samples	Positive
Broilers	22,484	10.2%
Market hogs	8,483	7.0%
Cows/bulls	3,695	2.1%
Steers/heifers	2,088	0.3%
Ground beef	50,515	3.7%
Ground chicken	735	14.5%
Ground turkey	3,192	29.2%

Value of Test Results

The usefulness of sampling as applied in certain, more recent foodborne pathogen analysis situations has been hotly debated. For highly infectious human pathogens, it is virtually impossible to sample sufficient volumes of food to assure the total absence of pathogens. Moreover, there is real danger in assuming that if a food is sampled for pathogen X, and that pathogen is not found, the food is safe. Because sampling cannot assure safety, other means to do so must be found or applied.

Relying on negative test results as an indicator of the food's safety creates a disincentive to pursue additional safety measures. Consumers with a false sense of security may relax their vigilance with regard to food safety in preparation and handling. Industry and regulatory agencies may hesitate to adopt new technologies that provide a superior level of food safety, mistakenly believing that the cost would not be justified because negative test results indicated present processing technologies were fully controlling the hazard.

Validity is defined as the ability of the test to do what it is intended to do—in this case, to detect the target microorganism if it is present, and to not detect it if it is absent. Two commonly used measures of test validity are sensitivity and specificity. Sensitivity is the probability of a sample testing positive if contamination is truly present, while specificity is the probability of a sample testing negative if the organism is truly absent. Both of these measures are inherent to the test itself, and fortunately, most tests currently available for foodborne pathogens are highly sensitive and highly specific, frequently in the range of 95% for both measures.

The predictive value of testing is an essential concept in the attempt to understand the value of testing. Positive predictive value is the probability that the product is indeed contaminated given that the test is positive, while the negative predictive value is the probability that the product is free of contamination given a negative test result. While predictive value determinations depend on the inherent sensitivity and specificity of the test, they also depend upon the prevalence of contamination (see Fig. 10).

In the case of a contaminant that has a high frequency of occurrence in a given food, testing may have considerable value because of the higher probability that the contaminant will be present in a representative sample, and, given adequate test sensitivity and specificity, the predictive value of positive and negative test results will be high (i.e., in excess of 90%). Therefore, the test is reasonably predictive of the true nature of contamination.

However, considering that the prevalence of contamination by nearly all foodborne pathogens is quite low in most food commodities, the combined effect of low sampling plan efficacy and low positive predictive value means that the value of testing is relatively minimal. The chance of obtaining a sample that has the pathogen of interest is quite small, and nearly all presumptively positive tests will be confirmed as negative after additional testing. This means a tremendous expenditure for testing with very little value with respect to detecting contamination and potentially significant costs associated with product recalls and holds. These costs are inevitably passed on to the consumer, in exchange for little public health benefit.

The relationship between contamination of food, pathogen test results, and public health impact is indeed a complex one. In an ideal world, perhaps a better criterion of testing efficacy would be based on the ability of the test to accurately predict disease. For this to work, a number of critical factors would need to be in place. First would be the recognition that pathogen contamination may occur as nonrandom, rare events that require very large samples to provide any confidence of finding positives if they exist. The second is the positive/negative predictive value of the tests. Third is time-to-results and cost of tests. Fourth is converting true positive results into a prediction of public health impact. And fifth is having to deal with the public health impact of false-negative results, which even for a test with 95% sensitivity, will occur for 5% of contaminated samples anyway. In essence, when we establish a zero-tolerance standard, it is more based on our inability to predict the no effect level than it is our unwillingness to accept even a single illness.

Fig. 10. Predictive Value (PV) of Test Results

$$PV(+) = \frac{(\text{prevalence})(\text{sensitivity})}{(\text{prevalence})(\text{sensitivity}) + (1\text{-prevalence})(1\text{-specificity})}$$

Contaminant/food combination of relatively high prevalence

Campylobacter jejuni in raw poultry:
60% prevalence;
Test sensitivity and specificity of 95%

$$PV(+) = \frac{(0.60)(0.95)}{(0.60)(0.95) + (0.40)(0.05)}$$

PV(+) = 0.966, or 96.6% of the time the test is positive, the sample is truly contaminated

Contaminant/food combination of relatively low prevalence

E. coli O157:H7 in raw beef:
1% prevalence;
Test sensitivity and specificity of 95%

$$PV(+) = \frac{(0.01)(0.95)}{(0.01)(0.95) + (0.99)(0.05)}$$

PV(+) = 0.161, or only 16.1% of the time the test is positive, the sample is truly contaminated

involves 13 analytical units weighing 25g each and a very sensitive analytical method. Aside from the low prevalence, there is strong evidence indicating that this pathogen is not randomly distributed within production lots of ground beef from large commercial grinding operations.

The criteria for most infectious agents involve 2-class sampling plans and presence/absence testing. The stringency of the sampling plan is determined by the number of samples analyzed and the number of allowable positive samples. Sampling plans that do not allow any positive sample units have been used for a variety of pathogens (e.g., salmonella and *L. monocytogenes* in RTE foods, and *E. coli* O157:H7 in raw ground beef). Some have referred to sampling plans that do not allow any positives as "zero tolerance." In reality, the zero tolerance can be made more or less stringent by increasing or decreasing the number of samples. Thus, a sampling plan could specify 5, 10, 20 or more samples. Although 25g analytical units are normally used, sampling plan stringency also could be increased by increasing the size of the sample unit, for example, to 50g.

Microbiological Criteria

Historically, attempts have been made to apply microbiological criteria for the purpose of classifying foods as either microbiologically acceptable, or microbiologically unacceptable. In 1985, the Food and Nutrition Board of the National Research Council (FNB/NRC) addressed the subject of microbiological criteria, and found that such criteria were of limited use, particularly if safety assurance is the goal, and that HACCP should be applied wherever possible for safety assurance (FNB/NRC, 1985). The HACCP systems envisioned by the FNB/NRC did not rely on end-product testing for pathogens, but rather, if analyzed microbiologically at all, analyses were performed at points along the food's production chain, particularly to verify that CCPs were under control.

Microbiological criteria considered by FDA generally include standard plate count, coliform counts, yeast and mold counts, and *E. coli* (generic) counts. Coliforms and *E. coli* were believed to be indicators of possible fecal contamination, and therefore, it is possible that food containing either bacteria at a prescribed level was unsafe.

The "zero tolerance" for *L. monocytogenes* in RTE foods was established as a safety-related criterion. The "zero tolerance" actually means that *L. monocytogenes* must be absent from two 25g samples of foods under FDA inspection. The total absence requirement was derived at a time when there were no effective methods for finding *L. monocytogenes* in food (or any environment outside of the human or animal). There was no understanding of the very widespread existence of *L. monocytogenes* throughout the environment, including food processing environments, nor an appreciation of the number of foods in which the bacteria historically had been present. Thus, contemporary knowledge about human exposure suggests that many humans are routinely exposed to the bacteria with no consequence to health, although *L. monocytogenes* does cause illness in sensitive subpopulations. The "zero tolerance" has acted as a disincentive for the application of quantitative (enumerative) methods, and thus, the body of human exposure data is incomplete.

Today, there is growing acceptance of the need for management systems based on GMPs and HACCP to control food safety hazards. Microbiological testing is sometimes valuable in verifying the effectiveness of GMP and HACCP systems and validating CCPs within HACCP systems. There may be a role for testing certain ingredients when they can influence the safety of a finished product, but because the testing of ingredients faces the same weakness as end-product testing, auditing of suppliers' control programs has increased to provide greater assurance.

In addition to product testing, environmental testing may be necessary. Salmonella and *L. monocytogenes* have the ability to become established as residents in food processing establishments. Environmental sampling programs assess the degree of control and indicate when corrective actions are needed. They may or may not indicate a safety problem in the finished product.

The optimal regulatory approach to environmental testing would use our understanding of basic human nature to encourage and reward diligence. Depending on the type of food and the processing conditions, it should be expected that these pathogens will be periodically introduced into the food processing environment by various pathways. To prevent the pathogens from becoming established and multiplying, the sampling program should aggressively look for these pathogens. Finding a positive sample should be treated as a success, because corrective actions can then be applied and consumer protection assured. Treating a positive sample as a failure and applying a penalty decreases the desire to detect the pathogens, discouraging the aggressive nature of the environmental sampling program.

Testing Methods

Disease surveillance and control efforts will benefit greatly from new pathogen detection methods that offer greater precision, rapid results, and decreased cost. However, efforts to adapt these new technologies to the challenges in the food environment are ongoing.

Many methods of detection are currently available for foodborne pathogens, but food microbiologists must often choose between enumeration and identification without the option of both. Enumerative methods are usually based on the ability of the normal healthy bacterial cells to multiply in a nutrient-rich medium. Although selective agents are sometimes added to favor the growth of a specific group of organisms, most of these methods are still reasonably nonspecific. With respect to pathogen identification, methods have historically relied on cultural enrichment to increase the numbers of the target microorganism and allow resuscitation of injured cells. When followed by selective and differential plating, these methods provide discrimination of the target organism from the background microflora, but are non-enumerative. For both enumerative and non-enumerative methods, the combined effect of low levels of contamination and the need for cultural growth results in lengthy assays, frequently extending beyond four days for even preliminary results.

Most rapid method developments have sought to shorten detection time by replacing the selective and differential plating steps with more rapid technologies such as ELISA and DNA hy-

Testing for Mycotoxins

In the United States, raw agricultural commodities are routinely screened for mycotoxins using specific FDA guidelines that are based on human risk assessments. The testing methods used must be validated by the AOAC International (Gaithersburg, Md.), which ensures reliability and reproducibility.

Some methods, such as commercial enzyme-linked immunosorbent assays (ELISAs), are particularly useful for rapid screening of commodities and take as little as 5 minutes to complete (Pestka et al., 1995). Because corn is susceptible to aspergilli that produce aflatoxins, it is screened at the grain elevator and rejected if it exceeds the FDA guideline. Thus, aflatoxin-contaminated corn is identified and diverted early in the food processing chain. A similar approach is linked with an indemnification program for peanuts, which are also highly susceptible to aflatoxin contamination. Analogous guidelines have been set for other mycotoxins (trichothecene deoxynivalenol and the fumonisins), and rapid tests are available.

In general, careful monitoring of weather conditions and field testing for mycotoxins will identify years in which there is increased potential for contamination in specific commodities. Screening efforts can be increased and targeted toward possible problematic materials and regions, such as aflatoxins in corn and peanuts during an extended drought in the southeastern United States.

bridization, but because these methods remain hampered by less than optimal assay detection limits, lengthy cultural enrichment steps are still necessary. Furthermore, cultural confirmation for presumptively positive results is generally required for regulatory purposes. The limiting factor in making these methods truly rapid is predominantly the lengthy incubation time required to increase cell numbers.

Enzymatic nucleic acid amplification methods such as the polymerase chain reaction (PCR) offer several potential advantages for the rapid and reliable detection of microbial pathogens in foods. The primary advantage of this technology is the theoretical replacement of cultural enrichment with specific nucleic acid sequence enrichment, thereby decreasing total detection time. There are many reports of PCR-based assays for the detection of foodborne pathogens and several companies market these systems, all of which are currently in evaluation for AOAC approval.

In reality, rapid molecular detection methods for pathogens in food products remain in the developmental stages. The significant methodological hurdles yet to be addressed include the need to: (1) test larger, realistic sample volumes (at least 25 ml or g) instead of the small volumes (10-50 microliters) used in molecular-based assays; (2) account for the effect of residual food components that inhibit PCR enzymatic reactions; (3) detect low levels of contaminating pathogens; (4) assure detection of viable (infectious) pathogens; and (5) confirm molecular amplification products with more lengthy DNA hybridization assays (Bej and Mahbubani, 1994).

With respect to the first three challenges, the application of rapid methods could perhaps be improved if pathogens were separated, concentrated, and purified from the food matrix before detection (Swaminathan and Feng, 1994). None of the various bacterial concentration methods, including the most widely used immunomagnetic separation, (Sharpe, 1997) is ideal, highlighting the need to increase research in this area. With respect to detection of viable pathogens, naked DNA and the DNA of dead cells can persist for long periods of time (Herman, 1997). Even the more stable 16S rRNA is not an ideal indicator of pathogen viability (McKillip et al., 1998). Although mRNA may be considered a more promising target (Sheridan et al., 1998), key assay design issues would need to be addressed. Finally, methods to simplify or even eliminate post-amplification confirmation assays (McKillip and Drake, 2000; Norton and Batt, 1999; Sharma and Carlson, 2000) are expensive and have not been widely applied to food matrices (Koo and Jaykus, 2000).

When taken together, the promise of emerging molecular detection methods, including biosensors (deBoer and Beumer, 1999) and microarray technology (Epstein and Butow, 2000), must be tempered with an appreciation for the complexity of the food matrix.

Surveillance for Foodborne Hazards and Illness

One way to identify emerging pathogens is surveillance of foodborne illness. Not only can scientists track the spread and frequency of a pathogen by looking for its victims, they can quickly spot changes in virulence or exposure. Surveillance data can be used for quick outbreak response and also as the basis for qualitative and quantitative risk assessment. New scientific tools have significantly increased the speed and depth of surveillance information gathering, making it more effective.

Purposes and Mechanisms

Surveillance involves the systematic collection of data with analysis and dissemination of results. Surveillance systems may be passive or active, national or regional in scope, or based on a sentinel system of individual sites. Traditionally, human foodborne disease surveillance has been conducted for three reasons: (1) to identify, control, and prevent outbreaks of foodborne disease, (2) to determine the causes of foodborne disease, and (3) to monitor trends in occurrence of foodborne disease.

By identifying outbreaks and their causes quickly, surveillance can result in early intervention to address hazards in the food supply. Officials may be able to remove contaminated products from retail shelves (e.g., identification of *Salmonella* Agona in contaminated cereal (CDC, 1998c)) or rectify inappropriate food handling procedures (e.g., undercooking of meats or cross-contamination of vegetables from raw chicken).

The cumulative information obtained through surveillance and outbreak investigation can reveal the magnitude and trends of foodborne disease, helping policy makers identify optimal prevention strategies (Borgdorff and Motarjemi, 1997). Additionally, improved understanding of disease and hazard etiology can help researchers anticipate or recognize new problems, such as toxins in one food that could pose a problem in other foods or toxins that are

Outbreak Investigations and New Foodborne Pathogens

On April 29, 1991, local public health officials were notified of an outbreak of foodborne illness among persons who celebrated "Secretary's Day" at a local restaurant (Hedberg et al., 1997). Seventeen (89%) of 19 members of the index group developed diarrhea and cramps 11 to 122 hours (median, 56 hours) after their meal. Fewer than half of cases reported nausea, myalgia, fever, or vomiting. Duration of illness ranged from 4 to 7 days (median, 5 days). Similar illnesses were also reported among other restaurant patrons and among five (15%) of 34 food handlers at the restaurant.

The restaurant served a large hotel and conference center and featured an elaborate buffet with a variety of fresh fruits, vegetables, salads, and gourmet food items that combined cooked and uncooked foods. The apparent high attack rate of illness in the index group and reports of illnesses among food handlers led to early concern that the restaurant was experiencing a large outbreak of viral gastroenteritis. However, as the clinical and epidemiologic features of the outbreak emerged from interviews with patrons, it appeared typical of previously described outbreaks caused by enterotoxigenic *E. coli* (ETEC).

The recognition that the outbreak may have been caused by an uncommon foodborne pathogen led to extensive efforts to obtain stool from ill patrons to confirm the etiology. A lactose-negative non-motile *E. coli* O39 was isolated from 10 of 22 cases. No *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Vibrio*, or *Plesiomonas* species were isolated from ill patrons. Although the clinical and epidemiologic features of the outbreak suggested ETEC, the outbreak-associated O39 strain did not possess ETEC heat-labile (LT) or heat-stable (ST) enterotoxins. Extensive testing of the outbreak-associated strain by a battery of gene probes detected the presence of intimin (*eae*), an adherence factor associated with enteropathogenic (EPEC) and enterohemorrhagic (EHEC) *E. coli* and EAST1 (*astA*), a heat-stable enterotoxin associated with enteroaggregative adherent (EaggEC) *E. coli*. Thus, the outbreak strain did not fit neatly into any of the recognized categories of diarrheagenic *E. coli* and would not have been identified as a pathogen had it not been implicated in this outbreak.

The majority of diarrheagenic *E. coli* virulence factors are encoded on pathogenicity islands, transmissible plasmids, bacteriophage, or transposons. Horizontal transmission of virulence factors led to the development of highly virulent *E. coli* O157:H7 which has emerged as a major foodborne disease of public health importance. This same genetic plasticity could lead to emergence of other combinations of virulence factors. Prompt and thorough epidemiologic investigation of outbreaks will be needed to identify these novel emerging pathogens and to further our understanding of their public health significance.

newly recognized as a human health hazard (see sidebar above).

HACCP systems rely on accurate knowledge of potential hazards (NACMCF, 1998). Many of these hazards—specific agents, food ingredients, or agent/food interactions—were originally identified as a result of foodborne disease surveillance. Because food sources and foodborne disease agents are constantly changing, hazard analysis is an ongoing process that requires continuous support from public health surveillance of foodborne disease.

Foodborne disease surveillance can also supply important feedback on the effectiveness of control strategies. For example, during the 1980s, the increased occurrence of sporadic *S.* Enteritidis infections and outbreaks in New England led to the identification of a new problem with *S.* Enteritidis contamination of grade A shell eggs (St. Louis et al., 1988). In the United States, USDA and FDA have worked with the egg industry to develop and implement a number of control strategies (Hogue et al., 1997). The incidence of *S.* Enteritidis infections in FoodNet sites declined 48% during 1996 -1999, suggesting that these control strategies are beginning to work (CDC, 2000a, b).

Current Surveillance Programs

Foodborne disease surveillance consists of four primary components: (1) identifying and reporting outbreaks, (2) monitoring for specific pathogens, (3) determining risk-factors for sporadic cases of infection with common foodborne pathogens, and (4) studying the population to track gastrointestinal illness, including trends in the requests for health care, food consumption and personal prevention measures.

Improving pathogen-specific surveillance has been a major focus of the National Food Safety Initiative. Serotype-specific surveillance of *Salmonella* conducted by state health departments and CDC's Public Health Laboratory Information System (PHLIS) has identified several large, multi-state outbreaks of salmonellosis. These outbreaks, caused by cantaloupes, tomatoes, and alfalfa sprouts, were spotted because of unusual, time-related clusters of cases caused by an uncommon *Salmonella* serotype. Epidemiological investigation of these cases identified the source.

PHLIS has developed an automated surveillance outbreak detection algorithm (SODA), originally developed to address *Salmonella*, that uses the 5-year mean number of cases from the same geographic area and week of the year to look for unusual case clusters (Hutwagner et al., 1997). *S.* Stanley and *S.* Agona infections initially identified by individual state health departments were discovered to be multi-state outbreaks based on disease clusters identified by SODA.

Because it compares current cases to 5-year means, SODA appears to be more effective at detecting case clusters of uncommon serotypes rather than common serotypes, such as *S.* Typhimurium. Although the Minnesota Department of Health reported 11 confirmed outbreaks of *S.* Typhimurium infection from 1996-1998, SODA detected only three. During the same time period, SODA identified nine weeks where the number of *S.* Typhimurium reports exceeded the 5-year average. Six of these notifications were due to epidemiologically unrelated *S.* Typh-

imurium isolates with different pulsed-field gel electrophoresis (PFGE) patterns. One flagged the occurrence of two simultaneous outbreaks and the other two were due to a single outbreak that extended over time (Bender et al., 2001).

Molecular subtyping schemes, such as PFGE, can improve the investigation of outbreaks by distinguishing unrelated sporadic cases from the main outbreak-associated strain. The ability to distinguish specific subtypes among relatively common organisms, such as *E. coli* O157:H7 and *S.* Typhimurium, is the basis of the National Molecular Subtyping Network (PulseNet), which takes advantage of recent advances in both molecular biology and information technology. Highly reproducible PFGE patterns are generated for a pathogen implicated in an illness, and the PFGE patterns can be electronically shared between participating laboratories. An outbreak of *E. coli* O157:H7 infections in Colorado was associated with consumption of a nationally distributed ground beef product. Within days, public health officials could compare the outbreak strain to PFGE patterns of *E. coli* O157:H7 isolates throughout the United States (CDC, 1997). PulseNet has the potential to be the "backbone" of a public health surveillance system that can provide truly national surveillance for a variety of foodborne pathogens in a manner timely enough to be an early warning system for outbreaks of foodborne disease (Hedberg et al., 2001).

PulseNet's usefulness is currently limited because not all public health laboratories are connected, not all clinical laboratories routinely submit isolates to public health laboratories, and many states do not have sufficient epidemiologic resources to investigate individual cases or clusters.

In contrast to PulseNet's widespread surveillance area, FoodNet is a sentinel-site, active surveillance project designed to track all diagnosed infections of important foodborne diseases and evaluate the laboratory, physician and patient practices that cause an individual case to be diagnosed. FoodNet's initial surveillance area (13.2 million residents of Minnesota, Oregon, and selected counties in California, Connecticut, and Georgia) was expanded in 2000 and 2001, adding sites in New York, Maryland, Tennessee, and Colorado that brought the population under surveillance to 33.1 million persons. FoodNet uses active surveillance, meaning public health authorities regularly contact clinicians and laboratories to obtain case reports.

Most foodborne disease surveillance uses passive reporting systems, in which reports are voluntarily submitted by health clinics and laboratories. This system depends on the clinician's ability to diagnose the illness and the willingness of clinicians and laboratory personnel to report the diagnoses to the appropriate public health authorities.

In general, active surveillance yields better data than passive systems but is more expensive and limited in scope. Because some cases of foodborne illness will remain unrecognized and go unreported, even active surveillance systems are inherently incomplete (Potter and Tauxe, 1997).

One of the most striking gaps in our foodborne disease surveillance is generation of data about individuals who have gastrointestinal illness but do not see a physician. Furthermore, physicians often treat mild to moderate gastrointestinal illness symptomatically and do not frequently culture specimens or conduct the wide range of diagnostic tests necessary to identify all foodborne agents.

Unknown Agents

Surveillance for foodborne diseases is based on detection of specific pathogens or the occurrence of illnesses, such as diarrhea, in defined groups. An outbreak may be recognized because a state public health laboratory detected the increased occurrence of a specific subtype of *E. coli* O157:H7, or because half of the people who attended a specific event developed vomiting and diarrhea shortly after the event. In the first case, surveillance is limited by what clinical laboratories routinely identify when processing human stool samples, whether by direct examination, culture, or use of non-culture diagnostic tests. In the second case, surveillance has a better chance of identifying foodborne agents that are not part of routine clinical microbiology, but it is still limited by the epidemiologic and laboratory resources available to public health departments.

Published estimates of foodborne disease occurrence highlight the limitations of our current surveillance system. Of the 76 million cases of foodborne illness estimated to occur each year in the United States, 82% are attributed to "unknown agents" (Mead et al., 1999). Of the 28 known foodborne pathogens included in this estimate, routine passive surveillance systems exist for only 17 (61%). For many of the others, scientists must infer their frequency from the occurrence of outbreaks or from a limited number of population-based studies that researched the causes of diarrhea. For example, clinical laboratories do not routinely identify Norwalk-like viruses (NLVs), and no surveillance program tracks cases of NLV infection. Yet estimates attribute 11% of all episodes of diarrheal illnesses to NLVs, based on a study from the Netherlands (Mead et al., 1999). The estimated proportion of foodborne illness that is caused by unidentified agents is bolstered by Centers for Disease Control and Prevention (CDC) data (Mead et al., 1999). No agent was identified in 1,873 (68%) of 2,751 confirmed foodborne outbreaks reported to CDC from 1993-1997 (Olsen et al., 2000).

A high percentage of the outbreaks attributed to "unknown etiology" are probably outbreaks of viral gastroenteritis that were not confirmed either because stool samples were not available for testing, or because public health laboratories did not perform the test necessary to detect viruses. For example, one retrospective study confirmed the presence of NLVs in 90% of a select group of outbreaks of non-bacterial gastroenteritis (Fankhauser et al., 1998). In Minnesota, officials used the clinical and epidemiologic appearance of the outbreak to link 120 (41%) of 295 confirmed foodborne outbreaks reported from 1981-1998 to NLVs, leaving only 26 outbreaks (9%) attributed to unknown agents (Deneen et al., 2000). The factors used in the classification included a median incubation period of between 24-48 hours, a 12-60 hour duration of symptoms, and a relatively high proportion of cases experiencing vomiting (Hedberg and Osterholm, 1993; Kaplan et al., 1982). In a retrospective review of foodborne outbreaks reported to CDC from 1982 to 1989, almost half (48%) of the 712 outbreaks reported as having an undetermined etiology met the epidemiologic criteria for outbreaks of NLV (Hall et al., 2001). Thus, although a high percentage of reported foodborne illnesses do not have an identified cause, it appears that many are potentially identifiable causes. Additional surveillance would help researchers more frequently identify the

agent responsible for cases of foodborne illness and provide more reliable estimates of the true prevalence of various foodborne pathogens.

Integrated Surveillance

The modern concept of public health surveillance, first articulated by Alexander Langmuir, views surveillance as a process (Foege, 1996). As it relates to food safety, the process is concerned not only with outcomes in the human population but also with the occurrence of foodborne hazards in all types of foods, their sources, and the various stages in their conversion to consumable food. Operationally, surveillance involves the systematic monitoring of disease and hazard reports—in animal and plant populations, food production and processing environments, foods and ingredients, and in human populations—through the systematic collection, analysis, and interpretation of outcome-specific data, closely integrated with the timely dissemination of these data to those responsible for preventing and controlling disease or injury (Thacker and Berkelman, 1988).

Foodborne disease surveillance has traditionally been viewed as a subset of public health surveillance. The links between surveillance for foodborne diseases in humans and surveillance for foodborne hazards in foods have only recently received increased attention. Foodborne hazard surveillance monitors the conditions that can lead to foodborne illnesses (Guzewich et al., 1997). For example, hazard surveillance systems can detect microbial pathogens at various facilities that handle food (e.g., farms, meat and poultry processors, and restaurants). Hazard surveillance typically involves the collection of data on foodborne hazards in food products and food sources, follow-up data when hazards are present at unusual levels, and information that helps define the sources of hazards in foods.

Animal health surveillance as it relates to food safety is a component of foodborne hazard surveillance. Comprehensive animal health surveillance systems were nonexistent until the National Animal Health Monitoring System (NAHMS) was implemented in 1983 (King, 1990). Current resources limit these surveillance programs; the NAHMS on-farm monitoring system does narrowly focused studies of a single species in a particular segment of the production process.

Integrating the information from an on-farm monitoring program such as NAHMS with processing data, retail food surveillance, residue and antimicrobial resistance monitoring, and subsequently with FoodNet information will be critical for the implementation of a true farm-to-table approach to food safety surveillance. Not only will the data be more reliable if a cohesive surveillance system monitors food from the farm to the table, but such a system will likely provide the impetus for a more comprehensive surveillance system in domestic animals (Bush et al., 1990).

The awareness of the need to monitor pathogens in healthy food animals is fairly recent, and monitoring pathogens in the food and water that food animals consume also may be appropriate (Tauxe, 1997). If farms show evidence of increasing pathogen prevalence, then prompt intervention might prevent the pathogens from eventually being consumed by humans.

Effective surveillance for food safety requires the coherent assembly of information from different sources. Integrating animal and environmental surveillance systems into established human surveillance systems will greatly increase our understanding of the epidemiology and sources of foodborne disease. In particular, an independent molecular subtyping system linked to PulseNet has great potential value for evaluating the potential public health significance of pathogens isolated all along the food processing continuum. For example, it

Animal Surveillance for *E. coli* O157:H7

Risk assessments for specific pathogens such as *E. coli* O157:H7 in ground beef require measurements of many parameters at every step from farm to table. Surveillance programs gather these data and reveal useful information about hazards. For example, *E. coli* O157:H7 is widely distributed throughout beef and dairy cattle herds in the United States (Hancock et al., 1998). NAHMS addresses emerging issues such as the association between calf management practices and the presence of *E. coli* O157:H7 in cattle herds (Garber et al., 1995; Losinger et al., 1995). The veterinary Diagnostic Laboratory Reporting System compiles and analyzes reports from state veterinary diagnostic laboratories to assess trends in infectious diseases among food animals (Salman et al., 1988).

To prepare for HACCP introduction in the meat industry, USDA conducted a series of baseline surveys of beef slaughter plants and the ground beef final product. At that time, only 4 (0.2%) of 2,081 steer and heifer carcasses and none of 2,112 cow and bull carcasses were contaminated with *E. coli* O157:H7. Of 563 ground beef samples, 78.6% were contaminated by nonpathogenic *E. coli* but none by *E. coli* O157:H7 (USDA/FSIS, 1996).

More recently, with the aid of much more sensitive detection methods, researchers found EHEC O157 (*E. coli* O157:H7 or O157:nonmotile) in the feces of 27.8% of cattle at a slaughter plant; 10.7% of hides were contaminated, and 43.4% of carcasses were contaminated before evisceration (Elder et al., 2000). Only 17.8% of carcasses were contaminated post-evisceration, and 1.8% of carcass tissues contained EHEC O157 after processing, which demonstrates the effectiveness of plant sanitation processes.

Despite this relative efficacy, USDA detected *E. coli* O157:H7 in ground beef samples at a rate of approximately 8.7 per 1,000 samples in 2001, and *E. coli* O157:H7 contamination of ground beef resulted in 25 recalls during 2001 (USDA/FSIS, 2002). Regulatory agencies and food processors need to work together to advance the scientific understanding of the persistence and transmission of these agents in food production environments.

would be extremely useful for a food processor to be able to evaluate whether a particular environmental strain of *Listeria* isolated from a processing environment had ever been associated with human infections. The technology exists to create such a system. Data privacy and regulatory penalties will need to be modified to encourage the food industry's full participation.

Future Methods: the Promise of Genomics

Since the advent of recombinant DNA technology, a better understanding of how and why pathogens do what they do has emerged. Progress has been steady. In many cases, one gene at a time has given up its secrets, and, in the process of doing so, has presented new puzzles to be solved. The science of genomics is simply the study of the genes of an organism and their function. It is now possible to sequence entire genomes, and this has been accomplished for some significant human pathogens. The process of whole genome sequencing has been accelerated by automation and the application of sophisticated computer technologies (informatics).

Data gathered to date on pathogenic bacteria have already provided revealing insights. For example, nearly one-half of the open reading frames (ORFs) sequenced have no known function. It is clear that we have just begun to understand how these bacteria survive and react to their environment. From comparisons among the complete sequences of bacteria, it is also clear that far more horizontal transmission of genetic material has occurred than previously thought. Horizontal transmission of genes can rapidly transform a commensal bacterium into a potential pathogen through the sharing of large numbers of virulence-related genes (pathogenicity islands) or genes encoding for antibiotic resistance.

Comparisons also enable the generation of hypotheses regarding a bacterium's virulence potential that can then be tested by other traditional laboratory approaches, or by further genetic manipulation. Comparative genomics also may lead to new approaches to phylogenetic classification. An adjunct to genomics is proteomics, the study of the complete protein complement of an organism. Although it might appear that these technologies have opened up a new level of complexity, it is believed by many scientists that this very complexity may yield new ways to control microorganisms that have not yet been conceptualized.

Another exciting area with genomics as the driving force relates to understanding the bacterium's global gene expression while varying the bacterium's environment. This has been made possible by the development of oligonucleotide "chips" or cDNA microarrays that enable "expression profiling," that is, the study of the messenger RNAs, and when and which ones are produced. Microarrays may help unravel the function of the numerous genes whose functions are not yet known. An excellent overview of these technologies was recently presented by Schwartz (2000).

Using DNA microarrays, also known as biochips, research scientists can analyze the transcription profiles of the whole genome for practically any microorganism of interest. For example, in organisms such as *Haemophilus influenzae* and *Streptococcus pneumoniae* scientists have used DNA biochips to map more than 100 genes and their expression profiles. The current detection range has been between one and five transcripts per cell, as confirmed by conventional methods such as Northern blot analysis. Scientists can learn what gene(s) are turned on or off under different conditions by putting (spotting) DNAs representing all ORFs in a bacterial genome and using differentially labelled cDNAs from both wild-type and mutant bacteria.

Microarray technology affords unprecedented opportunities and approaches to diagnostic and detection methods. For example, microarrays can be used to develop rapid identification systems for both pathogenic and spoilage bacteria, to conduct mutation analyses, and to investigate protein-DNA interaction. RNA of a related species can be studied using differential gene expression under less stringent hybridization conditions (referred to as virtual expression arrays). In addition, microarrays will be used in the future to detect organisms or foods modified using recombinant DNA biotechnology. For example, transcript mapping (imaging) of wild-type versus genetically modified organisms can monitor changes in risk-related factors such as virulence genes.

Pathogen identification

New pathogen identification technologies are faster, cheaper, more powerful and increasingly automated. No single method is appropriate for all circumstances, so selection of the best method is important. This technology is changing rapidly, and the following information provides a brief overview of the progress in this area and the future potential.

Until recently, efforts to determine bacterial relatedness relied on techniques that assessed one or more phenotypic markers. These methods include serotyping, phage typing, biotyping, antibiotic susceptibility testing and bacteriocin typing. Now, molecular typing methods can identify different clones (genetically identical organisms descended from a single common ancestor) at the bacterial species level. These molecular techniques are used to physically characterize bacteria based on their DNA composition (genotyping) or on production of proteins, fatty acids, carbohydrates, or other biochemical content (phenotyping or chemotyping).

As technology has evolved, many previously complex processes have been automated, miniaturized and linked to computer control centers that guide the operation, including data analysis. As the technologies have become widespread, researchers are now able to generate more timely data at a lower unit cost. Of particular interest to those involved in the biochemical analysis of microorganisms are procedures that have been adapted or are amenable to whole cell techniques, as they offer all of the conveniences of rapid and economical analysis. Huge libraries of customizable computer databases are now available to assist in pattern recognition for detection and identification of microorganisms based on analysis of whole cells, as well as individual genetic elements or chemical derivatives. The need for quick test results have driven these advancements. Automated methods are now available for detection, identification, typing and analysis of biological components or structural changes that occur due to environmental pressures or extraneous influences. Bench top versions of sophisticated devices allow for more portability and efficient use of laboratory space.

Genotyping Methods

Genotyping has many advantages over traditional typing procedures (Olive and Bean, 1999; Spratt, 1999; Tompkins, 1992; Versalovic et al., 1993). The major advantage lies in its ability to distinguish between two closely related strains. Oth-

er advantages of genotyping include: (1) DNA can always be extracted from bacteria so all strains are theoretically typeable; (2) analytical strategies for the genotypic methods are similar and can be applied to DNA from any source; (3) genotyping procedures do not generally require species-specific reagents and (4) the methods are amenable to automation and statistical data analysis (Arbeit, 1995; Bingen et al,. 1994). Combinations of different genotypic methods can be used to increase the discriminatory power of typing and fingerprinting analyses. Furthermore, selection of the appropriate typing method can allow analysis of groups of bacteria at the appropriate level and rate of change. To illustrate, ribotyping of *Vibrio cholerae* isolates responsible for the 1994 - 1995 cholera epidemic in Ukraine indicated that only a single strain arising from introduction into that country was responsible (Clark et al., 1998). Other more discriminatory methods were used to track the course of the epidemic. A combination of typing or fingerprinting methods may therefore be necessary to fully characterize bacterial populations important to public health.

The most common genotypic methods currently used include:

- chromosomal DNA restriction analysis,
- plasmid typing,
- DNA probe-based hybridizations (such as ribotyping)
- amplified fragment length polymorphism (AFLP)
- PFGE
- PCR-based methods (such as randomly-amplified polymorphic DNA (RAPD), repetitive sequence-based PCR (rep-PCR), PCR-ribotyping and PCR-restriction fragment length polymorphism (PCR-RFLP)), and
- sequence-based methods, including multilocus sequence typing, flagellar locus and flagellar short variable region sequencing (e.g., for *Campylobacter)*, and analysis of DNA sequences of a number of other genes.

PFGE has now been applied to a wide range of microorganisms and has become the genotypic method of choice for many scientists because it is very discriminating, reproducible and broadly applicable. PFGE has recently been used to help in the investigations of widespread foodborne outbreaks involving *Salmonella* (Bender et al., 2001; Van Beneden et al., 1999), *L. monocytogenes* (Graves and Swaminathan, 2001; Ojenivi et al., 2000; Proctor et al., 1995), *E. coli* O157:H7 (Barrett et al., 1994) and viruses (CDC, 2001), and is the method that is currently being used by CDC as the basis for its PulseNet system.

There is a trend to use new diagnostic assays to disclose the presence of pathogenic bacteria in foods or human patients without isolation of the organism. This presents a challenge to microbiologists, in that many of the typing/fingerprinting methods currently in use rely on large quantities of DNA isolated after amplification of the strain of interest. Methodologies based on DNA sequencing after PCR amplification directly from the source material may present at least a partial solution to the problems created by the absence of an isolate. The CDC PulseNet group has, for instance, recently provided funding to interested state laboratories for research into the appropriate genes to be sequenced to allow differentiation of bacterial pathogens of interest.

Biochemical and Chemical Methods

Numerous biochemical techniques offer an alternative to direct nucleic acid fingerprinting. Chemotaxonomy involves the application of chemical and physical manipulations to the analysis of the chemical composition of whole bacterial cells or their cellular components to arrive at some identification or taxonomic positioning. Even with accelerated advances in technology that have allowed for miniaturization and automation of analytical equipment, the bacterial growth period and chemical derivations prior to analysis still remain the ultimate limiting factor with respect to rapid analysis. Thus, methods that are amenable or adaptable to whole cell techniques and require only minute quantities of sample and/or in situ chemical derivations are of particular interest, including:

- mass spectrometry (MS), especially matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry
- pyrolysis (Py),
- gas or liquid chromatography (GC or LC), and
- infrared spectroscopy (IR).

Future Issues

Although genome-based typing methods are increasingly powerful tools in molecular epidemiology, several issues need to be addressed if these methods are to be incorporated more routinely. Firstly, one should not forget about the advantages of classifying organisms to the genus and species level, as well as doing serotyping and phage-typing for some bacteria, before interpreting banding patterns resulting from molecular typing. A good example of this is a 2001 salmonellosis outbreak associated with raw almonds that was detected only because both serotyping and phage-typing of salmonella isolates were done. As well, in the absence of a "gold standard" by which to judge a typing method (van Embden et al., 1993), careful standardization of and adherence to laboratory protocols is essential, if individual methods are to be accepted for classification of strains. The lack of reproducibility of certain techniques is another contentious issue. Consistent reproducibility is essential, if these methods are to be of value in the long-term analysis and categorizing of bacterial strains. Another extremely important issue is in the interpretation of minor (ca. 1 to 3) banding differences between strains. Some scientists argue that a single difference in the production of an enzyme or the shift of a single band on a gel is not enough to say that two isolates are different, and that clonality should be considered as a relative concept (Arbeit, 1995). In addition, strain relatedness should only be judged in the presence of other data, especially epidemiologic data.

The ultimate goal is an "ideal" molecular typing method; one that is easy to perform, cost-effective, relatively rapid, amenable to statistical analysis and automation, able to type all possible strains, reproducible, and properly balanced between increased discriminatory power and applicability. Rapid advances in typing methods based on whole organism DNA sequencing are helping us to approach such an ideal method. In the future, the use of molecular typing in foodborne disease investigations will assist us in identifying the source of many more outbreaks, will lead to the earlier detection of outbreaks, and will be beneficial in identifying and eliminating areas of persistent contamination in food plants.

Next Steps in Food Safety Management

Foodborne illness in the United States is a major and complex problem that is likely to become a greater problem as we become a more global society. To adequately address this complex problem, we need to develop and implement a well conceived strategic approach that quickly and accurately identifies hazards, ranks the hazards by level of importance, and identifies approaches that have the greatest impact on reducing hazards, including strategies to address emerging hazards that were previously unrecognized.

Because certain elements of pathogen evolution are inherently unpredictable, it is impossible to predict, with absolute accuracy, the emerging microbiological food safety issues of the future. However, our knowledge of the current issues and the complex factors that drive changes in microbiological food safety do provide us with a good sense of likely trends. With this knowledge and understanding, we can target our research and surveillance efforts to spot emerging issues as they arise and be prepared to respond quickly and appropriately. Such a response requires a flexible regulatory framework that is based on science.

Recent scientific advances have provided tremendous insight into each of the three factors in foodborne illness, both separately and in combination. Knowledge of the pathogens themselves and their interaction with the microbial environment creates opportunities for both prevention and control. Foodborne illness surveillance systems can benefit from enhanced understanding of pathogen evolution to spot new problems quickly, and, in turn, the outbreak investigation data can provide further insight into the forces that drive pathogen evolution. Policies based on risk assessment and Food Safety Objectives (FSOs) will enable us to better consider the impact of changing population demographics and consumer behaviors. This science-based approach to food safety will require that the education of food safety professionals become more multidisciplinary. These broadly educated professionals will work in partnership with a wide range of experts across government, industry and academia.

To achieve the maximum benefits, our food safety efforts and policies must be carefully prioritized, both in terms of research and in application of controls. As scientific advances provide a better picture of pathogenicity, we must decide whether to focus our efforts on those pathogens that cause many cases of moderate illness or instead focus on those pathogens with the greatest severity, despite the relatively few number of cases. In the move toward making decisions based on risk, our food safety policies need to weigh these issues, and communicate information about risk to all stakeholders, including the public.

Strategic Prioritization to Reduce Foodborne Disease

In an ideal world, gaps in data would be quickly filled by data from high quality research. In reality, our research needs are far greater than our willingness to fund research. To maximize our resources, prioritization of research is essential. Similarly, our efforts at control and prevention should focus first on areas with the greatest impact on public health.

Resource Priorities

The Centers for Disease Control and Prevention (CDC) estimates that 76 million cases of foodborne illness occur annually in the United States. A large number of known pathogens are responsible for 14 million cases; for the other 62 million cases, the pathogen causing the illness is not known. Of the known pathogens, Norwalk-like viruses (9,280,000 cases), *Campylobacter* species (1,963,000 cases), and nontyphoid *Salmonella* (1,332,000 cases) are responsible for most illness, while *Trichinella spiralis*, *Vibrio cholerae*, and *Vibrio vulnificus* are the known pathogens responsible for the fewest cases of illness (approximately 50 cases each) (Mead et al., 1999).

With the large number of pathogens responsible for foodborne illnesses and the apparent lack of a single, all encompassing solution to foodborne disease, how should a public health organization determine its priorities and distribute its resources to have the greatest impact on food safety? The reality is that public health priorities have always been influenced by a crisis like a recent outbreak or by the concerns of special interest groups. However, as a society, we need to balance these influences with a more systematic approach that allocates scarce resources to have the greatest impact on food safety.

Instead, a more strategic approach is needed. Ranking hazards based on quantitative hazard analysis—to identify, in order of importance, those pathogens of principal concern to public health—provides a scientifically based approach for resource allocation. Criteria for such hazard ranking must be established. Examples of suitable criteria include: incidence and severity of illnesses, number and predisposing conditions of high-risk populations, principal risk factors associated with illness, and prevalence and virulence of the pathogen.

Efforts to prioritize public health problems based on more objective criteria have been conducted in the past and may serve as a model for food safety (Murray and Lopez, 1996).

Difficulties exist, however, in weighing various components of public health impact when conducting quantitative hazard analysis for ranking pathogens. For example, *Salmonella* species are responsible for an estimated 1.34 million cases of illness and 553 deaths, with a mortality rate of 0.04%, via a variety of foods of animal and plant origin. Most cases involve mild diarrhea of only a few days duration. The young and elderly populations are at greatest risk for severe symptoms. On the other hand, *V. vulnificus* causes an estimated 47 cases of foodborne illness and 18 deaths annually, principally via raw oysters. Forty percent of the cases involve fulminating septice-

mia that results in death. The population at greatest risk is people with high levels of serum iron. These two very different foodborne diseases illustrate the many factors that must be considered in prioritizing the hazard ranking. Severity of illness, while important, may or may not be the most important factor in the ranking.

Each agent responsible for foodborne illness has unique characteristics that influence its transmission or ability to cause illness (Doyle et al., 1997). Transmission of Norwalk-like viruses is controlled by preventing contamination of food by human feces, whereas transmission of *Campylobacter jejuni* is often controlled by preventing contamination of carcasses by poultry feces. Because there are so many different factors influencing pathogen contamination of foods, no single solution can be broadly applied to eliminate foodborne illness; each agent must be addressed on an individual basis with different procedures for control applied depending on the pathogen.

Creating such a policy framework will not be an easy task. Tailored regulatory responses that react to newly recognized hazards with the best science available at the time may be criticized as premature or arbitrary regulatory enforcement that creates uneven economic burdens within the food industry. But the alternative is waiting until there is significant scientific information and applying it in a uniform manner to all foods, whether they pose public health hazards or not. Although this approach may be politically easier, it fails to maximize public health protection.

Strategic Control Measures

Because each pathogen must be addressed individually, a strategic approach to applying control measures is necessary. Within a strategic approach, intervention strategies identify points at which control measures will have the greatest influence on providing safe foods. To identify and rank these points, microbial risk assessments are conducted. The risk assessments involve systematically collecting and analyzing exposure and dose-response data. Case-control studies and other epidemiologic research approaches are helpful in identifying risk factors in sporadic infections and outbreaks.

The U.S. Department of Agriculture (USDA) and the Food and Drug Administration (FDA) have used this general approach to develop critical food safety information regarding the control of pathogens in specific foods. For example, risk assessments of *Salmonella* Enteritidis in eggs, *Escherichia coli* O157:H7 in ground beef, *V. parahaemolyticus* in raw molluscan shellfish, and *Listeria monocytogenes* in ready-to-eat (RTE) foods have been drafted. When sufficient data are available, quantitative risk assessments can: identify what foods are of greatest risk and contribute most to specific foodborne illnesses, estimate the levels of pathogens in foods that are unsafe, and identify what points within the food continuum have the greatest influence on exacerbating or preventing foodborne illnesses.

Examples of the use of case-control studies to identify risk factors for sporadic illnesses include *E. coli* O157:H7, *Campylobacter*, and *Cryptosporidium*. Major risk factors associated with sporadic cases of *E. coli* O157:H7 infection in the United States are eating undercooked ground beef and visiting a farm. Risk factors for *E. coli* O157:H7 infection in Scotland are handling/preparing raw food (40%), being involved in gardening/garden play (36%), living on or visiting a farm (20%), having direct/indirect contact with animal manure (17%), having private water supplies (12%), and recent failures with high coliform counts of water supplies (12%) (Coia et al., 1998). Risk factors for sporadic *Campylobacter* infections in the United States, identified in a case-control study of six FoodNet sites from January 1998 through March 1999 involving 1,463 patients with *Campylobacter* infection and 1,317 controls included: foreign travel, eating undercooked poultry, eating chicken or turkey cooked outside the home, eating non-poultry meat cooked outside the home, eating raw seafood, drinking raw milk, living on or visiting a farm, contact with farm animals, and contact with puppies (Friedman et al., 2000). Risk factors associated with cryptosporidiosis cases in Minnesota from July 1-December 31, 1998, were swimming in public pools (e.g., hotel or school pools), drinking well water, visiting a farm, living on a farm for those less than age 6, and exposure to cattle and to manure for those not living on a farm (Soderlund et al., 2000). The underlying vehicle largely responsible for transmitting these pathogens to humans is contaminated manure.

The vast quantities of manure produced each year as a by-product of animal agriculture present a challenge. Cattle, hogs, chickens and turkey produced an estimated 1.37 billion tons of manure in 1997 (U.S. Senate Agriculture Committee, 1998). Because many of the most prominent foodborne pathogens in the United States, including *C. jejuni*, *Salmonella*, and *E. coli* O157:H7, are carried by livestock and are principally transmitted to foods by fecal contamination, the amount of manure created in the United States is a growing environmental threat.

Manure-related food safety issues on the near term horizon include issues related to fresh produce and organic produce in particular. For example, recent outbreaks of *E. coli* O157:H7 infection and salmonellosis have been associated with organically produced alfalfa and clover sprouts and mesclun lettuce. Use of contaminated cow manure is a major concern. The lack of an established, proven composting protocol to assure elimination of pathogens and prevent recontamination contributes to this concern. Another issue on the horizon is the importation of fruits and vegetables from countries with poor agricultural practices, i.e., use of contaminated irrigation water, improper preparation and application of manure as fertilizer, and harvesting and washing produce under unsanitary conditions.

Food irradiation has received considerable attention as a means to address food safety issues. The suggestion, however, that irradiation is a single solution to eliminating most pathogens associated with fresh or RTE foods lacks foundation. For some foods, irradiation results in foods with unacceptable sensory characteristics (Olson, 1998). Irradiation is a tool with broad applicability, but it is not a comprehensive solution for all infectious foodborne hazards in all foods.

Emerging Pathogens

Unfortunately, pathogens can be addressed only after they evolve. Consider, for example, the relatively recent identification of *E. coli* O157:H7. *E. coli* O157:H7 received relatively little attention from food safety scientists and the medical community for more than a decade after its discovery. It was not until 1993, following a large outbreak involving more than 700 patients infected by eating undercooked fast-food hamburgers, that this pathogen rose to promi-

nence as a major food safety issue (Doyle et al., 1997). USDA established a "zero tolerance" policy for *E. coli* O157:H7 in ground beef, the first rule to "outlaw" the presence of a pathogen in a raw food (Griffin, 1998). Although some improvement has been made, the policy clearly has not resolved the problem, as illnesses associated with ground beef continue to occur. An estimated 73,500 cases of *E. coli* O157:H7 infection (both food- and nonfood-related) occur annually in the United States. Many outbreaks are associated with swimming in recreational lakes, drinking contaminated water, handling animals, and consuming contaminated foods, including alfalfa sprouts, lettuce, unpasteurized apple juice, coleslaw, and undercooked ground beef (Doyle et al., 1997; Griffin, 1998).

Although the emergence of foodborne pathogens similar to *E. coli* O157:H7 cannot be anticipated, a well-conceived plan should be in place to address these issues as they arise. Essential information needed to assess the significance and likely impact of the pathogen as an agent of foodborne disease is often unavailable for a hazard analysis. Scientists need to know the pathogen's reservoir, prevalence, virulence, ability to survive in different environments, and association with human illness. Also, sensitive methods to detect the pathogen are often lacking. A framework is needed to identify and prioritize the information required for a hazard analysis and a subsequent quantitative microbial risk assessment. Public health agencies should be prepared to quickly obtain the essential information to complete a hazard analysis and, depending on the degree of hazard and available data, a risk assessment.

Outbreak Investigation

A comprehensive system of foodborne disease surveillance must include a system for detecting and rapidly responding to potential outbreaks. Outbreaks caused by specific foodborne pathogens, such as *Salmonella* and *E. coli* O157:H7, may be identified by detecting unusual case clusters or increased occurrence of cases by routine surveillance of cases reported by medical clinics and clinical laboratories. Recently, molecular subtyping of isolates by pulsed-field gel electrophoresis (PFGE) has increased both the sensitivity and specificity of pathogen-specific surveillance for detecting outbreaks caused by relatively common pathogens, such as *Salmonella* Typhimurium (Bender et al., 2001). Routine subtyping and transmission of subtype patterns through electronic communication networks, such as PulseNet, creates the potential to detect widely dispersed outbreaks that might not be recognized in any individual state (Swaminathan et al., 2001). Investigation of these outbreaks is required to determine the source and mode of transmission of the outbreak-associated strain.

In addition to detecting outbreaks through laboratory-based surveillance, outbreaks may also be recognized because of the occurrence of similar illnesses among persons who attended an event or establishment together. Many of these outbreaks are recognized before a causative agent has been diagnosed. Thus, investigation of these outbreaks must be conducted to identify the agent as well as the source and mode of transmission.

Outbreak investigations require the close collaboration of epidemiologists, environmental health specialists and public health laboratories. Collection of stool samples and interviews of ill persons and healthy comparison groups must be conducted rapidly. Epidemiologists must coordinate their activities with the public health laboratories to maximize the potential to isolate the agent. Information collected by epidemiologists can help guide environmental health evaluation of an establishment and interviews of food service workers. Results of environmental health evaluations can further guide epidemiologic investigations. Epidemiologic data need to be analyzed and interpreted in light of the results of laboratory tests and environmental investigations. Strategies for outbreak control and prevention need to be identified and implemented as soon as can be justified by the results of the investigation. Depending on the scope of the outbreak and nature of the response, coordination with other state and local agencies, FDA, USDA, and CDC may be needed.

Most outbreak investigations are initiated by local or state health departments. Because outbreak investigations are complex activities that need to be rapid, thorough, and well-coordinated, CDC issued a report (CDC/NCID/DBMC, 2000) intended to assist state and local health departments assess their outbreak response capacities and to help guide them in developing and strengthening their foodborne disease surveillance programs. The core components required for outbreak investigations include: epidemiology, food protection programs, and public health laboratories. The essential element to improving foodborne outbreak investigations is the capacity to respond quickly and comprehensively to the occurrence of suspected foodborne illness.

National Initiatives

Foodborne illness has no easy solutions. However, major strides can be made by developing and implementing a well-conceived strategic approach that prioritizes the hazards and defines the strategies that will most effectively reduce hazards. This approach must include a strategy to address emerging hazards. This strategic approach should be a national initiative that includes state, local, and international involvement, and perhaps reorganizes existing federal food safety agencies and programs.

The importance of an expanded surveillance system that covers animal health and the environment cannot be overstated. The additional information from an expanded and coordinated surveillance system would enable a broader vision of the flow of pathogens and potential pathogens throughout the food chain, and it would fill some important data gaps in risk assessment. Coupled with the new genetic tools, potential foodborne pathogens may be detected before they cause confirmed human illness. While it may be politically or economically impractical to respond vigorously to a likely pathogen before cases are identified and linked to the food/pathogen combination, prior knowledge of the potential pathogen will decrease response time and enable a more appropriate first response. Expanded surveillance will require state and local participation, coupled with leadership and coordination at the national level. Thus, expanded surveillance should be part of any national initiative. These activities also are consistent with international efforts being planned by the World Health Organization (WHO) towards establishing a coordinated, expanded, worldwide surveillance system (Archer, 2001).

Strategies for the Future

The complex interrelationship of the pathogen, host, and microbial ecology ensures a role for everyone in food safety

management: government, industry, and consumers. A flexible, science-based approach that relies on all parties to fulfill their role is our best weapon against emerging microbiological food safety issues.

Role of Government and Industry

Developing a strategic, science-based approach that prioritizes our resources will not be an easy task. Quantitative risk assessment must be based on data, but our current system does not effectively encourage data generation and sharing. The regulatory framework must be structured to allow the food industry to generate and share data and information with the regulatory agencies. In addition, a science-based program will necessarily involve acceptance of some level of risk, because zero risk is not achievable. Using the FSO approach will enable us to translate our public health goals into achievable standards that are based on science.

Addressing consumer attitudes will present a substantial challenge. Naturally, we all desire the minimum possible risk that can be reasonably achieved. Achieving consensus on an appropriate level of risk will be difficult. Risk communication and modification of perception and behavior will need to be considered an important part of any move to a risk-based food safety policy.

Food manufacturers must accept their role in microbiological food safety and achieving public health goals. Rapid response to a new food safety issue may require investing money for controls before the scientific data are complete. In exchange for flexibility, food manufacturers must be willing to work as partners with regulatory officials, sharing scientific information and data to develop appropriate food safety policies.

Developing and implementing these new, risk-based policies will require food safety professionals with a broad understanding of many scientific disciplines and subjects. Changes in how we educate food safety professionals will ensure they have the knowledge and the skills to maximize the effectiveness of new tools and methods.

Data Sharing and Cooperation

For risk assessments that are be based on the best data available and translate into the soundest science-based decisions possible, ways need to be found to access data from food manufacturers. This is far from simple under current conditions. From their own quality assurance (in-line, and environmental) and/or finished product monitoring programs, manufacturers gather huge amounts of data. If they were available, these data could provide valuable exposure information to risk assessors and information on the prevalence of pathogens in various food processing environments. Food manufacturers currently do not often share such data or even collect potentially useful information, because of potential regulatory ramifications or for product liability reasons. Ways must be found to collect and share this information in a penalty-free manner.

For example, food producers concerned with *L. monocytogenes* are hesitant to test below the genus level, such that data show only *Listeria* spp. While somewhat useful, further speciation and subtyping could yield even more useful data, but the finding of *L. monocytogenes* in a finished, RTE food would result in regulatory action under current policy of both FDA and USDA. Knowing how the presence of other *Listeria* species in food or the processing environment relates to the possible presence of *L. monocytogenes* is thus not achievable.

Recent studies question whether all subspecies of *L. monocytogenes* are virulent, or of equal virulence (Wiedmann et al., 1997). Perhaps this finding, when further developed, will help define more appropriate policies that foster collection of good and meaningful data. Additionally, knowing that the presence of *L. monocytogenes* alone may not necessarily mean the food is potentially harmful may be an incentive to manufacturers to speciate further, and to apply methods that determine or indicate virulence or lack of virulence.

Interdisciplinary Research. A growing area in federal research funding has been the formation of interdisciplinary teams to examine complex problems. This shift from more traditional projects with a single researcher has reached all the major federal funding agencies. Some of these new interdisciplinary programs have been highly successful, most notably in the areas of vaccine development, epidemiologic surveillance, and genome sequencing. These different program structures have created new paradigms for the generation and sharing of data that provide added benefit for detecting the emergence of pathogens, spotting new transmission patterns, or predicting the effects of new production technologies.

Pre-harvest Safety. In the last 10 years, several teams have been formed to focus on the microbiological aspects of pre-harvest food safety. These teams typically address animal production but recently have also targeted produce. The main emphasis of these teams has been to define the existing problems, typically with the use of epidemiologic surveys, and to then develop and test intervention strategies. A good example is the efforts to develop pre-harvest interventions for *E. coli* O157:H7 in the beef production industry. Such teams usually comprise veterinary microbiologists, veterinarians, food microbiologists, animal scientists, and epidemiologists. The studies usually use epidemiologic surveillance methods to identify potential intervention points in current animal production methods.

These systematically designed studies generate large microbial strain sets. Currently, these strains are often logged and stored, without generating much additional data, save for occasional heroic efforts to perform modest genotyping studies. However, such strain sets and the associated samples hold much information about the impact of different factors on populations of pathogens and commensal organisms. This information could be mined in collaboration with population geneticists and genome researchers to examine the relationships between microbial population landscapes, genome evolution, and ecology.

Sanitation Assurance. Surveillance studies of pathogens and indicator organisms in food production facilities are a part of industry sanitation programs. These programs are designed to identify in-house events, catching potential hazards before they develop or become established in the production line. A carefully designed sampling regimen, coupled with the appropriate statistical methods for data mining, could provide a tremendous amount of information regarding the nature of hazardous events and the identification of previously unknown hazards. Moreover, inclusion of high-throughput genome studies on populations of pathogens or indicator organisms would again provide a wealth of added information regarding evolution and ecology of microorganisms in food production settings. In this context, combining information from several different producers could provide public

A Cooperative Approach to the Safety of Sprouts

Outbreaks of foodborne illness associated with the consumption of raw vegetable sprouts are a recently emerged food safety issue addressed using a combination of industry and regulatory action.

Sprouts can harbor large populations of microorganisms, because the conditions used for sprouting seeds also promote rapid microbial growth. If present on the seeds, pathogens grow to high levels. In the 1980s and 1990s, consumption of fresh, uncooked vegetable sprouts became popular, and commercial sprout suppliers developed broad distribution systems. The number of reported illnesses increased significantly. In 1997-1998 alone, at least 7 documented outbreaks of *Salmonella* and *E. coli* O157:H7 infections were caused by consumption of various types of raw vegetable sprouts. One of these outbreaks, which occurred in Japan, was the largest outbreak of *E. coli* O157:H7 ever recorded (Taormina et al., 1999).

The responsiveness and coordinated efforts of our institutions are critical factors in understanding and gaining control of an emerging food safety issue. In the case of sprouts, federal and state government agencies worked cooperatively with industry and academic sectors to respond. CDC and FDA met with sprout industry representatives in 1995 to discuss food safety concerns. In 1997, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) was asked to review available data and to formulate science-based recommendations to enhance the safety of sprouts.

A 1998 public meeting gathered sprout suppliers and trade organizations, consumer groups, academic research scientists, federal and state government research scientists, and public health officials to discuss the current scientific data and possible food safety strategies. At that meeting, participants shared information about epidemiologic and outbreak data, agricultural and sprouting practices, pathogen detection methodology, and disinfection and control measures. In that same year, the California Environmental Protection Agency, acting on a request from the California Department of Health Services, issued a special local need registration that allowed seeds to be treated with 20,000 ppm calcium hypochlorite to kill pathogens. Federal and state agencies issued consumer advisories to warn at-risk populations (children, elderly, and those with compromised immune systems) to avoid consumption of raw sprouts. Also in 1998, the National Center for Food Safety and Toxicology, a food safety research consortium comprised of industry, FDA and academic participants, formed a Sprouts Task Force to identify data gaps and prioritize short-term research goals.

All of these activities, along with the NACMCF report (1999), formed the scientific underpinnings for regulatory guidance (FDA, 1999) with recommendations for the sprout industry that were based on the best available scientific information at the time. To reduce safety risks associated with sprouts, the guidance documents promoted the combined approaches of good agricultural and manufacturing practices, seed disinfection, and rapid pathogen testing of spent irrigation water.

health officials with further information about geographic and other factors associated with the emergence and spread of populations of problematic organisms.

Food Safety Education

New integrative educational approaches that directly link the basic and applied sciences will be necessary to effectively train the future food safety professional. For instance, tomorrow's food safety professional must be knowledgeable in basic sciences such as microbiology and toxicology, yet also understand the entire food safety continuum and be able to address issues wherever they occur along that continuum. And because microbiology and toxicology are intimately tied to newer disciplines such as molecular biology, genomic sciences, and mathematical modeling, extensive subdiscipline training remains crucial.

This new approach will focus on preparation of professionals who, in addition to expertise in their primary discipline, also are grounded in supporting food safety areas such as veterinary, agronomic, environmental and public health practices. Although it is difficult for a single campus to provide such broad training, yet through creative collaborations, new food safety curricula already are developing. These programs make aggressive use of distance learning technologies; emphasize critical thinking skills, professional development and ethics training; provide practical field experience through summer internships; and devote special attention to diversity issues to create a highly trained and well-represented work force (Jaykus and Ward, 1999). Food science departments around the country are uniquely positioned to offer the strongest and most comprehensive leadership in the development of the integrated graduate food safety education programs that will provide the larger, multidisciplinary workforce needed to address emerging food safety issues.

Role of Consumer Understanding

For the last 30 years, the dominant food safety message has been that the United States has the world's safest food supply. As a result, most consumers believe that there is in place an extensive system of controls applied throughout the food production and distribution system, guaranteed ultimately by government oversight, and that this system protects them against well-recognized and emerging foodborne disease. One of the consequences of this confidence is that food safety problems may be seen only as defects of the system to be fixed by strengthening the system of controls and

government action, rather than also as problems with a strong component of consumer-based risk reduction or risk avoidance.

Studies indicate that 80% of consumers think food safety problems are mainly due to failures in food processing, food distribution and food preparation in restaurants; in other words, consumers believe the failures are occurring in the most regulated parts of the food safety system where they have little direct responsibility. Relatively few consumers perceive food safety problems due to actions in the home or in supermarkets—the final stage of the food safety system—or on farms—the beginning of the system (Levy, 1997; Penner et al., 1985; Williamson, 1991). To achieve a truly farm-to-table approach to maximizing food safety, it is important to consider potential contributions from all segments of the food chain.

Since 1993, the Food Marketing Institute Trends Survey has asked consumers an open-ended question about the greatest threats to food safety to measure top of the mind awareness of different possible sources of food safety problems. The number of respondents who mentioned improper quality control/shipping/handling and storage rose from 9 percent in 1993 to 34 percent in 1997. During the same time period, the number of people mentioning food preparation declined from 12 percent to 2 percent.

Consumer Behavior

Research indicates that people consider themselves fairly knowledgeable about food safety guidelines, and for the most part they are. However, as in other areas of health and safety, knowledge and awareness does not always translate into behavioral changes. Between 1988 and 1993, indicators of concern about food increased significantly, suggesting an emerging public awareness and interest in food safety problems. At the same time, data suggest that unsafe food consumption and preparation behavior actually increased (Levy, 1997).

Behavioral surveillance systems can provide data identifying people or groups in which behaviors associated with foodborne diseases are more common and who are at higher risk for foodborne illness, thus assisting in the development of food safety education programs (Yang et al., 1998). Further, surveillance data can be used to evaluate the progress of education programs (Altekruse et al., 1999; Yang et al, 1998). Data collected through the Behavioral Risk Factor Surveillance Systems during 1995-1996, which included 19,356 survey participants, showed that several high risk food handling, preparation, and consumption behaviors were common, and some varied by gender, age, race/ethnicity, education and income. For example, 50.2% of respondents reported eating undercooked eggs, and 19.7% reported eating undercooked hamburgers. All high risk food handling, preparation, and consumption behaviors were more prevalent in men than in women. The prevalence of reported consumption of undercooked hamburgers decreased with age, increased with education, and increased with income.

Decisions about behavior frequently are guided by risk perception rather than risk awareness (Frewer et al., 1994). Defining risk as "hazard + outrage," Sandman (1997) stated that when people misperceive hazards it is often because they are outraged. Sandman noted that generally, even when the hazard is serious, the public is apathetic and the least dangerous hazard often generates the greatest outrage. He said, "Too often, experts focus on the hazard and ignore the outrage while the public focuses on the outrage and ignores the hazard." Under these circumstances, the hazard cannot be mitigated without addressing their outrage. Sandman suggested that experts determine why the outrage is high and what can be done to lower it so that people want to hear or acknowledge the extent of the hazard. As an example, Sandman stated that "consumers know how to cook and generally will get angry if you tell them how to do something they already know about."

If people do not recognize and accept their role in food safety problems, behavior change is unlikely. One way to break through public misconceptions is to describe the magnitude of food safety problems and challenge people's understanding of themselves as experts. New data from the FoodNet surveillance system may be the best way to challenge people's understanding of themselves as experts (Levy, 1997).

Consumer Education

Recent federal initiatives have sought to improve the safety of the U.S. food supply using a farm-to-table approach, recognizing that food safety is not only the responsibility of the federal government, but is the shared responsibility of all components of the food system from primary producers to consumers. Consumer education about risk reduction will be a valuable component of an FSO program. Consumers will need to understand their role in preventing foodborne illness.

Numerous sources provide a wealth of information about food safety and other food-related issues in many formats to meet the needs of various audiences. Different information sources serve different needs, and the effectiveness is not equal.

Consumer Information Sources. Surveys indicate that people get most of their information about food safety from electronic and print news media; additional information sources include labels and food packages, regulatory agencies, and cookbooks (Hingley, 1997; Levy, 1997).

A national educational campaign of the Partnership for Food Safety Education (a public-private partnership of the federal government, food industry, and consumer organizations), FightBAC!™, was created in 1996 to conduct broad-based food safety education designed to reach people of all ages. The FightBAC!™ campaign has produced multiple educational tools used through many information channels, i.e., public service announcements, the Internet, point of purchase materials, and school and community outreach.

The National Food Safety Information Network formed in 1998 by FDA's Center for Food Safety and Applied Nutrition (CFSAN) and USDA's Food Safety Inspection Service (FSIS) and National Agricultural Library reaches consumers with information on food-related issues and safe food handling via USDA's Meat and Poultry Hotline, CFSAN's Outreach Information Center, USDA/FDA's Foodborne Illness Educational Information Center, the foodsafety.gov web site, EdNet (food safety educators' network), and the Foodsafe listserve. Considered the "gateway to government food safety information," the www.foodsafety.gov web site provides the public access to advice pertaining to specific population subgroups (e.g., children, people with immune diseases) as well as product specific advice (e.g., refrigerated RTE foods). CFSAN and FSIS also have conducted public awareness campaigns.

For example, CFSAN developed materials (press kits, consumer brochure, video news release, and public service announcement) explaining the risk that unpasteurized or untreated juices may pose to vulnerable populations. The materials were targeted to a variety of audiences (senior citizen groups, day care centers, elementary schools, and PTA offices). Similarly, FSIS launched a "Thermy the Thermometer" campaign in 2000 to encourage use of thermometers to ensure sufficient cooking of meat and poultry.

Consumer Trust in Information. Consumers place different levels of trust in information from different sources. A survey of more than 1000 Americans conducted by the University of Kentucky found that more consumers "completely trusted" the accuracy of the information from government publications and food labels than from any other source (Buzby and Ready, 1996). Of those respondents who trusted food safety information from government publications, 10.8% trusted the accuracy completely. Of those who trusted food safety information on food packaging and labels, 10.2 percent did so completely. Consumers' complete trust of store brochures and advertisements was lower than other sources, 3% and 1.4% respectively. The authors stated that it is not surprising that these were the least trusted of the seven sources of food-safety information, because people may feel that advertisers have incentives to make positive claims about their products.

Addressing the data on how competing motivations, risk perception, and taste preferences affect hamburger preparation, Ralston et al. (2000) reported that several information channels appear to be effective for communicating the risks of unsafe food preparation. Respondents who said they get their information from magazines, television, cookbooks, or government hotlines had 15-17% higher risk motivation, i.e., were more risk averse, than those who did not cite these sources of information. Respondents who said that they get information from labels did not have a higher risk motivation index after accounting for other factors that also increase awareness. Consumers who cited brochures as their information source had lower risk motivation than respondents who did not. Ralston et al. (2000) noted that it is difficult to separate the effects of different forms of information because consumers are exposed to several information sources simultaneously and information sources may interact in their effect on perceptions. The authors concluded that the results show that consumers who are more aware of risk from undercooked hamburger are more likely to adopt safe behavior and thus contribute to a reduction of foodborne disease.

Risk Communication

Risk communication is a necessary and critical tool to appropriately define issues and to produce the best risk management decisions (FAO/WHO, 1998). Risk communication has been defined as the interactive exchange of information and opinions concerning risk and risk management among risk assessors, risk managers, consumers and other interested parties (CAC, 1997b). Others have added risk-related factors to the definition, reflecting a wider risk communication concept (FAO/WHO, 1998).

Several factors play a role in understanding and communicating risk: whether a risk is voluntary or involuntary, whether the distribution of risk and benefit is equitable, the degree of personal control, the individual dread of the adverse event, the catastrophic potential of the event, and the extent of trust in the risk managers (Covello et al., 1988; NRC, 1989; Sandman, 1987). A Consultation of the Food and Agricultural Organization (FAO) of the United Nations and WHO (FAO/WHO, 1998) described these principles of risk communication: (1) know the audience, (2) involve the scientific experts, (3) establish expertise in communication, (4) be a credible source of information, (5) share responsibility, (6) differentiate between science and value judgment, (7) assure transparency, and (8) put the risk in perspective. Barriers to effective risk communication can occur within the risk analysis process (e.g., inadequate access to vital information and inadequate participation of interested parties) or in a broader context (e.g., differing perceptions among participants, limited understanding of the scientific process, lack of credibility of the information source, and societal characteristics). Elements of effective risk communication include:

- the nature of the risk (e.g., its magnitude and severity);
- the nature of the benefits (e.g., who benefits and in what ways);
- uncertainties in risk assessment (e.g., the methods used to assess the risk and weaknesses in the available data); and
- risk management options (e.g., action taken to control the risk and action individuals may take to reduce personal risk) (FAO/WHO, 1998).

A National Research Council Committee on Risk Perception and Communication (NRC, 1989) addressed ways to improve risk communication. The committee noted that it is a mistake to expect the public to always want simple answers about risk; often, at least part of the public desires considerable detailed information about risks. The committee concluded that successful risk communication improves or increases the base of accurate information that decision makers use, whether they are government officials, industry managers, or individual citizens, and, at the same time, satisfies those involved that they are adequately informed within the limits of available knowledge. Further, the committee explained that because risk communication is tightly linked to the management of risks, solutions to the problems of risk communication often entail changes in risk management and risk analysis. In moving toward risk-based food safety policies, risk communication with all interested parties, including risk assessors, risk managers, and the public, will be an important part of the process (NRC, 1989).

The FAO/WHO Consultation also identified several considerations for framing risk communication strategies. From an international perspective, addressing the critical role of effective communication in determining equivalence of food control measures in different countries is a consideration. From an industry perspective, labeling is a consideration. The consultation recommended that if consumer food handling, storage or other practices can assist in controlling a foodborne illness or disease outbreak, clear instructions using unambiguous language should be presented. The effectiveness of labeling as a risk management/communication strategy, however, needs further study. The consultation recommended that labeling—which has been used extensively to convey information such as product composition, nutrition, weights and measures, and health-related warnings—should not be used as a substitute for

consumer education. The consultation also stated that because national governments are responsible for food quality and safety and are the primary sources for risk communication with the public on food safety issues, the capability to effectively communicate risks should be one of the highest priorities for these agencies.

It is important that risk communication involve effective dialogue, a two-way exchange, among interested parties (NRC, 1989). Effective dialogue goes beyond passively providing access to the risk message formation process, e.g., via pro forma public hearings, to including early in the process all interested and affected groups and comprehending the range of potentially contending viewpoints (NRC, 1989).

Addressing the importance of broad participation in communication of interested and affected parties, a National Research Council (NRC) Committee on Risk Characterization reported (NRC, 1996) that deliberation is intimately connected with and as important as analysis in understanding risks. The committee stated that analysis and deliberation can be thought of as two complementary approaches to gaining knowledge of the world, forming understandings on the basis of knowledge and reaching agreement among people. Defined by the committee as any formal or informal process for communication and for raising and collectively considering issues, deliberation is important in risk decision-making for its role in considering conflicts of values and interests. A variety of techniques are used for deliberation and public participation. These include citizen advisory committees and task forces, alternative dispute resolution, citizens juries and panels, surveys, focus groups, interactive technology-based approaches, and combinations of methods (NRC, 1996).

Risk communication takes place at local, national, and international levels. On an international scale, risk communication on food safety occurs within Codex Alimentarius Commission, its subsidiary bodies, and its United Nations parent organizations, FAO and WHO, and their expert advisory groups. The Sanitary and Phytosanitary Measures Agreement of the World Trade Organization encourages harmonization and places a strong emphasis on risk communication principles of transparency and consistency in the development and application of food safety measures (FAO/WHO, 1998) and refers to Codex standards as international benchmarks for nations.

Anticipating the Future: Food Safety Issues on the Horizon

Looking ahead, and considering the content of this report, several food safety issues are likely to come to the forefront in the next decade.

Globalization of the Food Supply

FDA electronically screened all 2.7 million entries of imported foods under its jurisdiction in fiscal year 1997, and physically inspected 1.7%, or 46,000 entries. FSIS visually inspected all 118,000 entries of imported meat and poultry under its jurisdiction in calendar year 1997, and conducted further physical examination of about 20% of entries. These numbers of entries will only increase in the future, and this brings into question how the regulatory agencies will handle the increases most effectively and efficiently. The shortcomings of sampling and analysis for the ever increasing list of pathogens, natural toxins, or pesticide and industrial chemicals suggest that different approaches must be sought. HACCP is gaining recognition worldwide as a desirable system of safety assurance, but for international trade, mutual recognition of HACCP systems must be sought.

The demand for year-round fresh fruits and vegetables is firmly established in the United States. Again, the volume of fresh produce being offered for entry into the United States will only continue to grow, as will the variety of produce offered. Without mutual understanding and application of good agricultural practices, resulting in mutual recognition of systems to assure safety, regulatory agencies will be further stressed.

Alternative Processing Technologies and Novel Foods

Novel foods and alternative processing technologies will continue to appear. With each new introduction, we must consider the possible consequences, intended and unintended, within the food system. Some technologies will reduce or eliminate microbiological hazards inherently present in current food safety systems. For example, treatment of a raw vegetable with a new chemical disinfectant might eliminate concern over certain pathogens of manure origin. However, this treatment also might inadvertently select for unidentified microorganisms that were previously inconsequential but that become hazardous without normal microbial competition to keep their numbers in check. The complex relationship between various factors cannot be overlooked.

Similarly, the introduction of any novel food requires a full analysis to assess the potential microbial hazards. The hazard analysis must be broad enough to consider all the intrinsic and extrinsic conditions that influence pathogens known to be associated with foods, ingredients or processes related to the novel food being introduced. The analysis also must consider microorganisms unique to the new situation that pose a threat to safety of the novel food.

Similar considerations are essential with the introduction of any alternative technology or combination of various alternative technologies and/or preservatives. It is essential to identify the pathogens that are most resistant to the alternative technology, determine mechanisms of inactivation or control including required conditions and kinetics, identify validation procedures, and describe critical process factors.

Scientists continue to be challenged to adequately address all the parameters associated with the introduction of a novel food or alternative processing technology. Once developed, new technologies must be appropriately regulated to ensure their proper application and acceptance by the public.

Increases in Organic Foods

Organic foods are becoming mainstream items in most grocery stores, and it is likely that this segment of the fresh produce industry will continue to grow. With or without facts to back up the assumption, consumers assume organic produce is more nutritionally complete and safer than conventionally grown produce. Recent outbreaks of salmonellosis and *E. coli* O157:H7 infection associated with organically produced sprouts and mesclun lettuce grown and distributed in the United States are evidence of an emerging problem (Griffin, 1998; Hilborn et al., 1999). Cow ma-

nure is a well-documented vehicle for *Salmonella* and *E. coli* O157:H7, and its use in produce production must be controlled to prevent contamination. With an estimated 1.2 billion tons of manure produced by cattle annually in the United States (U.S. Senate Agriculture Committee, 1998), this voluminous source of foodborne pathogens is likely to be an influential factor in the transmission of foodborne illness for the foreseeable future. Methods are needed to reduce the shedding of pathogens in livestock and poultry and to identify effective procedures for eliminating pathogens in manure before they contaminate the environment and food.

Changes in Food Consumption

The global trade in food stuffs is only one force in people's changing dietary patterns. Certainly the variety of available food has expanded drastically and will continue to do so. Ethnic foods are increasingly popular, and the percentage of foods prepared and/or consumed outside the home will continue to rise. Fruits and vegetables are likely to constitute a greater portion of the average diet, and the consumer demand for "fresh" products will lead to more minimally processed foods. Our control and prevention methods will need to be adapted to these changing dynamics.

At-Risk Subpopulations

It is likely that the number of persons at higher risk for foodborne disease agents will continue to increase with time. The population of the United States is aging, and clearly aging is a risk factor for more serious outcomes from agents such as *Salmonella*. As people live longer, they may develop more chronic underlying illnesses that predispose them to foodborne illness. Increasingly complex combinations of drugs to treat various conditions in the elderly can have unpredictable effects on susceptibility. Formerly fatal conditions, such as loss of major organ function, are now survivable thanks to organ transplants. The numbers of transplant recipients will likely increase with time, but it is important to remember that these individuals may be among the most susceptible populations to certain foodborne pathogens.

Pathogen Evolution

Microbial evolution has always happened and will always happen. Bacteria, for example, have an enormous capacity for mutation, integration of new genetic material, and recombination of genetic material in order to assure survival. Bacteria can sense and react to their environment and genetically change themselves in response. Unfortunately, newly evolved pathogens are first recognized when they cause an outbreak of illness. Using new technologies and genomics, perhaps surveillance of food animals and the environment for newly emerging microorganisms with pathogenic potential will become a reality in the future, and there will be no need to wait for human illness. Another hope for the future is a better understanding of how human activities affect foodborne pathogens. For example, does the cross protection afforded a pathogen by exposure to an environmental insult have a negative impact on further processing? Genomics may also provide a better snapshot of how a microorganism manifests virulence, and even help determine why and what to do about it.

Consumer Understanding

Although consumers are only a small part of the food safety chain, as consumers we all need to take responsibility for our contribution to food safety. Those that have not already done so must accept that zero risk is not a reality. These two concepts may be difficult for some consumers to accept. Education and risk communication will be necessary to provide consumers with a more accurate perception of food safety risks and to encourage behavior modification, where needed.

Integrated Food Safety System

A farm-to-table food safety system must involve many interested parties working together toward a common goal. When properly applied, the FSO approach would incorporate input from all stakeholders in developing the appropriate levels of protection. Although regulatory oversight is necessary to monitor and enforce the performance of the food safety system, food manufacturers must play an important role as well, because they have first-hand information about food safety hazards and the production environment. A partnership environment will enhance data sharing and provide a solid scientific basis for policies. An ideal system identifies hazards, institutes appropriate controls in a flexible manner through FSOs, and monitors the operation of the system. The challenge is to build a system that applies science in a predictable, consistent, and transparent manner to enable harmonization within and between countries.

Conclusions

History has demonstrated that science, when appropriately applied through food safety management policies, can dramatically improve food safety. The past century produced numerous examples: refrigeration of perishable foods, pasteurization of milk, and commercial canning of low acid foods. Our current level of food safety is the result of effective implementation on the part of industry, government, and consumers. More recent approaches, such as the development of Hazard Analysis and Critical Control Point (HACCP) systems, continue to be refined as we strive to further improve food safety.

This report articulates the science behind microbiological food safety, especially as it relates to emerging microbiological hazards. Interpretation and analysis of this scientific information pro-

nure is a well-documented vehicle for *Salmonella* and *E. coli* O157:H7, and its use in produce production must be controlled to prevent contamination. With an estimated 1.2 billion tons of manure produced by cattle annually in the United States (U.S. Senate Agriculture Committee, 1998), this voluminous source of foodborne pathogens is likely to be an influential factor in the transmission of foodborne illness for the foreseeable future. Methods are needed to reduce the shedding of pathogens in livestock and poultry and to identify effective procedures for eliminating pathogens in manure before they contaminate the environment and food.

Changes in Food Consumption

The global trade in food stuffs is only one force in people's changing dietary patterns. Certainly the variety of available food has expanded drastically and will continue to do so. Ethnic foods are increasingly popular, and the percentage of foods prepared and/or consumed outside the home will continue to rise. Fruits and vegetables are likely to constitute a greater portion of the average diet, and the consumer demand for "fresh" products will lead to more minimally processed foods. Our control and prevention methods will need to be adapted to these changing dynamics.

At-Risk Subpopulations

It is likely that the number of persons at higher risk for foodborne disease agents will continue to increase with time. The population of the United States is aging, and clearly aging is a risk factor for more serious outcomes from agents such as *Salmonella*. As people live longer, they may develop more chronic underlying illnesses that predispose them to foodborne illness. Increasingly complex combinations of drugs to treat various conditions in the elderly can have unpredictable effects on susceptibility. Formerly fatal conditions, such as loss of major organ function, are now survivable thanks to organ transplants. The numbers of transplant recipients will likely increase with time, but it is important to remember that these individuals may be among the most susceptible populations to certain foodborne pathogens.

Pathogen Evolution

Microbial evolution has always happened and will always happen. Bacteria, for example, have an enormous capacity for mutation, integration of new genetic material, and recombination of genetic material in order to assure survival. Bacteria can sense and react to their environment and genetically change themselves in response. Unfortunately, newly evolved pathogens are first recognized when they cause an outbreak of illness. Using new technologies and genomics, perhaps surveillance of food animals and the environment for newly emerging microorganisms with pathogenic potential will become a reality in the future, and there will be no need to wait for human illness. Another hope for the future is a better understanding of how human activities affect foodborne pathogens. For example, does the cross protection afforded a pathogen by exposure to an environmental insult have a negative impact on further processing? Genomics may also provide a better snapshot of how a microorganism manifests virulence, and even help determine why and what to do about it.

Consumer Understanding

Although consumers are only a small part of the food safety chain, as consumers we all need to take responsibility for our contribution to food safety. Those that have not already done so must accept that zero risk is not a reality. These two concepts may be difficult for some consumers to accept. Education and risk communication will be necessary to provide consumers with a more accurate perception of food safety risks and to encourage behavior modification, where needed.

Integrated Food Safety System

A farm-to-table food safety system must involve many interested parties working together toward a common goal. When properly applied, the FSO approach would incorporate input from all stakeholders in developing the appropriate levels of protection. Although regulatory oversight is necessary to monitor and enforce the performance of the food safety system, food manufacturers must play an important role as well, because they have first-hand information about food safety hazards and the production environment. A partnership environment will enhance data sharing and provide a solid scientific basis for policies. An ideal system identifies hazards, institutes appropriate controls in a flexible manner through FSOs, and monitors the operation of the system. The challenge is to build a system that applies science in a predictable, consistent, and transparent manner to enable harmonization within and between countries.

Conclusions

History has demonstrated that science, when appropriately applied through food safety management policies, can dramatically improve food safety. The past century produced numerous examples: refrigeration of perishable foods, pasteurization of milk, and commercial canning of low acid foods. Our current level of food safety is the result of effective implementation on the part of industry, government, and consumers. More recent approaches, such as the development of Hazard Analysis and Critical Control Point (HACCP) systems, continue to be refined as we strive to further improve food safety.

This report articulates the science behind microbiological food safety, especially as it relates to emerging microbiological hazards. Interpretation and analysis of this scientific information pro-

vides insight into food safety policies and management practices. At the simplest level, foodborne illness can be reduced to three factors: the pathogen, the host, and the environment in which they interact. Any efforts to improve food safety must address these factors.

Managing microbiological food safety is a complex task. Microbiological hazards are ever-changing, and the amount and complexity of data and the residual unknowns are growing at a rapid rate. Each new scientific advance gives us the opportunity to add to our knowledge of foodborne illness using new techniques and researching new questions. At the same time, human susceptibilities are increasing, and our ability to link food to adverse health outcomes is improving. The human health and economic consequences of emerging microbiological food safety issues are immense. To address these circumstances, food safety policies should be developed as part of national initiatives, with input from all stakeholders.

International coordination of food safety efforts should be encouraged. Globalization of the food supply has contributed to changing patterns of food consumption and foodborne illness. Global food trade has the potential to introduce pathogens to new geographic areas. In addition, we have generally less knowledge about the growing conditions and processing and distribution practices for imported foods than for foods produced domestically.

Scientific research has resulted in significant success in improving food safety, but the current science underpinning the safety of our food supply is not sufficient to protect us from all the emerging issues associated with the complexity of the food supply. The body of scientific knowledge must be further developed, with our research efforts carefully prioritized to yield the greatest benefit. Food safety and regulatory policies must be based on science and must be applied in a flexible manner to incorporate new information as it becomes available and to implement new technologies quickly. The food industry, regulatory agencies and allied professionals should develop partnerships to improve food safety management.

Human foodborne disease surveillance will continue to be very important to: (1) identify outbreaks of foodborne disease so they can be controlled and prevented; (2) determine the causes of foodborne disease; (3) improve control strategies; and (4) monitor trends in occurrence of foodborne disease. Comprehensive, coordinated surveillance activities must be expanded to include animal health and the production and processing environment. Integrating animal and environmental surveillance systems into established human surveillance systems will greatly increase our understanding of the epidemiology and sources of foodborne disease.

Enhanced surveillance will provide data that can be used in risk assessment, which is appropriately becoming a foundation for selecting food safety management options. One of the primary limiting factors for quantitative risk assessment is the quality and sufficiency of available data. As an example, there is little information available about the relationship between the quantity of pathogen ingested and resulting frequency and severity of adverse health effects, especially for susceptible subpopulations. Risk assessment is an iterative process, and assessments must be updated as additional information becomes available. As risk assessments are refined with better methods and improved data, their new conclusions must be shared broadly with all stakeholders to enlighten the public debate over appropriate levels of protection.

Our scientific understanding of the microbiology of foodborne pathogens continues to improve. Scientists are only just beginning to understand the factors that cause a particular microbial strain to be pathogenic while other strains of the same microorganism are not, the ways by which some microorganisms adapt and evolve to become pathogenic, and the mechanisms pathogens employ to adapt to differing environments. Further research is essential to understand microbial ecology and virulence sufficiently well to anticipate future microbial hazards and construct barriers to disease.

Some pathogenic microorganisms are significantly more virulent than others. Virulence may vary within species, subspecies, and even different strains. Understanding the many different virulence factors that microorganisms use to cause illness offers opportunities to develop better controls and therapeutics. Further research will enable scientists to classify pathogens based on specific virulence factors rather than based on name, serotype or other traits unrelated to virulence. This research will improve our evaluation of safety, which currently is focused on microbes that may or may not be pathogenic.

Recent advances in genomics have contributed to the further understanding of virulence at the genus level (e.g., *Salmonella*) and at the level of specific strains within a species (e.g., *Escherichia coli* O157:H7). Genomics also has greatly facilitated our understanding of the continuous and sometimes rapid evolution of pathogens.

Adverse changes in the microbial environment and ecology may cause bacteria to experience stress. Although many bacteria die, some may survive, because bacteria have elaborate systems to adapt to environmental stress. In addition to tolerance of the original stress, the surviving microorganisms also may be tolerant to other unrelated stresses, and these tolerant microorganisms may demonstrate increased virulence. Understanding these response mechanisms will provide the information necessary to refine food processing conditions or to develop other appropriate intervention strategies that enhance food safety.

Improved analytical systems are needed to gather better data about pathogens in the food production environment to improve our understanding of the microbial ecology in these situations. Sensitive quantitative methods are necessary for assessing pathogen growth, survival, and inactivation, as well as for accurate risk assessments.

New processing and packaging technologies offer the potential for continued improvement in the organoleptic quality of foods, extended shelf life, and enhanced microbiological safety. However, these new processes and packaging technologies may change the microbial ecology, resulting in potential positive and negative effects that must be assessed along the entire food chain. Even an apparently insignificant change in the microbial environment can trigger a food safety concern because of the complexity of the microbial environment and the interrelationship of various factors.

Combinations of food manufacturers' efforts, regulatory programs, and consumer actions have driven down rates of certain foodborne diseases, but continued efforts are necessary. Although not easy to accomplish, it is

critically important that regulatory policies be based on the best science currently available. Regulatory policies based on sampling and testing may incorrectly imply an absence of pathogens, causing some individuals to assume that it is unnecessary to engage in proper food selection and handling practices. Given the characteristics of some foods, available technologies, and our desire for year-round availability of a diverse array of foods, it is unlikely that the marketplace can be made free from the presence of pathogenic microorganisms at all times.

The large-scale production of some ready-to-eat (RTE) foods consistently free of *Listeria monocytogenes* appears practically impossible. A great deal of progress has been made during the past quarter century to reduce the levels and frequency of contamination of ready-to-eat foods during their manufacture, but consistently assuring the absence of *Listeria* has remained out of reach. This bacterium is commonly present in the environment and is constantly reintroduced into the processing environment on raw ingredients and via other means. *Listeria* survives well in the manufacturing and retail environment, and it grows at refrigerator temperatures. At present, a "limit-of-detection standard" exists for *L. monocytogenes* in an RTE food such that its mere detection is grounds for legal action against the company distributing the food and is the legal basis for removing the food from the marketplace by regulatory agencies. A true farm-to-table food safety system would consider downstream handling and consumption patterns and epidemiologic characteristics of cases; such a system would not destroy foods that are unlikely to cause illness in the general population. In addition, some subtypes of *L. monocytogenes* found in or on foods have not been associated with illness, and additional research may demonstrate that some subtypes are not pathogenic.

Current technologies are also unlikely to consistently satisfy the demand for large volumes of fresh fruits and vegetables that are free of harmful microorganisms. Because these raw agricultural commodities are often consumed without cooking, effective interventions are needed that will diminish or prevent the presence of pathogens. Policies that result in minimizing contamination and preventing illness from residual levels of pathogens will be more likely to achieve public health goals than policies that haphazardly interdict some small percentage of contaminated produce.

Although a great deal of progress has been made in minimizing contamination of animal carcasses during slaughter, the occasional presence of pathogens on meat and poultry carcasses is largely unavoidable. Prescribed microbial control processes and regulatory standards—in combination with a number of other important risk-reducing measures, including educational programs—apparently have minimized the risk of *E. coli* O157:H7 infections. Some segments of the marketplace are successfully using strict purchase specifications as part of sophisticated quality control programs. Unfortunately, this combination of factors is not in place for all parts of the marketplace. Continuing to focus on "limit-of-detection pathogen standards" for some raw meats in the absence of effective measures in other parts of the farm-to-table continuum is more likely to shift the risk to other parts of the marketplace (such as those with less strict purchase specifications) than it is to achieve public health goals. Greater attention to preventing cross-contamination and undercooking may have more impact on the public's health than further reductions in the already small numbers of *E. coli* O157:H7 occasionally present in raw ground beef.

Improving the scientific basis of food safety programs will depend on further understanding the pathogenicity of microorganisms, including the infectious dose of foodborne pathogens under a variety of conditions, and a further reinforcement and implementation of proper hygienic and food handling practices of those responsible for preventing foodborne disease, including food producers and processors, public health professionals, retail food preparers, and consumers.

Regulatory agencies should work with other public health officials and interested parties, including industry and consumers, to establish Food Safety Objectives (FSOs). FSOs offer a means to convert public health goals into values or targets that can be used by regulatory agencies and food manufacturers. FSOs, which can be applied throughout the food chain, specify the level of hazard that would be appropriate at the time a food is consumed. FSOs would enable food manufacturers to design processes that provide the appropriate level of control and that could be monitored to verify effectiveness. Establishing FSOs is a societal issue that will require inclusive participation of all sectors of society.

The FSO approach can be used to integrate risk assessment and current hazard management practices into a framework that achieves public health goals in a science-based, flexible manner. FSOs help translate the outcome of risk assessment into something that can be used with HACCP programs. The FSO approach will be successful when directly intertwined with a food processor's good manufacturing practices (GMPs) and HACCP systems.

Although HACCP is a science-based management tool, HACCP may not be an appropriate approach for all circumstances. It is not possible to have a valid HACCP plan when a scientific analysis does not identify any point that meets the critical control point criteria. HACCP implementation must remain flexible to incorporate the scientific knowledge and data available in a product- and process-specific manner that best meets FSOs.

The application of HACCP to primary production is particularly limited, because all the HACCP principles generally cannot be achieved. Well-defined, science-based good agricultural practices should be further developed for specific commodities where appropriate. Additional research will be necessary to better understand the microbial ecology in these agricultural environments and to formulate science-based recommendations for pathogen control.

Routine microbiological testing is useful for some purposes but not for others. It can focus on pathogens of interest or on nonpathogenic microorganisms whose presence indicates conditions favorable to the presence of pathogens. Testing is useful for surveillance and HACCP verification purposes. It also is used for validating and revalidating processes.

However, microbiological testing of finished product can be misleading, because negative results do not ensure safety. Testing has statistical limitations based on the amount of product sampled, the percentage of product that is contaminated, and the uniformity of the distribution of contamination through-

out the food. As the amount of contamination in the food decreases, the food safety emphasis should focus on further controlling processing conditions through the application of science-based HACCP plans.

Because fresh produce undergoes very little processing, preventing contamination is the primary emphasis for ensuring the microbiological safety of fresh fruits and vegetables. Thus, careful consideration must be given to growing conditions—including soil, water, manure, livestock, wildlife, pets, environmental pollution and effluent/sewage, and humans—and their effect on food safety throughout the food chain.

Many of the most prominent foodborne pathogens in the United States are carried by livestock and are principally transmitted to food by fecal contamination. Manure, a significant vehicle for pathogens, is a growing source of fertilizer. Use of manure fertilizer is an increasing environmental concern because it may contaminate water for drinking, irrigation, and recreation. Manure also is applied with or without composting to the soil used to grow food crops. Manure used in the production of food crops is of special concern because the available scientific information is insufficient to ensure that foodborne pathogens are killed by current agricultural practices. Intensive farming practices can contribute to the rapid spread of human and animal pathogens by creating more concentrated environments for pathogens to multiply and evolve and by generating larger quantities of subsequently contaminated food.

An examination of the science reveals that foodborne illness is caused by a complex combination of factors that must be managed on a continual basis. To achieve our public health goals, everyone along the farm-to-table continuum must take responsibility for their role in food safety management.

Foodborne disease is widely recognized for acute effects on the gastrointestinal tract but also includes other effects throughout the body. In addition, foodborne pathogens may cause chronic disease, which may occur independently or accompany an acute illness. Many of these chronic diseases have only recently been linked to foodborne pathogens. In addition, a growing proportion of foodborne illnesses are due to viruses, and improvements are needed in testing methods for viral pathogens in patients and in foods.

The range of pathogens associated with foodborne illness continues to increase as new information identifies pathogen/food associations. When new food vectors are identified, risk management decisions must consider the best approach to control and prevention. Application of controls during food production and processing may be necessary, although some hazards may be better addressed at the consumer level through modification of exposure or susceptibility.

The person who serves as the host for the foodborne pathogen is a major factor in the occurrence and character of foodborne disease. The individual's health, food consumption habits, and sanitation practices all directly affect the risk of foodborne illness. Hygiene, food preparation, and food handling and storage practices contribute to pathogen exposure. Food selection also contributes to the likelihood of exposure. In addition, an individual's underlying health can have a significant impact on susceptibility to disease when exposed.

An important contributor to microbial pathogenicity and human illness is the changing human population and its behavior. The elderly portion of the U.S. population continues to grow, and large numbers of individuals have conditions necessitating the use of immunosuppressive drugs or drug combinations with unknown effects, potentially increasing their susceptibility to foodborne illnesses. Many factors cause changes in the immune system function, such as age, health conditions (e.g., AIDS, cancer), pregnancy, nutritional status, and antacid/medication use. Factors that suppress the immune system increase the risk of foodborne illness.

The increased understanding of intestinal microflora and the immune system is providing opportunities for intervention strategies, such as probiotics, to facilitate human health and decrease susceptibility to foodborne illnesses.

The combination of proper hygiene and sanitation related to food handling and preparation, appropriate methods of refrigeration and freezing, and thorough cooking of foods comprise a very effective approach to preventing foodborne illness. After GMPs and HACCP provide adequately safe foods, the individuals preparing the food must use proper knowledge, attitudes, skills and practices to achieve food safety.

Consumers are sometimes inattentive to their personal ability to reduce the risk of foodborne illness. The public health community has the responsibility to discuss risk reduction strategies with consumers. Current risk communication is inadequate, and some consumer perceptions and behaviors are not consistent with reasonable expectations regarding the safety of some foods. Communication with consumers to improve food choices and handling practices will be an essential component of strategies for the further reduction of foodborne illness. This approach has been successful in the education of sensitive populations, an activity that will necessarily continue in the future.

Scientific data are a very substantial limiting factor in enhancing food safety. Further research will continue to help resolve complex problems and to provide information to improve the delivery of safe foods. Appropriate and aggressive data collection throughout the food production and processing system is essential for valid risk assessments and the resulting food safety improvements. Procedures must be implemented to obtain data from food manufacturers in "penalty-free" environments so the data can be properly evaluated by public officials and the results made available to all interested parties.

It is difficult to conceive of a food safety system that responds effectively and efficiently to emerging microbiological food safety concerns that does not permit rapid changes in approach based on advances in science. Flexibility to respond to new information and new hazards will require unfettered data sharing. In addition, such a system cannot rely on the use of prescribed microbial control processes but instead must emphasize validation and verification of the methods used to assure food safety.

The complex interrelationship of the pathogen, host, and microbial ecology ensures a role for everyone in food safety management—industry, regulatory agencies, public health officials, and consumers. A flexible, science-based approach that relies on all parties to fulfill their role is our best weapon against emerging microbiological food safety issues.

References

A

AAP. 1998. Prevention of rotavirus disease: Guidelines for use of rotavirus vaccine. American Academy of Pediatrics. Pediatr. 102: 1483-1491.

AAP. 2000. Potentially contaminated food products. In "Red Book: Report of the Committee on Infectious Diseases." 25th Ed. ed. L.K. Pickering. pp. 770-771. American Academy of Pediatrics, Elk Grove Village, Ill.

Abbas, A.K., Lichtman, A.H., and Pober, J.S. 1997. Cellular and Molecular Immunology. Saunders, Philadelphia, Pa.

Abee, T. and Wouters, J.A. 1999. Microbial stress response in minimal processing. Intl. J. Food Microbiol. 50: 65-91.

Ackers, M., Mahon, B., Leahy, E., Damrow, T., Hutwagner, L., Barrett, T., et al. 1996. An outbreak of *Escherichia coli* infections associated with leaf lettuce consumption, Western Montana. Proc. of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, (Abstr. K43) Sep. 15-18, New Orleans, La. ASM Press. Washington D.C.

Albrecht, J.A. 1995. Food safety knowledge and practices of consumers in the U.S.A. J. Consumer Studies Home Econ. 19: 119-34.

Allos, B.M. 1997. Association between *Campylobacter* infection and Guillain-Barré syndrome. J. Infect. Dis. 175: 125S-128S.

Altekruse, S.F., Street, D.A., Fein, S.B., and Levy, A.S. 1995. Consumer knowledge of foodborne microbial hazards and food-handling practices. J. Food Protect. 59: 287-294.

Altekruse, S.F., Yang, S., Timbo, B.B., and Angulo, F.J. 1999. A multi-state survey of consumer food-handling and food-consumption practices. Am. J. Prev. Med. 16: 216-221.

Altuvia, S., Almirón, M., Huisman, G., Kolter, R., and Storz, G. 1994. The *dps* promoter is activated by OxyR during growth and by IHF and ss in stationary phase. Molec. Microbiol. 13: 265-272.

Anderson, D.M. 1994. Red tides. Sci. Am. 271: 62-68.

Angulo, F., Johnson, K., Tauxe, R., and Cohen, M. 2000. Significance and sources of antimicrobial-resistant nontyphoidal *Salmonella* infections in the United States. Microbial Drug Resistance 6(1): 77-83.

Anonymous. 1996. Salmonella typhimurium infections in humans. Commun. Dis. Rep. 6(41): 356.

Anonymous. 2000. Significance of coliforms and generic *Escherichia coli* on fresh and minimally processed produce. NFPA White Paper. National Food Processors Association, Washington, D.C.

Arbeit, R.D. 1995. Laboratory procedures for the epidemiologic analysis of microorganisms. In: "Manual of Clinical Microbiology," 6th Ed. ed. P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken. pp. 190-208. ASM Press. Washington, D.C.

Archer, D.L. 1996. Preservation microbiology and safety: Evidence that stress enhances virulence and triggers adaptive mutations. Trends Food Sci. Technol. 71: 91-95.

Archer, D.L. 2001. WHO holds food safety strategic planning meeting. Food Technol. 55(5): 22.

Ayres, J.C. 1955. Microbiological implications in the handling, slaughtering, and dressing of meat animals. Adv. Food Res. 6: 109-161.

B

Baden, D.A., Fleming, L.E., and Bean, J.A. 1995. Marine toxins. Handbook of Clinical Neurology 21: 141-175.

Baer, J.T., Vugia, D.J., Reingold, A.L., Aragon, T., Angulo, F.J., and Bradford, W.Z. 1999. HIV infection as a risk factor for shigellosis. Emerg. Infect. Dis. 5(6): 820-823.

Baggesen, D.L., Sandvang, D., and Aarestrup, F.M. 2000. Characterization of Salmonella enterica serovar typhimurium DT104 isolated from Denmark and comparison with isolates from Europe and the United States. J. Clin. Microbiol. 38: 1581-1586.

Baird-Parker, A.C. 1990. Foodborne salmonellosis. The Lancet 336: 1231-1234.

Baird-Parker, A.C. 2000. The production of microbiologically safe and stable foods. Chpt. 1 In "The Microbiological Safety and Quality of Food," Vol. 1, pp. 3-18. Aspen Publishers, Inc., Gaithersburg, Md.

Banks, J.G. and Board, R.G. 1983. The incidence and level of contamination of British fresh sausages and ingredients with salmonellas. J. Hygiene 90: 213-223.

Barkate, M.L., Acuff, G.R., Lucia, L.M., and Hale, D.S. 1993. Hot water decontamination of beef carcasses for reduction of initial bacterial numbers. Meat Sci. 35: 397-401.

Barker, C. and Park, S.F. 2001. Sensitization of *Listeria monocytogenes* to low pH, organic acids, and osmotic stress by ethanol. Appl. Environ. Microbiol. 67: 1594-1600.

Barrett, T.J., Lior, H., Green, J.H., Khakhria, R., Wells, J.G., Bell, B.P., Greene, K.D., Lewis, J., and Griffin, P.M. 1994. Laboratory investigation of a multistate foodborne outbreak of *Escherichia coli* O157: H7 by using pulsed field gel electrophoresis and phage typing. J. Clin. Microbiol. 32(12): 3013-3017.

Barton, C.C., Ganey, P.E., and Roth, R.A. 2000. Lipopolysaccharide augments aflatoxin B1-induced liver injury through neutrophil-dependent and -independent mechanisms. Toxicol. Sci. 58: 208-215.

Barton, C.C., Barton, E.X., Ganey, P.E., Kunkel, S.L., and Roth, R.A. 2001. Bacterial lipopolysaccharide enhances aflatoxin B1 hepatotoxicity in rats by a mechanism that depends on tumor necrosis factor alpha. Hepatol. 33: 66-73.

Bauman, H.E. 1992. Introduction to HACCP. In "HACCP Principles and Applications." Ed. M.D. Pierson and D.A. Corlett, Jr., Van Nostrand Reinhold. New York, N.Y.

Bäumler, A.J., Hargis, B.M., and Tsolis, R.M. 2000. Tracing the origins of Salmonella outbreaks. Science 287: 50-2.

BC/USDC. 1996. Per capita utilization of selected commercially produced fresh fruits and vegetables: 1970 to 1994. Statistical Abstract of the United States, 116th Ed. p. 148. Bureau of the Census/U.S. Dept. of Commerce. U.S. Government Printing Office. Washington, D.C.

Bean, N.H., Griffin, P.M., Goulding, J.S., and Ivey, C.B. 1990. Foodborne disease outbreaks, 5-year summary, 1983-1987. CDC Surveill. Summ. 39: 15-57.

Bean, N.H., Goulding, J.S., Lao, C., and Angulo, F.J. 1996. Surveillance for foodborne disease outbreaks-United States, CDC Surveill. Summ. 45: 1-66.

Bean, N.H., Goulding, J.S., Daniels, M.T., and Angulo, F.J. 1997. Surveillance for foodborne disease outbreaks-United States, 1988-1992. J. Food Protect. 60: 1265-1286.

Becker, L.A., Cetin, M.S., Hutkins, R.W., and Benson, A.K. 1998. Identification of the gene encoding the alternative sigma factor s^B in *Listeria monocytogenes* and its role in osmotolerance. J. Bacteriol. 180: 4547-4554.

Becker, K.M., Moe, C.L., Southwick, K.L., and MacCormack, J.N. 2000. Transmission of Norwalk virus during a football game. N. Eng. J. Med. 343: 1223-1227.

Bej, A.K. and Mahbubani, M.H. 1994. Detection of Food borne Microbial Pathogens. Chpt. X In "PCR Technology: Current Innovations". p. 341-365. CRC Press, Boca Raton, Fla.

Beletshachew, S., Yang, S., Cieslak, P., Vugia, D., Marcus, R., Koehler, J., Deneen, V., Angulo, F., and the FoodNet Working Group. 2000. Prevalence of high-risk food consumtion and food-handling practices among adults: A multistate survey, 1996 to 1997. J. Food Protect. 63: 1538-1543.

Beltrametti, F., Kresse, A.U., and Guzmán, C.A. 1999. Transcriptional regulation of the *esp* genes of enterohemorrhagic *Escherichia coli*. J. Bacteriol. 181: 3409-3418.

Bender, J.B., Hedberg, C.W., Boxrud, D.J., Besser, J.M., Wicklund, J.H., Smith, K.E., and Osterholm, M.T. 2001. Molecular subtype surveillance of *Salmonella typhimurium*, Minnesota 1994-1998. N. Engl. J. Med. 344: 189-195.

Benson, A.K., and Haldenwang, W.G. 1993. Bacillus subtilis sB is regulated by a binding protein (RsbW) that blocks its association with core RNA polymerase. Proc. Natl. Acad. Sci. 90: 2330-2334.

Bergthorsson, U. and Ochman, H. 1998. Distribution of chromosome length variation in natural isolates of *Escherichia coli.* Molec. Biol. Evol. Jan. 15(1): 6-16.

Bernet, M.F., Brassart, D., Neeser, J.R., and Servin, A.L. 1994. *Lactobacillus acidophilus* LA1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. Gut 35: 483-489.

Besser, R.E., Lett, S.M., Weber, J.T., Doyle, M.P., Barrett, T.J., Wells, J.G., and Griffin, P.M. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. J. Am. Med. Assn. 269: 2217-2220.

Besser, T.E., Goldoft, M., Pritchett, L.C., Khakhria, R., Hancock, D.D., Rice, D.H., Gay, J.M., Johnson, W., and Gay, C.C. 2000. Multiresistant *Salmonella* Typhimurium DT104 infections of humans and domestic animals in the Pacific Northwest of the United States. Epidemiol. Infect. 124: 193-200.

Beuchat, L.R. 1996. Pathogenic microorganisms associated with fresh produce. J. Food Protect. 59: 204-216.

Beuchat, L.R. and Ryu, J.-H. 1997. Produce handling and processing practices. Emerg. Infect. Dis. 3: 459-465.

Bhat, R.V., Beedu, S.R., Ramakrishna, Y., and Munshi, K.L. 1989. Outbreak of trichothecene mycotoxicosis associated with consumption of mould-damaged wheat production in Kashmir Valley, India. Lancet 1: 35-37.

Bidawid, S., Farber, J.M., and Sattar, S.A. 2000. Inactivation of hepatitis A virus (HAV) in fruits and vegetables by gamma irradiation. Intl. J. Food Microbiol. 57: 91-97.

Bille, J. 1988. Anatomy of a foodborne listeriosis outbreak. Foodborne Listeriosis. Proc. of a Symposium. Sept. 7, pp. 30-35. Wiesbaden, Germany.
Bingen, E.H., Denamur, E., and Elion, J. 1994. Use of ribotyping in epidemiological surveillance of nosocomial outbreaks. Clin. Microbiol. Rev. 7(3): 311-327.
Blais, B.W., Turner, G., Sooknanan, R., and Malek, L.T. 1997. A nucleic acid sequence-based amplification system for detection of Listeria monocytogenes hlyA sequences. Appl. Environ. Microbiol. 63: 310-313.
Bloomfield, S.F., Stewart, G.S.A.B., Dodd, C.E.R., Booth, I.R., and Power, E.G.M. 1998. The viable but non-culturable phenomenon explained? Microbiol. 144: 1-3.
Bogdanos, D.P., Mieli-Vergani, G., and Vergani, D. 2000. Virus, liver and autoimmunity. Dig. Liver Dis. 32(5): 440-446
Bondy, G.S. and Pestka, J.J. 2000. Immunomodulation by fungal toxins. J. Toxicol. Environ. Health B. Crit. Rev. 3(2): 109-143.
Borgdorff, M.W. and Motarjemi, Y. 1997. Surveillance of Foodborne Diseases: What Are the Options? World Health Statistics Quarterly 50: 12-23.
Bower, C.K. and Daeschel, M. A. 1999. Resistance responses of microorganisms in food environments. Int. J. Food Microbiol. 50: 33-44.
Breeuwer, P. and Abee, T. 2000. Assessment of viability of microorganisms employing fluorescence techniques. Intl. J. Food Microbiol. 55: 193-200.
Brown, J.R. and Doolittle, W.F. 1997. Archaea and the prokaryote-to-eukaryote transition. Microbiol. Mol. Biol. Rev. 61(4): 456-502.
Buchanan, R.L. 1997a. Identifying and controlling emerging foodborne pathogens: Research needs. Emerg. Infect. Dis. 3(4): 517-521.
Buchanan, R.L. 1997b. National Advisory Committee on Microbiological Criteria for Foods: Principles of risk assessment for illnesses caused by foodborne biological agents. J. Food Protect. 60: 1417-1419..
Buchanan, R.L., Edelson, S.G., and Boyd, G. 1999. Effects of pH and acid resistance on the radiation resistance of enterohemorrhagic *Escherichia coli.* J. Food Protect. 62: 219-228.
Buchko, S.J., Holley, R.A., Olson, W.O., Gannon, V.P., and Veira, D.M. 2000. The effect of different grain diets on fecal shedding of *Escherichia coli* O157: H7 by steers. J. Food Protect. 63: 1467-1474.
Buchmeier, N.A. and Heffron, F. 1990. Induction of *Salmonella* stress proteins upon infection of macrophages. Science. 248: 730-732.
Burke, S. 1985. "Human Anatomy and Physiology for the Health Sciences." 2nd Ed. John Wiley and Sons, New York, N.Y.
Burkholder, J.M., Glasgow, H.B., and Hobbs, C.W. 1995. Fish kills linked to a toxic ambush-predator dinoflagellate: Distribution and environmental conditions. Marine Ecology Progress Series 124: 43-61.
Burnett, S.L., Chen, J., and Beuchat, L.R. 2000. Attachment of *Escherichia coli* O157:H7 to the surfaces and internal structures of apples as detected by confocal scanning laser microscopy. Appl. Environ. Microbiol. 66: 4679-4687.
Bush, E.J., Lautner, E., McKean, J., and Miller, L.M. 1990. SwineFutures Project, Final Report. 91-51-048. U.S. Dept. Agriculture/Animal and Plant Health Inspection Service, Washington, D.C.
Buzby, J.C. and Ready, R.C. 1996. Do consumers trust food-safety information? Food Rev. Jan.-Apr., pp. 46-49.

C

CAC. 1997a. Principles for the establishment and application of microbiological criteria for foods. In "General Requirements (Food Hygiene): Supplement to Volume 1B – 1997," 2nd Ed., p. 27. CAC/GL 21-1997. Joint FAO/WHO Food Standards Prgoramme. Codex Alimentarius Commission, Rome.
CAC. 1997b. Report of the 22nd Session of the Codex Alimentarius Commission. Joint FAO/WHO Food Standards Programme. ALINORM 97/37. Codex Alimentarius Commission, Rome.
CAC. 1997c. Hazard Analysis and Critical Control Point (HACCP) System and Guidelines for Its Application. In "General Requirements (Food Hygiene): Supplement to Volume 1B-1997," 2nd Ed., pp. 33-46. Annex to CAC/RCP1-1969, Rev. 3(1997). Joint FAO/WHO Food Standards Programme. Codex Alimentarius Commission, Rome.
CAC. 1999. Principles and guidelines for the conduct of microbiological risk assessment. CAC/GL-30. Codex Alimentarius Commission, Rome.
Canada, J.C. and Strong, D.H. 1964. *Clostridium perfringens* in bovine livers. J. Food Sci. 29: 862-864.
CAST. 1994. Foodborne pathogens: Risks and Consequences. Council for Agricultural Science and Technology, Ames, Ia.
CAST. 2001. Johne's disease in cattle. Issue Paper No. 17. Council for Agricultural Science and Technology, Ames, Ia.
Castillo, A., Lucia, L.M., Goodson, K.J., Savell, J.W., and Acuff, G.R. 1999. Decontamination of beef carcass surface tissue by steam vacuuming alone and combined with hot water and lactic acid sprays. J. Food Protect. 62: 146-151.
Catterall, W.A. 1985. The voltage sensitive sodium channel: A receptor for multiple neurotoxins. In "Toxic Dinoflaellates." Ed. D.M. Anderson, A.W. White, and D.G. Baden. pp. 329-342. Elsevier, New York, N.Y.
CDC. 1997. *Escherichia coli* O157:H7 infections associated with eating a nationally distributed commercial brand of frozen ground beef patties and burgers-Colorado, 1997. Centers for Disease Control and Prevention, Atlanta, Ga. MMWR 46: 777-778.
CDC. 1998a. Multistate surveillance for food-handling, preparation, and consumption behaviors associated with foodborne diseases: 1995 and 1996 BRFSS food-safety questions. Centers for Disease Control and Prevention, Atlanta, Ga. MMWR. 47(SS-4): 33-54.
CDC. 1998b. Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters-Pacific Northwest, 1997. Centers for Disease Control and Prevention, Atlanta, Ga. MMWR. 47: 457-462.
CDC. 1998c. Multistate outbreak of salmonella serotype agona infections linked to toasted oats cereal-United States, April-May, 1998. Centers for Disease Control and Prevention, Atlanta, Ga. MMWR. 47(22): 462-464.
CDC. 1999a. Foodborne diseases active surveillance network (FoodNet): Population survey atlas of exposures: 1998-1999. Centers for Disease Control and Prevention, Atlanta, Ga.
CDC. 1999b. Outbreak of *Vibrio parahaemolyticus* infection associated with eating raw oysters and clams harvest from Long Island Sound-Connecticut, New Jersey, and New York, 1998. Centers for Disease Control and Prevention, Atlanta, Ga. MMWR. 48: 48-51.
CDC. 1999c. Outbreaks of *Shigella sonnei* infection associated with eating fresh parsley: United States and Canada. Centers for Disease Control and Prevention, Atlanta, Ga. MMWR. 48: 285-9.
CDC. 1999d. PulseNet: The national molecular subtyping network for foodborne disease surveillance. Centers for Disease Control and Prevention, Atlanta, Ga. FoodNet News 1(3): 3.
CDC. 2000a. Preliminary FoodNet data on the incidence of foodborne illnesses- selected sites, 1999. Centers for Disease and Prevention, Atlanta, Ga. MMWR. 49: 201-205.
CDC. 2000b. Erratum. Centers for Disease and Prevention, Atlanta, GA. MMWR. 49: 286.
CDC/NCID/DBMC. 2000. Essential epidemiology and laboratory components of a state foodborne disease prevention and control program. Letter to state and territorial epidemiologists and state and territorial public health laboratory directors from Director, Division of Bacterial and Mycotic Diseases. National Center for Infectious Disease/Centers for Disease Control and Prevention. Feb. 7. Atlanta. Ga.
CDC. 2001. Norwalk-like viruses: Public health consequences and outbreak management. Centers for Disease Control and Prevention, Atlanta, Ga. MMWR. 50(RR09): 1-18
CDC. 2002. Toxoplasmosis. A Fact Sheet of the Centers for Disease Control and Prevention, Atlanta, Ga. (http://www.cdc.gov/ncidod/dpd/parasites/toxoplasmosis/factsht_toxoplasmosis.htm).
Celum, C.L., Chaisson, R.E., Rutherford, G.W., Barnhart, J.L., and Echenberg, D.F. 1987. Incidence of salmonellosis in patients with AIDS. J. Infect. Dis. Dec. 156(6): 998-1002.
Cerda, H., Delincee, H., Haine, H., and Rupp, H. 1997. The DNA comet assay as a rapid screening technique to control irradiated food. Mut. Res. 375: 167-181.
Cetinkaya, Y., Falk, P., and Mayhall, C.G. 2000. Vancomycin-resistant enterococci. Clin. Microbiol. Rev. 13: 686-707.
Cha, J.J., Srivastava, R., Vakharia, V.N., Rao, G., and Bentley, W.E. 1999. Green fluorescent protein as a noninvasive stress probe in resting *Escherichia coli* cells. Appl. Environ. Microbiol. 65: 409-414.
Cheville, A.M., Arnold, K.W., Buchrieser, C., Cheng, C.-M., and Kaspar, C.W. 1996. *rpoS* regulation of acid, heat, and salt tolerance in *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 62: 1822-1824.
Chomel, B.B., DeBess, E.E., Mangiamele, D.M., Reilly, K.F., Farver, T.B., Sun, R.K., and Barrett, L.R. 1994. Changing trends in the epidemiology of brucellosis in California from 1973-1992: A shift to foodborne transmission. J. Infect. Dis. 170: 1216-1223.
Christman, M.F., Storz, G., and Ames, B.N. 1989. OxyR, a positive regulator of hydrogen peroxide-inducible genes in *Escherichia coli* and *Salmonella typhimurium*, is homologous to a family of bacterial regulatory proteins. Proc. Natl. Acad. Sci. 86: 3484-3488.
Clark, C.G., Kraveta, A.N., Dendy, C., Wang, G., and Tyler, K.D. 1998. Investigation of the 1994-5 Ukrainian *Vibrio cholerae* epidemic using molecular methods. Epidemiol. Infect. 121: 15-29.
Clemmer, D.I., Hickey, J.L.S., Bridges, J.F., Schliessmann, D.J., and Shaffer, M.F. 1960. Bacteriologic studies of experimental air-borne salmonellosis in chicks. J. Infect. Dis. 6: 197-210.
Cohen, B.A. 1999. Neurological manifestations of toxoplasmosis in AIDS. Semin. Neurol. 19(2): 201-11.
Coia, J.E., Sharp, J.C., Campbell, D.M., Curnow, J., and Ramsay, C.N. 1998. Environmental risk factors for sporadic *Escherichia coli* O157 infection in Scotland: Results of a descriptive epidemiology study. J. Infect. 36(3): 317-21.
Collins, J.L., Small, M.L., Kann, L., Pateman, B.C., Gold, R.S., and Kolbe, L.J. 1995. School health education, J. Sch. Health 65: 302-11.
Colwell, R.R., Brayton, P.R., Grimes, D.J., Roszak, D.B., Huq, S.A., and Palmer, L.M. 1985. Viable but non-culturable *Vibrio cholerae* and related pathogens in the environment: Implications for release of genetically engineered microorganisms. Bio/Technol. 3: 817-820.
Cook, K.A., Boyce, T., Langkop, C., Kuo, K., Swartz, M., Ewert, D., et al. 1995. Scallions and shigellosis: A multistate outbreak of food traced to imported green onions [Abst.]. Proc. of the 44th Annual Conference of the Epidemic Intelligence Service. Mar. 27-31, Atlanta, Ga, p. 36. Centers for Disease Control and

Prevention, Atlanta, Ga.
Cook, K.A., Swerdlow, D., Dobbs, T., Wells, J., Puhr, Hlady G., Wells, J.G., Barrett, T.J., Phur, N.D., Lanrette, G.A., Bodager, D.W., Toth, B.L., Genese, C.A., Highsmith, A.K., Pilot, K.E., Finelli, L., and Swerdlow, D.L. 1996. Outbreak of *Salmonella* serotype hartford infections associated with unpasteurized orange juice. J. Am. Med. Assoc. 280(17): 1504-1509.
Covello, V.T., Sandman, P.M., and Slovic, P. 1988. "Risk Communication, Risk Statistics, and Risk Comparisons: A Manual for Plant Managers." Chemical Manufacturers Association, Washington, D.C.
Coulombe, R.A. 1993. Biological action of mycotoxins. J. Dairy Sci. 76: 880-91.
Cuthbert, J.A. 2001. Hepatitis A: Old and new. Clin. Microbiol. Rev. 14: 38-58.

D

Daifas, D.P., Smith, J.P., Blanchfield, B., and Austin, J.W. 1999a. Growth and toxin production by *Clostridium botulinum* in English-style crumpets packaged under modified atmospheres. J. Food Protect. 62: 349-55.
Daifas, D.P., Smith, J.P., Blanchfield, B., and Austin, J.W. 1999b. Challenge studies with *Clostridium botulinum* in high moisture bakery products packaged under modified atmospheres. Food Austral. 51: 507-511.
Daly, A.K., Cholerton, S., Armstrong, M., and Idle, J.R. 1994. Genotyping for polymorphisms in xenobiotic metabolism as predictor of disease susceptibility. Environ. Health Perspect. 102: S44-S61.
Daniels, R.W. 1998. Home Food Safety. Food Technol. 52: 54-56.
Daniels, R., Daniels, B., Gilmet, P., and Noonan, D. 2000. Audits International 2000 Home Food Safety Study Report. Audits Intl., Northbrook, Ill.
D'Aoust, J.W. 1985. Infective dose of *Salmonella* Typhimurium in cheddar cheese. Am. J. Epidemiol. 122(4): 717-720.
D'Aoust, J-Y. 1989. *Salmonella*. In "Foodborne Bacterial Pathogens." pp. 327-445. ed. M.P. Doyle, Marcel Dekker, New York, N.Y.
Darlow, H.M., Bale, W.R., and Carter, G.B. 1961. Infection of mice by respiratory route with *Salmonella typhimurium*. J. Hyg. 59: 303-308.
Davidson, P.M. 1997. Chemical preservatives and natural antimicrobial compounds. Chpt. 29, In "Food Microbiology: Fundamentals and Frontiers," ed. M.P. Doyle, L.R. Beuchat, T.J. Montville. pp. 520-556. ASM Press, Washington, DC.
Davidson, P.M. 1999. Potential for emergence of resistance to antimicrobials used in the food industry. Proc. of Symposium on Food Microbiology. pp. 19-21. International Life Sciences Institute/ILSI North America Technical Committe on Food Microbiology. (http://www.ilsi.org/file/iamfes99.pdf)
Davies, R.H. and Wray, C. 1997. Distribution of *Salmonella* on 23 pig farms in the U.K. In Proc. of the 2nd International Symposium on the Epidemiology and Control of Salmonella in Pork. pp. 137-141. Copenhagen.
Dawson, D.J. and Sartory, D.P. 2000. Microbiological safety of water. Brit. Med. Bull. 56: 74-83.
Day, W.A. and Maurelli, A.T. 2001. *Shigella flexneri* LuxS quorum-sensing system modulates virB expression but is not essential for virulence. Infect. Immun. 69(1): 15-23.
De Bethizy, J. and Hayes, J.R. 1994. Metaolism: A determinant of toxicity. In "Principles and Methods of Toxicology" 3rd Ed., ed. A.W. Hayes. Raven Press, New York, N.Y.
De Blackburn, W.C. and McCarthy, J.D. 2000. Modifications to methods for the enumeration and detection of injured *Escherichia coli* O157:H7 in foods. Intl. J. Food Microbiol. 55: 285-290.
De Boer, E. and Beumer, R.R. 1999. Methodology for detection and typing of foodborne microorganisms. Intl. J. Food Microbiol. 50: 119-130.
De Kievit, T.R. and Iglewski, B.H. 2000. Bacterial quorum sensing in pathogenic relationships. Infect. Immunol. 68: 4839-4849.
Del Torre, M., Stecchini, M.L., and Peck, M.W. 1998. Investigation of the ability of proteolytic *Clostridium botulinum* to multiply and produce toxin in fresh Italian pasta. J. Food Protect. 61: 988-993.
Deming, M.S., Tauxe, R.V., Blake, P.A., Dixon, S.E., Fowler, B.S., Jones, T.S., Lockamy, E.A., Patton, C.M., and Sikes, R.O. 1987. *Campylobacter* enteritis at a university: Transmission from eating chicken and from cats. Am. J. Epidemiol. 126: 526-534.
Deneen, V.C., Hunt, J.M., Paule, C.F., James, R.l., Johnson, R.G., Raymond, M.J., and Hedberg, C.W. 2000. The impact of foodborne calicivirus disease: The Minnesota experience. J. Infect. Dis. 181(Suppl. 2): S281-283.
DePaola, A., Flynn, P.A., McPhearson, R.M., and Levy, S.B. 1988. Phenotypic and genotypic characterizations of tetracycline and oxytetracycline resistant *Aeromonas hydrophila* for altered channel catfish (*letalurus punctatus*) and their environments. Appl. Environ. Microbiol. 54: 1861-1863.
Desenclos, J.A., Klontz, K.C., Wolfe, L.E., and Hoecherl, S. 1991. The risk of *Vibrio* illness in the Florida raw oyster eating population, 1981-1988. Am. J. Epidemiol. 134: 290-297.
De Roever, C. 1998. Microbiological safety evaluations and recommendations on fresh produce. Food Control 9(6): 321-347.
Dickson, J.S. and Anderson, M.E. 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems. J. Food Protect. 55: 133-140.
Dickson, J.S. and Kunduru, M.R. 1995. Resistance of acid adapted salmonellae to organic acid rinses on beef. J. Food Protect. 58: 973-976.
Diehl, J.F. 1995. "Safety of irradiated foods," 2nd Ed. Marcel Dekker, New York, N.Y.
Diez-Gonzalez, F., Callaway, T.R., Kizoulis, M.G., and Russell, J.B. 1998. Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. Science 281: 1666-1668.
Dorsa, W.J., Cutter, C.N., Siragusa, G.R., and Koohmaraie, M. 1996. Microbial decontamination of beef and sheep carcasses by steam, hot water spray washes and a steam-vacuum sanitizer. J. Food Protect. 59: 127-135.
Downes, T. 1989. Food packaging in the IFT era: Five decades of unprecedented growth and change. Food Technol. 43(9): 228-240.
Doyle, M. 1998. Extending the shelf life of refrigerated foods: For better or worse? Food Technol. 52(2): 20.
Doyle, M.P. 2000. Reducing foodborne disease: What are the priorities? Nutrition 16(7/8): 647-694.
Doyle, M.P., Beauchat, L.R., and Montville, T.J. 1997. "Food Microbiology: Fundamentals and Frontiers." pp. 327-336. ASM Press, Washington, D.C.
Dubey, J.P., Brake, R.J., Murrell, K.D., and Fayer, R. 1986. Effect of irradiation on the viability of *Toxoplasma gondii* cysts in tissues of mice and pig. Am. J. Vet. Res. 47: 518-522.
Dubois, A. 1995. Spiral bacterial in the human stock: The gastric helicobacters. Emerg. Infect. Dis. 1(3): 79-85.
DuPont, H.L., Chappell, C.L., Sterling, C.R., Okhuysen, P.C., Rose, J.B., and Jakubowski, W. 1995. The infectivity of *Cryptosporidium parvum* in healthy volunteers. N. Eng. J. Med. 332: 855-859.

E

Eaton, D.A., Gallagher, E.P., Bammler, T.K., and Kunze, K.L. 1995. Role of cytochrome P4501A2 in chemical carcinogenesis: Implications for human variability in expression and enzyme activity. Pharmacogen. 5: 259-274.
Eberhart-Phillips J., Besser, R.E., Tormey, M.P., Koo, D., Feikin, D., Araneta, M.R., Wells, J., Kilman, L., Rutherford, G.W., Griffin, P.M. Baron, R., and Mascola, L. 1996. An outbreak of cholera from food served on an international aircraft. Epidemiol. Infect. 116: 9-13.
EC. 2000. Possible links between Crohn's disease and paratuberculosis. Report of the Scientific Committee on Animal Health and Animal Welfare, Adopted Mar. 21. SANCO/B3/R16/2000. Directorate-General Health and Consumer Protection. European Commission.
Elder, R.O., Keen, J.E., Siragusa, G.R., Barkocy-Gallagher, G.A., Koohmarie, M., and Laegreid, W.W. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci. 97: 2999-3003.
Elwood, C.M. and Garden, O.A. 1999. Gastrointestinal immunity in health and disease. Vet. Clin. North Am. Small Anim. Pract. 29(2): 471-500, vi-vii.
Engberg, J., Aarestrup, F.M., Taylor, D.E., Gerner-Smidt, P., Nachamkin, I. 2001. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: Resistance mechanisms and trends in human isolates. Emerg. Infect. Dis. 7: 24-34.
EPA. 1998. What you should know about *Pfiesteria piscicida*: A Fact Sheet. pp. 1-6. U.S. Government Printing Office, Washington, D.C.
EPA. 2000. Proposed regulations to address water pollution from concentrated animal feeding operations. Report No. 833-F-00-016. Office of Water, Environmental Protection Agency, Washington, D.C.
EPA/AID. 1992. Manual: Guidelines for Water Reuse. U.S. Environmental Protection Agency / Agency for International Development. (http://www.fsis.usda.gov/oppde/rdad/frpubs/san_update1.pdf).
Epstein, P. 1998. Marine Ecosystems: Emerging Diseases as Indicators of Change. pp. 4-75. Health, Ecological, and Economic Dimensions (HEED) of Global Change Program, Harvard University, Boston, Mass.
Epstein, C.B. and Butow, R.A. 2000. Microarray technology: Enhanced versatility, persistent challenge. Current Opinions in Biotechnol. 11(1): 36-41.
Erickson, J.W. and Gross, C.A. 1989. Identification of the sE subunit of *Escherichia coli* RNA polymerase: A second alternate s factor involved in high-temperature gene expression. Genes Dev. 3: 1462-1471.
Ervik, A., Tehorsen, B., Eriksen, V., Lunestad, B.T., and Samuelsen, O.B. 1994. Impact of administering antibacterial agents on wild fish and blue mussels (*Mytilus edulis*) in the vicinity of fish farms. Disease of Aquatic Organisms18: 45-51.

F

Fairchild, T.M. and Foegeding, P.M. 1993. A proposed nonpathogenic biological indicator for thermal inactivation of *Listeria monocytogenes*. Appl. Environ. Microbiol. 59: 1247-1250.
Fairweather, N.F., Chatfiled, S.N., Charles, I.G., Roberts, M., Lipscombe, M., Jiang Li, L., Strugnell, D., Comerford, S., Tite, J., and Dougan, G. 1990. Use of live attenuated bacteria to stimulate immunity. Res. Microbiol. 141: 769-773.
Faith, N.G., Shere, J.A., Brosch, R., Arnold, K.W., Ansay, S.E., Lee, M.-S., Luchansky, J.B., and Kaspar, C.W. 1996. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. Appl. Environ. Microbiol. 62: 1519-1525.

Fang, F. C., Libby, S.J., Buchmeier, N.A., Loewen, P.C., Switala, J., Harwood, J., and Guiney, D.G. 1992. The alternative s factor KatF (RpoS) regulates *Salmonella* virulence. Proc. Natl. Acad. Sci. 89: 11978-11982.

Fankhauser, R.L., Noel, J.S., Monroe, S.S., Ando, T., and Glass, R.I. 1998. Molecular epidemiology of "Norwalk-like viruses" in outbreaks of gastroenteritis in the United States. J. Infect. Dis. 178: 1571-1578.

FAO. 1995. Code of conduct for responsible fisheries. Food and Agriculture Organization of the United Nations. Fisheries Department. p. 41. Rome.

FAO/WHO. 1998. Application of risk communication to food standards and safety matters. An expert consultation of the Food and Agricultural Organization of the United Nations and the World Health Organization, Feb. 2-6, Rome.

FAO/WHO. 2001. Risk characterization of *Salmonella* spp. in eggs and broiler chickens and *Listeria monocytogenes* in ready-to-eat foods. A Joint FAO/WHO Consultation on Risk Assessment of Microbiological Hazards in Food. Apr. 30 - May 4. Food and Agriculture Organization of the United Nations/World Health Organization, Rome.

Farber, J.M. and Pagotto, F. 1992. The effect of acid shock on the heat resistance of *Listeria monocytogenes*. Lett. Appl. Microbiol. 15: 197-201.

Farber, J.M., Gendel, S.M., Tyler, K.D., Boerlin, P., Landry, W.L., Fritschel, S.J., and Barrett, T.J. 2001. Typing and Differentiation. Chpt. 11 In "Compendium of Methods for the Microbiological Examination of Foods," 4th Ed., ed. F. P. Downes and K. Ito, American Public Health Association, Washington, D. C.

Farkas, J. 1998. Irradiation as a method for decontaminating food: A review. Int. J. Food Microbiol. 44: 189-204.

Fayer, R., Graczyk, T.K., Lewis, E.J., Trout, J.M., and Farley, C.A. 1998. Survival in infectious *Cryptosporidium parvuum* oocysts in seawater and Eastern oysters (*Crassostrea virginica*) in the Chesapeake Bay. Appl. Environ. Microbiol. 64: 1070-1074.

FDA. 1995. Procedures for the safe and sanitary processing and importing of fish and fishery products; Final Rule. Fed. Reg. 60: 65095-65202.

FDA. 1998. "Bacteriological Analytical Manual," 8th ed. AOAC International, Gaithersburg, Md.

FDA. 1999. Guidance for industry: Reducing microbial food safety hazards for sprouted seeds and guidance for industry: Sampling and microbial testing of spent irrigation water during sprout production. Fed. Reg. 64: 57893-57902.

FDA. 2000. Report of the FDA Retail Food Program Database of Foodborne Illness Risk Factors. September 10. Food and Drug Administration, Washington, D.C.

FDA. 2001. Good agricultural practices meeting. Food and Drug Administration, Orlando, Fla., June 28.

FDA/CFSAN. 1998. Guide to minimize microbial food safety hazards for fresh fruits and vegetables. U.S. Department of Health and Human Services, Food and Drug Administration/Center for Food Safety and Applied Nutrition. Washington, D.C. (http://www.foodsafety.gov/~dms/prodguid.html).

FDA/CFSAN. 2001. Draft risk assessment on the public health impact of *Vibrio parahaemolyticus* in raw molluscan shellfish. Food and Drug Administration/Center for Food Safety and Applied Nutrition. (http://www.cfsan.fda.gov/~dms/vprisk.html).

FDA/CFSAN. 2002. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook: The "Bad Bug Book." Food and Drug Administration/Center for Food Safety and Applied Nutrition, Washington, D.C. Accessed Jan. 29, 2002. (http://www.cfsan.fda.gov/~mow/intro.html)

FDA/CFSAN, USDA/FSIS, CDC. 2001. Draft assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Food and Drug Administration/Center for Food Safety and Applied Nutrition, U.S. Dept. of Agriculture/Food Safety and Inspection Service, and Centers for Disease Control and Prevention. (http://www.FoodSafety.gov/~dms/lmrisk.html).

FDA/CVM. 1997. CVM policy on competitive exclusion products. Feb. 21. Food and Drug Administration/Center for Veterinary Medicine. Washington, D.C.

Fedorka-Cray, P.J., Kelley, L.C., Stabel, T.J., Gray, J.T., and Laufer, J.A. 1995. Alternate routes of invasion may affect pathogenesis of *Salmonella typhimurium* in swine. Infect. and Immun. 63: 2658-2664.

Fedorka-Cray, P., Gray, J.T., and Wray, C. 2000. Salmonella infections in pigs. In "Salmonella in Domestic Animals." pp. 191-207. ed. C. Wray and A. Wray. CABI Publishing, Wallingford.

Feinman, S.E. 1998. Antibiotics in animal feed- Drug resistance revisited. ASM News 64: 24-30.

Feng, P., Lampel, K.A., Karch, H., and Whittam, T.S. 1998. Genotypic and phenotypic changes in the emergence of *Escherichia coli* O157:H7. J. Infect. Dis. 177: 1750-1753.

Finelli, L., Swerdlow, D., Mertz, K., Ragazzoni, H., and Spitalny, K. 1992. Outbreak of cholera associated with crab brought from an area with epidemic disease. J. Infect. Dis. 166: 1433-6.

Finlay, B.B. and Cossart, P. 1997. Exploitation of mammalian host cell functions by bacterial pathogens. Science 276: 718-725.

Finlay, B.B. and Falkow, S. 1997. Common themes in microbial pathogenicity revisited. Microbiol. Mol. Biol. Rev. 61: 136-69.

Fleming, L.E., Easom, J., Baden, D., Rowan, A., and Levin, B. 1999. Emerging harmful algal blooms and human health: *Pfiesteria* and related organisms. Toxicol. Pathol. 27(5): 573-581.

FNB/NRC. 1985. An evaluation of the role of microbiological criteria for foods and food ingredients. Subcommittee on Microbiological Criteria, Committee on Food Protection, Food and Nutrition Board, National Research Council. National Academy Press, Washington, D.C.

Foege, W.H. 1996. Alexander D. Langmuir: His Impact on Public Health. A Tribute to Alexander D. Langmuir. Am. J. Epidemiol. 144(8 Suppl): S11-50.

Foster, J.W. and Hall, H.K. 1990. Adaptive acidification tolerance response of *Salmonella typhimurium*. J. Bacteriol. 172: 771-778.

Francis, G.A. and O'Beirne, D. 1998. Effects of storage atmosphere on *Listeria monocytogenes* and competing microflora using a surface model system. Intl. J. Food Sci. Technol. 33: 465-476.

Frewer, L.J., Shepherd, R., and Sparks, P. 1994. The interrelationship between perceived knowledge, control and risk associated with a range of food-related hazards targeted at the individual, other people and society. J. Food Safety 14: 19-40.

Friedman, C.R., Neimann, J., Wegener, H.C ., and Tauxe, R.V. 1992. Epidemiology of *Campylobacter jejuni* Infections in the United States and Other Industrial Countries. In "*Campylobacter jejuni*: Current Status and Future Trends. ed. I. Nachamkin, M.J. Blaser, L.S. Tompkins, pp. 121-138. ASM Press, Washington, D.C.

Friedman, C., Reddy, S., Samual, M., Marcus, R., Bender, J., Desai, S., Shiferaw, B., Helfrick, D., Carter, M., Anderson, B., Hoekstra, M., and the EIP Working Group. 2000. Risk factors for sporadic *Campylobacter* infections in the United States: A case-control study on FoodNet sites. 2nd International Conference on Emerging Infectious Diseases, 2000. July 16-19. Abstr. No. 63, pp. 149-150. Atlanta, Ga. (http://www.cdc.gov/foodnet/pub/iceid/2000/friedman_c.htm).

Fujita, M., Tanaka, K., Takahashi, H., and Amemura, A. 1994. Transcription of the principal sigma-factor genes, *rpoD* and *rpoS*, in *Pseudomonas aeruginosa* is controlled according to the growth phase. Molec. Microbiol. 13: 1071-1077.

Fuller, R. 1989. A review: Probiotics in man and animals. J. Appl. Bacteriol. 66: 365-378.

Fuller, R. 1992. "Problems and prospects." In Probiotics, The Scientific Basis, ed. R. Fuller. Chapter 14, pp. 377-386. Chapman & Hall, London.

G

Gahan, C.G.M. and Hill, C. 1999. The relationship between acid stress responses and virulence in *Salmonella typhimurium* and *Listeria monocytogenes*. Intl. J. Food Microbiol. 50: 93-100.

GAO. 1998. "Federal Efforts to Ensure the Safety of Imported Foods are Inconsistent and Unreliable." GAO/RCED-98-103, USGAO2. General Accounting Office. Washington, D.C.

GAO. 1999. "Fresh Produce: Potential Implications of Country-of-Origin Labeling." GAO/T-RCED-99-200. General Accounting Office. Washington, D.C.

Garber, L.P., Wells, S.J., Hancock, D.D., Doyle, M.P., Tuttle, J., Shere, J.A., and Zhao, T. 1995. Risk factors for fecal shedding of *Escherichia coli* O157: H7 in dairy calves. J. Am. Vet. Med. Assn. 207: 46-49.

Garber, L., Wells, S., Schroeder-Tucker, L., and Ferris, K. 1999. Factors associated with fecal shedding of verotoxin-producing *Escherichia coli* O157 on dairy farms. J. Food Protect. 62: 307-312.

Gebreyes, W.A., Turner, M.B., Funk, J.A., Altier, C., and Davies, P.R. 1999. *Salmonella* prevalence, serotypes, and patterns of antimicrobial resistance in cohorts of nursery and finishing pigs. In "Proc. of the 3rd International Symposium on the Epidemiology and Control of Salmonella in Pork", pp. 250-251. Washington, D.C.

Gibbens, J.C., Pascoe, S.J., Evans, S.J., Davies, R.H., and Sayers, A.R. 2001. A trial of biosecurity as a means to control *Campylobacter* infection of broiler chickens. Prev. Vet. Med. 48: 85-99.

Gill, C.O., McGinnis, D.S., Bryant, J., and Chabot, B. 1995. Decontamination of commercial, polished pig carcasses with hot water. Food Microbiol. 12: 143-149.

Glasgow, H.B., Burkholder, J.M., Schmechel, D.E., Tester, P.A., and Rublee, P.A. 1995. Insidious effects of a toxic estuarine dinoflagellate on fish survival and human health. J. Toxicol. Environ. Health 46: 501-522.

Goepfert, J.M. 1976. The aerobic plate count, coliform and *Escherichia coli* content of raw ground beef at the retail level. J. Milk Food Technol. 39: 175-178.

Goldblith, S.A. 1989. 50 Years of progress in food science and technology: From art based on experience to technology based on science. Food Technol. 88-105, 286.

Gorman, B.M., Sofos, J.N., Morgan, J.B., Schmidt, G.R., and Smith, G.C. 1995. Evaluation of hand-trimming, various sanitizing agents, and hot water spray washing as decontamination interventions on beef brisket adipose tissue. J. Food Protect. 58: 899-907.

Gould, G.W. 2000a. Strategies for food preservation. Chpt. 2, In "The Microbiological Safety and Quality of Food." Vol. I. ed. B.M. Lund, T.C. Baird-Parker, and G.W. Gould, pp. 19-35. Aspen Publishers Inc., Gaithersburg, Md.

Gould, G.W. 2000b. New and emerging physical methods of preservation. Chpt. 13, In "The Microbiological Safety and Quality of Food." Vol. I. ed. B.M. Lund, T.C. Baird-Parker, and G.W. Gould, pp. 277-293. Aspen Publishers Inc., Gaithersburg, Md.

Grattan, L.M., Oldach, D., Perl, T.M., Lowitt, M.H., Matuszak, D.L., Dickson, C., Parrott, C., Shoemaker, R.C., Kauffman, C.L., Wasserman, M.P., Hebel, J.R., Charache, P., and Morris, J.G. 1998. Learning and

memory difficulties after environmental exposure to waterways containing toxin-producing *Pfiesteria* or *Pfiesteria*-like dinoflagellates. Lancet 352: 532-539.
Graves, D.J. 1999. Powerful tools for genetic analysis come of age. Trends Biotechnol. 17: 127-134.
Graves, L.M. and Swaminathan, B. 2001. PulseNet standardized protocol for subtyping Listeria monocytogenes by macrorestriction and pulsed-field gel electrophoresis. Intl. J. Food Microbiol. 65(1-2): 55-62.
Gray, J.T., Fedorka-Cray, P.J., Stabel, T.J., and Kramer, T.T. 1996. Natural transmission of *Salmonella choleraesuis* in swine. Appl. Environ. Microbiol. 62: 141-146.
Green, J., Brown, D., Cheesbrough, J., and Wright, P. 1999. The detection of environmental small round structured virus by nested reverse transcriptase polymerase chain reaction. (Abst. S3-8). International Workshop on Human Caliciviruses, Mar. 29-31, Atlanta, Ga.
Griffin, P.M. 1998. Epidemiology of Shiga toxin-producing *Escherichia coli* infections in humans in the United States. In "Escherichia coli and Other Shiga Toxin-Producing E. coli Strains." ed. J.B. Kaper and A.D. O'Brien. pp. 15-22. ASM Press, Washington, D.C.
Gross, C.A. 1996. Function and regulation of the heat shock proteins In "*Escherichia coli* and *Salmonella*." ed. F.C. Neidhardt. pp. 1382-1399. ASM Press, Washington, D.C.
Grossman, A.D., Erickson, J.W., and Gross, C.A. 1984. The *htpR* gene of *E. coli* is a sigma factor for heat shock promoters. Cell 38: 383-390.
Gruenewald, R., Blum, S., and Chan, J. 1994. Relationship between human immunodeficiency virus infection and salmonellosis in 20- to 59-year-old residents of New York City. Clin. Infect. Dis. 18(3): 358-363.
Guzewich, J.J., Bryan, R.L., and Todd, E.C.D. 1997. Surveillance of foodborne disease I. Purposes and types of surveillance systems and networks. J. Food Protect. 60: 555-556.

H

Haas, C.N. 1983. Estimation of risk due to low doses of microorganisms: A comparison of alternative methodologies. Am. J. Epidemiol. 118: 573-582.
Haas, G.J., Prescott, H.E., Dudley, E., Dik, R., Hintlian, C., and Keane, L. 1989. Inactivation of microorganisms by carbon dioxide under pressure. J. Food Safety 9: 253-265.
Hacker, J. and Kaper, J.B. 2000. Pathogenicity islands and the evolution of microbes. Ann. Rev. Microbiol. 54: 641-79.
Hacker, J., Blum-Oehler, G., Muhldorfer, I., and Tschape, H. 1997. Pathogenicity islands of virulent bacteria: Structure, function and impact on microbial evolution. Molec. Microbiol. 23: 1089-97.
Hall, J.A., Goulding, J.S., Bean, N.H., Tauxe, R.V., and Hedberg, C.W. 2001. Epidemiologic profiling: Evaluating foodborne outbreaks for which no pathogen was isolated by routine laboratory testing, United States: 1982-1989. Epidemiol. Infect. In press.
Hancock, D.D., Besser, T.E., Rice, D.H., Ebel, E.D., Herriott, D.E., and Carpenter, L.V. 1998. Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in Northwestern USA. Prevent. Vet. Med. 35: 11-19.
Harris, N.V., Weiss, N.S., and Nolan, C.M. 1986. The role of poultry and meats in the etiology of *Campylobacter jejuni/coli* enteritis. Am. J. Publ. Health 76: 407-411.
Hayes, E.B., Matte, T.D., O'Brien, T.R., McKinley, T.W., Logsdon, G.S., Rose, J.B., Ungar, B.L.P., Word, D.M., Pinsky, P.F., Cummings, M.L., Wilson, M.A., Long, E.G., Hurwitz, E.S., and Juranek, D.D. 1989. Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply. N. Eng. J. Med. 320: 1372-1376.
Hedberg, C.W. and Osterholm, M.T. 1993. Outbreaks of foodborne and waterborne viral gastroenteritis. Clin. Microbiol. Rev. 6: 199-210.
Hedberg, C.W., Savarino, S.J., Besser, J.M., Paulus, C.J., Thelen,V.M., Myers, L.J., Cameron, D.N., Barrett, T.J., Kaper, J.B., and Osterholm, M.T. 1997. An outbreak of foodborne illness caused by *Escherichia coli* O39:NM, an agent not fitting into the existing scheme for classifying diarrheageneic *E. coli*. J. Infect. Dis. 176: 1625-1628.
Hedberg, C.W., Smith, K.E., Besser, J.M., Boxrud, D.J., Hennessy, T.W., Bender, J.B., Anderson, F.A., and Osterholm, M.T. 2001. Limitations of pulsed-field gel electrophoresis for the routine surveillance of *Campylobacter* infections. J. Infect. Dis. 184: 242-243.
Hengge-Aronis, R. 1993. Survival of hunger and stress: The role of *rpoS* in early stationary phase gene regulation in *E. coli*. Cell 72: 165-168.
Hengge-Aronis, R. 1996. Back to log phase: ss as a global regulator in the osmotic control of gene expression in *Escherichia coli*. Molec. Microbiol. 21: 887-893.
Hengge-Aronis, R. 2000. The general stress response in *Escherichia coli*. In "Bacterial stress responses." ed. G. Storz and R. Hengge-Aronis. ASM Press, Washington, D.C.
Hennessy, T.W., Hedberg, C.W., Slutsker, L., White, K.E., Besser-Wiek, J.M., Moen, M.E., Feldman, J., Coleman, W.W., Edmonson, L.M., MacDonald, K.L., and Osterholm, M.T. 1996. A national outbreak of *Salmonella enteritidis* infections from ice cream. N. Eng. J. Med. 334: 1281-1286.
Herman, L. 1997. Detection of viable and dead *Listeria monocytogenes* by PCR. Food Microbiol. 14: 103-110.
Herman-Taylor, J., Bull, T.J., Sheridan, J.M., Cheng, J., Stellakis, M.L., and Sumar, N. 2000. Causation of Crohn's disease by *Mycobacterium avium* subspecies paratuberculosis, Can. J. Gastroenterol. 14(6): 521-39.
Herwaldt ,B.L., Ackers, M.L., and the Cyclospora Working Group. 1997. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. N. Engl J. Med. 336(22): 1548-1556.
Hilborn, E.D., Mermin, J.H., Mshar, P.A., Hadler, J.L., Voetsch, A., Wojtkunski, C., Swartz, M., Mshar, R., Lambert-Fair, M.A., Farrar, J.A., Glynn, M.K., and Slutsker, L. 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. Archiv. Intern. Med. 159: 1758-1764.
Hingley, A. 1997. Rallying the troops to fight foodborne illness. FDA Consumer Magazine. Nov./Dec. (www.fda.gov/fdac/features/1997/797-food.html)
Hite, B. 1899. The effect of pressure in the preservation of milk. WV Univ. Agric. Exp. Sta. Bull. No. 146: 1-67. Morgantown, W.V.
Hlady, W.G. and Klontz, K.C. 1996. The epidemiology of *Vibrio* infections in Florida, 1981-1993. J. Infect. Dis. 173: 1176-1183.
Hlady, W.G., Mulle, R.C., and Hopkins, R.S. 1993. *Vibrio vulnificus* from raw oysters: Leading cause of reported deaths from illness in Florida. J. Fla. Med. Assn. 80: 536-538.
Hogue, A., White, P., Guard-Petter, J., Schlosser, W., Gast, R., Ebel, E., Farrar, J., Gomez, T., Madden, J., Madison, M., McNamara, A.M., Morales, R., Parham, D., Sparling, P., Sutherlin, W., and Swerdlow, D. 1997. Epidemiology and control of egg-associated *Salmonella enteritidis* in the United States of America. Rev. Sci. Tech. 16: 542-53.
Hollinger, K., Wray, C., Evans, S., Pascoe, S., Chappell, S., and Jones, Y. 1998. *Salmonella typhimurium* DT104 in cattle in Great Britian. J. Am. Vet. Med. Assn. 213: 1732-1733.
Holmberg, S.D., Wells, I.G., and Cohen, M.L. 1984. Animal-to-human transmission of antimicrobial-resistant *Salmonella*: Investigations of U.S. outbreaks 1971-1983. Science 225: 833-835.
Hoover, D.G. 1993. Pressure effects on biological systems. Food Technol. 47(6): 150-155.
Hopkins, R.S., Olmsted, R., and Istre, G.R. 1984. *Campylobacter jejuni* infection in Colorado: Identified risk factors. Am. J. Publ. Health 74: 249-250.
Hosek, G., Leschinsky, D., Irons, S., and Safranek, T.J. 1997. Multidrug-resistant *Salmonella* serotypre typhimurium- United States, 1996. MMWR. 46: 308-310.
Howgate, P. 1997. Review of the public health safety of products from aquaculture. A report prepared for the Fish Utilization and Marketing Service, Fisheries Utilization Division, Food and Agricultural Organization of the United Nations. Presented at the Joint FAO/WHO Study Group on Food Safety Issues Associated with Products from Aquaculture. June 1997. Bangkok, Thailand.
Hovde, C.J., Austin, P.R., Cloud, K.A., Williams, C.J., and Hunt, C.W. 1999. Effect of cattle diet on *Escherichia coli* O157:H7 acid resistance. Appl. Environ. Microbiol. 65: 3233-3235.
Hueck, C.J. 1998. Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol. Molec. Biol. Rev. 62: 379-433.
Hughes, D.B. and Hoover, D.G. 1991. *Bifidobacteria*: Their potential for use in American dairy products. Food Technol. 45(1): 74-83.
Humphrey, T.J., Richardson, N.P., Statton, K.M., and Rowbury, R.J. 1993. Effects of temperature shift on acid and heat tolerance in *Salmonella enteritidis* phage type 4. Appl. Environ. Microbiol. 59: 3120-3122.
Hutwagner, L.C., Maloney, E.K., Bean, N.H., Slutsker, L., and Martin, S.M. 1997. Using laboratory-based surveillance data for prevention: An algorithm for detecting *Salmonella* outbreaks. Emerg. Infect. Dis. 3: 395-400.

I

ICMSF. 1986. "Microorganisms in Foods 2. Sampling for Microbiological Analysis: Principles and Specific Applications." 2nd Ed. International Commission on Microbiological Specifications for Food. University of Toronto Press, Toronto.
ICMSF. 1996. "Microorganisms in Foods 5. Microbiological Specifications of Food Pathogens." International Commission on Microbiological Specifications for Food. Blackie Academic and Professional, an imprint of Chapman and Hall, New York, N.Y.
ICMSF. 1997. Establishment of microbiological safety criteria for foods in international trade. International Commission on Microbiological Specifications for Food. World Health Statist. Quart. 50: 119-123.
ICMSF. 2002. "Microorganisms in Foods 7. Microbiological Testing in Food Safety Management." International Commission on Microbiological Specifications for Food. Kluwer Academic, N.Y.
IFT. 1989. Top 10 food science innovations 1939-1989. Food Technol. 43(9): 308.
IFT. 2000a. A Century of Food Science. IFT Research Report. Institute of Food Technologists, Chicago, Ill.
IFT. 2000b. Kinetics of microbial inactivation for alternative food processing technologies. A report of the Institute of Food Technologists for the Food and Drug Administration, Institute of Food Technologists, Chicago, Ill. J. Food Sci. 65(8): 1S-108S.
IFT. 2002. Analysis and evaluation of preventive control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. A report of the Institute of Food Technologists for the Food and Drug Administration, Institute of Food Tech-

nologists, Chicago, Ill. J. Food Sci. In press.
Ingram, M. 1964. Feeding meat animals before slaughter. Vet. Record 76: 1305-1315.
Ingram, M. 1977. The significance of the index and indicator organisms in foods. Presented at the 10th Intl. Symposium of the IUMS Committee on Food Microbiology and Hygiene, Sept. 5-10, Szczecin, Poland.
Ingram M. and Dainty, R.H. 1971. Changes caused by microbes in spoilage of meat. J. Appl. Bacteriol. 34: 21-39.
Iversen, A.M., Gill, M., Bartlett, C.L.R. 1987. Two outbreaks of foodborne gastroenteritis caused by a small round structured virus: Evidence of prolonged infectivity in a food handler. Lancet 2(8558): 556-558.

J

Jackson, L.S., De Vries, J.W., and Bullerman, L.B. 1996. "Fumonisins in food". Plenum Press, New York, N.Y.
Jacob, J.Q. 2000. The final stages of global eradication of polio. N. Eng. J. Med. 343: 806-807.
Jaenicke, R. 1981. Enzymes under extreme conditions. Ann. Rev. Biophys. Bioeng. 10: 1-67.
Jay, J.J. 2000. "Modern Food Microbiology". 6th ed. pp. 425-437. Aspen Publishers, Inc. Gaithersburg, Md.
Jaykus, L.A. 1996. The application of quantitative risk assessment to microbial food safety risks. CRC Crit. Rev. Microbiol. 22: 279-93.
Jaykus, L. 1997. Epidemiology and detection as options for control of viral and parasitic foodborne disease. Emerg. Infect. Dis. 3: 529-539.
Jaykus, L.A. 2000a. Detection of human enteric viruses in foods. In "Foodborne Disease Handbook: Viruses, Parasites, and HACCP." Vol. 2, 2nd Ed. ed. Y.H. Hui, S.A. Sattar, K.D. Murrell, W.K. Nip, P.S. Stanfield. pp. 137-163. Marcel Dekker, New York, N.Y.
Jaykus, L.A. 2000b. Enteric viruses as "emerging" agents of foodborne disease. Irish J. Agr. Food Res. 39: 245-255.
Jaykus, L. and Ward, D.R. 1999. An integrated approach: The future of graduate food safety education. Dairy Food Environ. Sanita. 19: 14-17.
Jaykus, L., DeLeon, R., and Toranzos, G.A. 2001. Detection of bacteria, viruses and parasitic protozoa in shellfish. In "Manual of Environmental Microbiology," 2nd Ed. ed. C.J. Hurst. In press.
Jenkins, D.E., Schultz, J.E., and Matin, A. 1988. Starvation-induced cross protection against heat or H_2O_2 challenge in *Escherichia coli*. J. Bacteriol. 170: 3910-3914.
Jenkins, D.E., Chaisson, S.A., and Matin, A. 1990. Starvation-induced cross protection against osmotic challenge in *Escherichia coli*. J. Bacteriol. 172: 2779-2781.
Jensen, L.B. and Hess, W.R. 1941. A study of ham souring. Food Res. 6: 272-326.
Jensen, G.L. and Greenless, K.J. 1997. Public health issues in aquaculture. OIE Scientific and Technical Review. 16(2): 641-651.
Johnson, F.H. and Campbell, D.H. 1945. The retardation of protein denaturation by hydrostatic pressure. J. Cell Comp. Physiol. 26: 4-7.
Johnson, K., Charles, I., Dougan, G., Pickard, D., O'Gaora, P., Costa, G., Ali, T., Miller, I., and Hormaeche, C. 1991. The role of a stress-response protein in *Salmonella typhimurium* virulence. Mol. Microbiol. 5: 401-407.
Josephson, E.S., Thomas, M.H., and Calhoun, W.K. 1978. Nutritional aspects of food irradiation: An overview. J. Food Proc. Preserva. 2: 299-313.
JSA. 1994. Guide to drug vaccine and pesticide use in aquaculture. Publication No. B-5085. Texas Agricultural Extension Service. Texas A&M University, College Station.

K

Kalow, W. 1993. Pharmacogenetics: Its biologic roots and the medical challenge. Clin. Pharmacol. Ther. 54: 235-241.
Kaplan, J.E., Gary, G.W., Baron, R.C., Singh, N., Schonberger, L.B., Feldman, R., and Greenberg, H.B. 1982. The epidemiology of Norwalk gastroenteritis and the role of Norwalk virus in outbreaks of acute nonbacterial gastroenteritis. Ann. Intern. Med. 96: 756-761.
Kaper, J.B. and O'Brien, A.D. 1998. "*Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains." ASM Press, Washington, D.C.
Kassenborg, H., Hedberg, C., Evans, M., Chin, G., Fiorentino, T., Vugia, D., Bardsley, M., Slutsker, S., and Griffin, P. 1998. Case-control study of sporadic *Escherichia coli* O157: H7 infections in 5 FoodNet sites (CA, CT, GA, MN, OR). (Abstr.) In "Program and Abstracts of the International Conference on Emerging Infectious Diseases," Atlanta, Mar. 8-11. American Society for Microbiology, Washington, D.C.
Kenny, B., DeVinney, R., Stein, M., Reinscheid, D.J., Frey, E.A., and Finlay, B.B. 1997. Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. Cell 91: 511-20.
Kerry, J., Hiney, M., Coyne, R., Cazabon, D., NicGabhainn, S., and Smith, P. 1994. Frequency and distribution of resistance to oxytetracycline in microorganisms isolated from marine fish farm sediments following therapeutic use of oxytetracycline. Aquaculture (123): 43-54.
Kilcast, D. 1994. Effect of irradiation on vitamins. Food Chem. 49: 157-164.
Kim, J., Nietfeldt, J., and Benson, A.K. 1999. Octamer based genome scanning distinguishes a unique subpopulation of *Eschericia coli* O157: H7 strains in cattle. Proc. Natl. Acad. Sci. 96: 13288-13293.
Kimmitt, P.T., Harwood, C.R., and Barer, M.R. 2000. Toxin gene expression by shiga toxin-producing *Escherichia coli*: The role of antibiotics and the bacterial SOS response. Emerg. Infect. Dis. 6(5): 458-465.
King, L.J. 1990. The National Health Monitoring System: Fulfilling a Commitment. Preven.Vet. Med. 8: 89-95.
King, W., Raposa, S., Warshaw, J., Johnson, A., Halbert, D., and Klinger, J.D. 1989. A new colorimetric nucleic acid hybridization assay for Listeria in foods. Intl. J. Food Microbiol. 8: 225.
Kinugasa, H., Takeo, T., Fukumoto, K., and Ishihara, M. 1992. Changes in tea components during processing and preservation of tea extract by hydrostatis pressure sterilization. Nippon Nogeikagaku Kaishi 66(4): 707-712.
Klontz, K.C., Timbo, B., Fein, S., and Levy, A. 1995. Prevalence of selected food consumption and preparation behaviors associated with increased risks of food-borne disease. J. Food Protect. 58: 927-930.
Kobayashi, S. and Ando, S. 2000. Reactive arthritis or Reiter's syndrome and B51-associated seroneagtive spondyloarthropathy. Intern. Med. 39(2): 89.
Kolter, R., Siegele, D.A., and Tormo, A. 1993. The stationary phase of the bacterial life cycle. Annu. Rev. Microbiol. 47: 855-874.
Koo, K. and Jaykus, L. 2000. A modified method to detect PCR products by 5' nuclease activity and an asymmetric fluorogenic probe set. BioTechniques 29: 690-694.
Korkeala, H., Stengel, G., Hyytia, E., Vogelsang, B., Bohl, A., Wihlman, H., Pakkala, P., and Hielm, S. 1998. Type E botulism associated with vacuum-packaged hot-smoked whitefish. Intl. J. Food Microbiol. 43: 1-5.
Kornacki, J.L. and Johnson, J.L. 2001. *Enterobacteriaceae*, coliforms, and *Escherichia coli* as quality and safety indicators. In "Compendium of Methods for the Microbiological Examination of Foods." ed. F.P. Downes and K. Ito. pp. 69-82. American Public Health Association, Washington, D.C.
Korzenik, J.R. and Dieckgraefe, B.K. 2000. Is Crohn's disease an immunodeficiency? A hypothesis suggesting possible early events in the pathogenesis of Crohn's disease. Dig. Dis. Sci. 45(6): 1121-29.
Krishnamachari, K.A.V.R., Bhat, R.V., Nagarajan, V., and Tilac, T.B.G. 1975. Hepatitis due to aflatoxicosis. Lancet 1: 1061-1063.
Kudva, I. T., C. W. Hunt, C. J. Williams, U. M. Nance, and Hovde, C. J. 1997. Evaluation of dietary influences on *Escherichia coli* O157:H7 shedding by sheep. Appl. Environ. Microbiol. 63: 3878-3886.
Kulick, I. and Giachino, P. 1997. The alternative sigma facter s^B in *Staphylococcus aureus*: Regulation of the *signB* operon in response to growth phase and heat shock. Arch. Microbiol. 167: 151-159.
Kushner, D.J. 1980. Extreme environments. In "Contemporary Microbial Ecology," ed. D.C. Ellwood, J.N. Hedger, M.J. Latham, et al., pp. 29-54. Academic Press, Inc., London.

L

Landini, P., Hajec, L.I., Nguyen, L.H., Burgess, R.R., and Volkert, M.R. 1996. The leucine-responsive regulatory protein (Lrp) acts as a specific repressor for the sS-dependent transcription of the *Escherichia coli aidB* gene. Molec. Microbiol. 20: 947-955.
Lange, R. and Hengge-Aronis, R. 1991. Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. Molec. Microbiol. 5: 49-59.
Langhendries, J.P., Detry, J., Van Hees J., Lamboray, J.M., Darimont, J., Mozin, M.J., Secretin, M.C., and Senterre, J. 1995. Effect of a fermented infant formula containing viable bifidobacteria on the fecal flora composition and pH of healthy full-term infants. J. Pediatr. Gastroenterol. Nutr. 21: 177-181.
Larsen, W.P., Hartzell, T.B., and Diehl, H.S. 1914. The effects of high pressure on bacteria. J. Infect. Dis. 22: 271-279.
Lawlor, K.A., Pierson, M.D., Hackney, C.R., Claus, J.R., and Marcy, J.E. 2000. Nonproteolytic *Clostridium botulinum* toxigenesis in cooked turkey stored under modified atmospheres. J. Food Protect. 63: 1511-1516.
Lawrence, J.G. and Ochman, H. 1998. Molecular archaeology of the *Escherichia coli* genome. Proc. Natl. Acad. Sci. 95: 9413-7.
Lee, L.A., Gerber, A.R., Lownsay, D.R., Smith, J.D., Carter, G.P., Puhr, N.D., Puhr, N.D., Parrish, C.M., Sikes, R.K., Finton, R.J., and Tauxe, R.V. 1990. *Yersinia enterocolitica* 0:3 infections in infants and children associated with household preparation of chitterlings. N. Eng. J. Med. 322: 984-7.
Lee, I. S., Lin, J., Hall, H.K., Bearson, B., and Foster, J.W. 1995. The stationary-phase sigma factor ss (RpoS) is required for a sustained acid tolerance response in virulent *Salmonella typhimurium*. Molec. Microbiol. 17: 155-167.
Lee, L., Arul, J., Lencki, R., and Castaigne, F. 1996. A review on modified atmosphere packaging and preservation of fresh fruits and vegetables: Physiological basis and practical aspects. Part 2. Packaging Technol. Sci. 9: 1-17.
Leistner, L. 2000. Use of combined preservative factors in foods of developing countries. Chpt. 14, In "The Microbiological Safety and Quality of Food." Vol. I. ed. B.M. Lund, T.C. Baird-Parker, and G.W. Gould, pp. 294-314. Aspen Publishers, Gaithersburg, Md.
Leung, C-K., Huang, Y-W., and Pansorbo, O.C. 1992. Bacterial pathogens and indicators in catfish and pond environments. J. Food Protect. 55: 424-427.
Levin, E.D., Schmechel, D.E., Burkholder, J.M., Glas-

gow, H.B., Deamer-Melia, N., Moser, V.C., and Harry, G.J. 1997. Persistent learning deficits in rats after exposure to *Pfiesteria piscicida*. Environ. Health Perspect. 105(12): 2-7.
Levine, P., Rose, B., Green, S., Ransom, G., and Hill, W. 2001. Pathogen testing of ready-to-eat meat and poultry products collected at federally inspected establishments in the United States, 1990 to 1999. J. Food Protect. 64: 1188-1193.
Levy, S.B. 1992. The antibiotic paradox. How Miracle Drugs are Destroying the Miracle. Plenum Press, New York, N.Y.
Levy, A. 1997. Overview of consumer studies of food safety knowledge, attitudes and behavior. Presented at Changing strategies-changing behavior: What food safety communicators need to know. June 12-13, Washington, D.C., U.S. Dept. of Agriculture and Food and Drug Administration, Washington, D.C. (http://www.fda.gov)
Levy, S.B., Fitzgerald, G.B., and Macone, A.B. 1976. Changes in the intestinal flora of farm personnel after introduction of tetracycline-supplemented feed on a farm. N. Eng. J. Med. 295: 583-588.
Leyer, G.J. and Johnson, E.A. 1992. Acid adaptation promotes survival of *Salmonella* spp. in cheese. Appl. Environ. Microbiol. 58: 2075-2080.
Leyer, G.J. and Johnson, E.A. 1993. Acid adaptation induces cross-protection against environmental stresses in *Salmonella typhimurium*. Appl. Environ. Microbiol. 59: 1842-1847.
Liao, C. and Sapers, G. 2000. Attachment and growth of *Salmonella* Chester on apple fruits and in vivo response of attached bacteria to sanitizer treatments. J. Food Protect. 63: 876-883.
Linton, A.H., Handley, B., Osborne, A.D., Shaw, B.G., Roberts, T.A., and Hudson, W.R. 1976. Contamination of pig carcasses at two abattoirs by *Escherichia coli* with special reference to O-serotypes and antibiotic resistance. J. Appl. Bacteriol. 41: 89-110.
Loewen, P.C., Hu, B., Strutinsky, J., and Sparling, R. 1998. Regulation in the *rpoS* regulon of *Escherichia coli*. Can. J. Microbiol. 44: 707-717.
Losinger, W.C., Wells, S.J., Garber, L.P., Hurd, H.S., and Thomas, LA. 1995. Management factors related to *Salmonella* shedding by dairy heifers. J. Dairy Sci. 78: 2474-2472.
Lou, Y. and Yousef, A.E. 1997. Adaptation to sublethal environmental stresses protects *listeria monocytogenes* against lethal preservation factors. Appl. Environ. Microbiol. 63: 1252-1255.
Lund, D. 1989. Food processing: From art to engineering. Food Technol. 43(9): 242-247.
Luo, X. 1994. Food poisoning caused by Fusarium toxins, pp. 129-136. International Life Sciences Institute, Chatuchak, Thailand.

M

MacKenzie, W.R., Hoxie, N.J., Proctor, M.E., Gradus, M.S., Blair, K.A., Petersen, D.E., Kazmierczak, J.J., Fox, K.R., Addiss, D.G., Rose, J.B., and Davis, J.P. 1994. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. N. Eng. J. Med. 331: 161-167.
Mackey, B.M. 2000. Injured Bacteria. In "The Microbiological Safety and Quality of Foods." ed. B.M. Lund, T.C. Baird-Parker and G.W. Gould. Aspen Publishers, Gaithersburg, Md.
Mahon, B.E., Ponka, A., Hall, W., Komatsu, K., Dietrich, S.E., Siitonen, A., Cage, G., Hayes, P.S.,Lambert-Fair, M.A., Bean, N.H., Griffin, P.M., and Slutsker, L. 1997. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seeds. J. Infect. Dis. 175: 876-82.
Malinverni, R., Glauser, M.P., Bille, J., and Rocourt, J. 1986. Unusual clinical features of an epidemic of listeriosis associated with a particular phage type. Eur. J. Clin. Microbiol. 5: 169-171.
Mallett, J.C., Beghian, L.E., Metcalf, T.G., and Kaylor, J.D. 1991. Potential of irradiation technology for improved shellfish sanitation. J. Food Safety 11: 231-245.
Manchester, A. and Clauson, A. 1995. 1994 spending for food away from home outpaces food at home. Food Review 18: 12-5.
Mandell, D., Bennett, G.L., and Dolin, J.E. 1995. "Bennett's Principles and Practice of Infectious Diseases." 4th Ed. W.B. Saunders and Co., New York, N.Y.
Manger, R.L., Leja, L.S., Lee, S.Y., Hungerford, J.M., Hokama, Y., Dickey, R.W., Granade, H.R., Lewis, R., Yasumoto, T., and Wekell, M.M. 1995. Detection of sodium channel toxins: Directed cytotoxicity assays of purified ciguatoxins, brevetoxins, saxitoxins, and seafood extracts. J. AOAC Intl. 78(2): 521-527.
Marks, P.J., Vipond, I.B., Carlisle, D., Deakin, D., Fey, R.E., and Caul, E.O. 2000. Evidence for airborne transmission of Norwalk-like virus (NLV) in a hotel restaurant. Epidemiol. Infect. 124: 481-487.
Marron, L., Emerson, N., Gahan, C.G., and Hill, C. 1997. A mutant of *Listeria monocytogenes* L028 unable to induce an acid tolerance response displays diminished virulence in a murine model. Appl. Environ. Microbiol. 63(12): 4945-4947.
Marth, E.H. 1998. Extended shelf life refrigerated foods: Microbiological quality and safety. A Scientific Status Summary by the Institute of Food Technologists Expert Panel on Food Safety and Nutrition. Chicago, Ill. Food Technol. 52(2): 57-62.
Masson, P. 1992. Protein denaturation of proteins. In "High Pressure and Biotechnology," ed. C. Balny, R. Hayashi, and P. Masson. pp. 89-99. John Libby Eurotext Ltd., London.
Matin, A., Auger, E.A., Blum, P.H., and Schultz, J.E. 1989. Genetic basis of starvation survival in nondifferentiating bacteria. Ann. Rev. Microbiol. 43: 293-316.
Mattick, K.L., Jorgensen, F., Legan, J.D., Lappin-Scott, H.M., and Humphrey, T.J. 2000. Habituation of *Salmonella* spp. at reduced water activity and its effect on heat tolerance. Appl. Environ. Microbiol. 66: 4921-4925.
Maule, A. 2000. Survival of verocytotoxigenic *Escherichia coli* O157: H7 in soil, water and on surfaces. J. Appl. Microbiol. 88 (Suppl): 71S-78S.
Mazotta, A.S. 1999. Problems and solutions to development of pathogen resistance to thermal processing. ISSI Meeting.
McDaniel, T.K., Jarvis, K.G., Donnenberg, M.S., and Kaper, J.B. 1995. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. Proc. Natl. Acad. Sci. 92: 1664-8.
McDermott, M.R. and Bienestock, J. 1979. Evidence for a common mucosal immunologic system I. Migration of B immunoblasts into intestinal, respiratory and genital tissues. J. Immunol. 122: 1892-1898.
McDougald, D., Rice, S.A., Weichart, D., and Kjelleberg, S. 1998. Nonculturability: Adaptation or debilitation? FEMS Microbiol. Ecol. 25: 1-9.
McFeters, G.A., Yu, F.P., Pyle, B.H., and Stewart, P.S. 1995. Physiological assessment of bacteria using fluorochromes. J. Microbiol. Meth. 21: 1-13.
McGhee, J.R., Mestecky, J., Dertzbaugh, M.T., Eldridge, J.H., Hirasawa, M., and Kiyono, H. 1992. The mucosal immune system: From fundamental concepts to vaccine development. Vaccine 10 (2): 75-88.
McGlynn, K.A., Rosvold, E.A., Lustbader, E.D., Hu, Y., Clapper, M.L., Zhouo, T., Wild, C.P., Xia, X.L., Baffoe-Bonnie, A., Ofori Adjei, D., Chen, G.-C., London, W.T., Shen, F.-M., and Buetow, K.W. 1995. Susceptibility to hepatocellular carcinoma is associated with genetic variation in the enzymatic detoxification of aflatoxin B1. Proc. Natl. Acad. Sci. 92: 2384-2387.
McKay, A.M. 1992. Viable but non-culturable forms of potentially pathogenic bacteria in water. Lett. Appl. Microbiol. 14: 129-135.
McKillip, J.L. and Drake, M.A. 2000. Molecular beacon-PCR detection of *Escherichia coli* 0157:H7 in milk. J. Food Protect. 63: 855-859.
McKillip, J.L., Jaykus, L.A., and Drake, M.A. 1998. rRNA stability in heat-killed and UV-irradiated enterotoxigenic *Staphylococcus aureus* and *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 64: 4264-4268.
McMullen, M., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devasting impact. Plant Dis. 81(12): 1340-1348.
Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., and Tauxe, R.V. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5: 607-625.
Mecsas, J., Rouviere, P.E., Erickson, J.W., Donohue, T.J., and Gross, C.A. 1993. The activity of s E, an *Eschericia coli* heat-inducible s-factor, is modulated by expression of outer membrane proteins. Genes Dev. 7: 2618-2628.
Meer, R.R. and Misner, S.L. 2000. Food safety knowledge and behavior of expanded food and nutrition program participants in Arizona. J. Food Protect. 63: 1725-1731.
Mermelstein, N.H. 2001. Sanitizing Meat. Food Technol. 55(3): 64-68.
Metchnikoff. E. 1908. "The prolongation of life." 1st Ed. Putnams Sons, New York,I N.Y.
Meyer, R.S., Cooper, K.L., Knorr, D., and Lelieveld, H.L.M. 2000. High-pressure sterilization of foods. Food Technol. 54(11): 67-68, 70, 72.
Midgley, J. and Desmarchelier, P. 2001. Pre-slaughter handling of cattle and Shiga toxin-producing *Escherichia coli* (STEC). Lett. Appl. Microbiol. 32: 307-311.
Miller, L.G. and Kaspar, C.W. 1993. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. J. Food Protect. 57: 460-464 [errata 57: 645].
Mitsuoka, T. 1989. "Microbes in the Intestine," pp. 24-25. Yakult Honsha Co., Ltd., Tokyo.
Mitsuoka, T. 1990. "A Profile of Intestinal Bacteria." Yakult Honsha Co., Ltd., Tokyo.
Miwa, N., Nishina, T., Kubo, S., Atsumi, M., and Honda, H. 1998. Amount of enterotoxigenic *Clostridium perfringens* in meat detected by nested PCR. Intl. J. Food Microbiol. 42: 195-200.
Moe, C.L. 1996. Waterborne transmission of infectious agents. In "Manual of Environmental Microbiology." ed. C.J. Hurst, G.R. Knudsen, M.J. McInerney, L.D. Stenzenbach, and M.V. Walter. ASM Press, Washington, D.C.
Moe, C.L., Rhodes, D., Pusek, S., Tseng, F., Heizer, W., Kapoor, C., Gilliam, B., Harb, M., Stewart, P., Miller, S., Sobsey, M., Herrmann, J., Blacklow, N., and Calderon, R. 1998. Determination of Norwalk virus dose-response in human volunteers. Poster # C-384, In "Abstracts of the 98th Annual Meeting of the American Society for Microbiology," Atlanta, Ga.
Moe, C.L., Sobsey, M.D., Stewart, P.W., and Crawford-Brown, D. 1999. Estimating the risk of human calicivirus infection from drinking water. Abstr. P4-6. In "Abstract Book of the International Workshop on Human Caliciviruses," Atlanta, Ga.
Molbak, K., Baggesen, D.L., Aarestrup, F.M., Ebbesen, J.M., Engberg, J., Frydendahl, K., Gerner-Smidt, P., Petersen, A.M., and Wegener, H.C. 1999. An outbreak of multidrug resistant, quinolone-resistant *Salmonella* enterica serotype Typhimurium DT104. N. Eng. J. Med. 341: 1420-1425.
Moleyar, V. and Narasimham, P. 1994. Modified atmosphere packaging of vegetables: An appraisal. J.

Food Sci. Technol. 31: 267-278.
Molin, G. 2000. Modified Atmospheres. In "The Microbiological Quality and Safety of Food," ed. B.M. Lund, T. C. Baird-Parker, and G.W. Gould. pp. 214-234. Aspen Publishers Inc., Gaithersburg, Md.
Monk, J.D., Beuchat, L.R., and Doyle, M.P. 1995. Irradiation inactivation of food-borne microorganisms. J. Food Protect. 58: 197-208.
Morita, M.T., Kanemori, M., Yanagi, H., and Yura, T. 2000. Dynamic interplay between antagonistic pathways controlling the sigma 32 level in *Escherichia coli*. Proc. Natl. Acad. Sci. 97(11): 5860-5865.
Mossel, D.A., Corry, J.E.L., Struijk, C.B., and Baird, R.M. 1995. Essentials of the microbiology of foods: a textbook for advanced studies. John Wiley and Sons. Chichester, England.
Mulvey, M.R., Switala, J., Borys, A., and Loewen, P.C. 1990. Regulation of transcription of *katE* and *katF* in *Escherichia coli*. J. Bacteriol. 172: 6713-6720.
Murray, C.J.L. and Lopez, A.D. 1996. The Global Burden of Disease: A Comprehensive Assessment of Mortality and Disability from Diseases, Injuries, and Risk Factors in 1990 and Projected to 2020. Vol. I. Harvard Sch. of Public Health, Harvard University Press, Cambridge, Mass.
Murray, C.J.L. and Lopez, A.D. 2001. The Global Epidemiology of Infectious Diseases. In "Global Burden of Disease and Injury." Harvard Univ. Press. Cambridge, Mass.
Musser, J.M. 1996. Molecular population genetic analysis of emerged bacterial pathogens: Selected insights. Emerg. Infect. Dis. 2: 1-17.

N

NACMCF. 1992. Hazard analysis and critical control point system. National Advisory Committee on Microbiological Criteria for Foods. Intl. J. Food Microbiol. 16: 1-25.
NACMCF. 1998. Hazard analysis and critical control point principles and application guidelines. National Advisory Committee on Microbiological Criteria for Foods. J. Food Protect. 61: 1246-1259.
NACMCF. 1999. Microbiological safety evaluations and recommendations on sprouted seeds. National Advisory Committee on Microbiology Criteria for Food. Int. J. Food Microbiol. 52: 123-153.
Naniche, D. and Oldstone, M.B. 2000. Generalized immunosuppression: How viruses undermine the immune response. Cell. Mol. Life Sci. 57(10): 1399-1407.
Narayan, K.G. 1966. Studies on clostridia incidence in beef cattle. Acta Veterinaria Acad. Sci. Hung. 16: 65-72.
NAS. 1991. "Seafood Safety." Committee on Evaluation of the Safety of Fishery Products, Food and Nutrition Board, Institute of Medicine. National Academies. Washington, D.C. National Academy Press. Washington, D.C.
Nataro, J.P. and Kaper, J.B. 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 11: 142-201.
Navin, T.R. and Juranek, D.D. 1984. *Cryptosporidiosis*: Clinical, epidemiologic, and parasitologic review. Rev. Infect. Dis. 6: 313-27.
Newby, T.J. 1984. Protective immune responses in the intestinal tract. In "Local Immune Responses of the Gut," ed. T.J. Newby and C.R. Stokes. pp. 143-198. CRC Press, Boca Raton, Fla.
Ngindu, A., Kenya, P.R., Ocheng, D.M., Omonde, T.N., Ngare, W., Gatei, D., Johnson, B.K., Ngira, J.A., Nandwa, H., Jansen, A.J., Kaviti, J.N., and Siongok, T.A. 1982. Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. Lancet 1: 1346-1348.
Nguyen-the, C. and Carlin, F. 2000. Fresh and processed vegetables. In "The Microbiological Safety and Quality of Food." ed. B.M. Lund, T.C. Baird-Parker, and G.W. Gould. pp. 620-684. Aspen Publishers, Inc. Gaithersburg, Md.
NIH/DMID. 1998. Crohn's disease: Is there a microbial etiology? Division of Microbiology and Infectious Diseases. National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, Md.
NLMB. 1994. Solving the *E. coli* 0157:H7 problem: A blueprint for industry action. Final Report of the Blue Ribbon Task Force. National Live Stock and Meat Board, National Cattlemens Beef Association, Centinnial, Colo.
NMFS. 2000. Fisheries of the U.S. Current Fisheries Statistics. 1999. U.S. Department of Commerce, NOAA, National Marine Fisheries Service. Silver Spring, Md.
Noga, E.J. 1997. Ichthyotoxicity of *Pfiesteria piscicida*. NIEHS Workshop on Hazardous Marine/Freshwater Microbes and Toxins, North Carolina Biotechnology Center, Research Triangle Park, Raleigh, N.C.
Noga, E.J. 2000. Culturing of *Pfiesteria* and production of *Pfiesteria* toxin(s). North Carolina State University Research Triangle Park, Raleigh, N.C.
Nogva, H.K., Bergh, A., Holck, A., and Rudi, K. 2000. Application of the 5'-nuclease PCR assay in evaluation and development of methods for quantitative detection of *Campylobacter jejuni*. Appl. Environ. Microbiol. 66: 4029-4036.
Norton, D.-M. and Batt, C.A. 1999. Detection of viable *Listeria monocytogenes* with a 5' nuclease PCR assay. Appl. Environ. Microbiol. 65: 2122-2127.
Norton, D.M., McCamey, M.A., Gall, K.L., Scarlett, J.M., Boor, K.J., and Wiedmann, M. 2001. Molecular studies on the ecology of *Listeria monocytogenes* in the smoked fish processing industry. Appl. Environ. Microbiol. 67: 198-205.
NRC. 1989. "Improving Risk Communication." Committee on Risk Perception and Communication. National Research Council. National Academy Press, Washington, D.C.
NRC. 1996. "Understanding Risk: Informing Decisions in a Democratic Society." Committee on Risk Characterization. Commission on Behavioral and Social Science and Education. National Research Council. National Academy Press, Washington, D.C.
Nurmi, E. and Rantala, M. 1973. New aspects of Salmonella infection in broiler production. Nature 241: 210-211.
Nurmi, E., Nuotio, L., and Schneitz, C. 1992. The competitive exclusion concept: Development and Future. Int. J. Food Microbiol. 15: 237-240.

O

Obiri-Dnaso, K., Paul, N., and Jones, K. 2001. The effects of UVB and temperature on the survival of natural populations and pure cultures of *Campylobacter jejuni, Camp. coli, Camp. lari* and urease-positive thermophilic campylobacters (UPTC) in surface waters. J. Appl. Microbiol. 90: 256-267.
Oh, M.K. and Liao, J.C. 2000. Gene expression profiling by DNA microarrays and metabolic fluxes in *Escherichia coli*. Biotechnol. Prog. 16: 278-286.
Ojeniyi, B., Christensen, J., and Bisgaard, M. 2000. Comparative investigations of Listeria monocytogenes isolated from a turkey processing plant, turkey products, and from human cases of listeriosis in Denmark. Epidemiol. Infect. 125(2): 303-308.
Okamura, N., Nakaya, R., Yokota, H., Yanai, N., and Kawashima, T. 1986. Interaction of *Shigella* with bifidobacteria. Bifidobacteria Microflora 5(1): 51-55.
Olive, D.M. and Bean, P. 1999. Principles and applications of methods for DNA-based typing of microbial organisms. J. Clin. Microbiol. 37(6): 1661-1669.
Oliver, J.D. and Wanucha, D. 1989. Survival of *Vibrio vulnificus* at reduced temperatures and elevated nutrient. J. Food Safety 10: 79-86.
Olsen, G.J., Overbeek, R., Larsen, N., Marsh, T.L., McCaughey, M.J., Maciukenas, M.A., Kuan, W.M., Macke, T.J., Xing, Y., and Woese, C.R. 1992. The ribosomal database project. Nucleic Acids Res. 11: 2199-200.
Olsen, S.J., MacKinon, L.C., Goulding, J.S., Bean, N.H., and Slutsker, L. 2000. Surveillance for foodborne-disease outbreaks-United States, 1993-1997. MMWR 49 (SS-1): 1-51.
Olson, D.G. 1998. Irradiation of food. A Scientific Status Summary published by Institute of Food Technologists Expert Panel on Food Safety and Nutrition, Chicago, Ill. Food Technol. 52(1): 56-62.
O'Mahony, M., Mitchell, E., Gilbert R.J., Hutchinson, D.N., Begg, N.T., Rodhouse, J.C., and Morris, J.E. 1990. An outbreak of foodborne botulism associated with contaminated hazelnut yoghurt. Epidemiol. Infect. 104(3): 389-395.
O'Neal, C.R., Gabriel, W.M., Turk, A., Libby, S.J., Fang, F., and Spector, M.P. 1994. RpoS is necessary for both the positive and negative regulation of starvation survival genes during phosphate, carbon, and nitrogen starvation in *Salmonella typhimurium*. J. Bacteriol. 176: 4610-4616.
Orr, R.V. and Beuchat, L.R. 2000. Efficacy of disinfectants in killing spores of *Alicyclobacillus acidoterrestis* and performance of media for supporting colony development of survivors. J. Food Protect. 63: 1117-1122.
Ortega, Y.R., Sterling, C.R., Gilman, R.H., Cama, V.A., Diaz, F. 1993. *Cyclospora* species-A new protozoan pathogen of humans. N. Eng. J. Med. 328: 1308-1312.
Owen, R.L. and Ermak, T.H. 1990. Structural specializations for antigen uptake and processing in the digestive tract. Springer Semin. Immunopathol. 12: 139-152.

P

Paci, E. 1995. Exploring new tourism marketing opportunities around the world. Proc. of the 11th General Assembly of the World Tourism Organization, Oct. 15. Cairo.
Pagan, R., Jordan, S., Benito, A., and Mackey, B. 2001. Enhanced acid sensitivity of pressure-damaged *Escherichia coli* 0157 cells. Appl. Environ. Microbiol. 67: 1983-1985.
Parashar, U.D., Dow, L., Fankhauser, R.L., Humphrey, C.D., Miller, J., Ando, T., Williams, K.S., Eddy, C.R., Noel, J.S., Ingram, T., Bresee, J.S., Monroe, S.S., and Glass, R.I. 1998. An outbreak of viral gastroenteritis associated with consumption of sandwiches: Implications for the control of transmission by food handlers. Epidemiol. and Infect. 121: 615-621.
Parker, C.T. and Thomas, D. 2000. Reiter's syndrome and reactive arthritis. J. Am. Osteopath. Assn. 100(2): 101-104.
Patterson, M.F. and Kilpatrick, D.J. 1998. The combined effect of high hydrostatic pressure and mild heat on inactivation of pathgens in milk and poultry. J. Food Protect. 61: 432-436.
Patterson, M.F., Quinn, M., Simpson, R., and Gilmour, A. 1995. Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. J. Food Protect. 58: 524-529.
Patterson, W., Haswell, P., Fryers, P.T., and Green, J. 1997. Outbreak of small round structured virus gastroenteritis arose after kitchen assistant vomited. Commun. Dis. Rep. 7(7): R101-3.
Paul, K.Y. and Morita, R.Y. 1971. Effects of hydrostatic pressure and temperature on the uptake and respiration of amino acids by a facultatively psychrophilic marine bacterium. J. Bacteriol. 108: 835.
Pauli, G.H. and Tarantino, L.M. 1995. FDA regulatory aspects of food irradiation. J. Food Protect. 58:

209-212.
Peck, M.W. and Fernandez, P.S. 1995. Effect of lysozyme concentration, heating at 90°C, and then icubation at chilled temperatures on growth from spores of non-proteolytic *Clostridium botulinum*. Let. Appl. Microbiol. 21: 50-54.
Penner, K., Kramer, C., and Frantz, G. 1985. Consumer food safety perceptions. MF774. Kansas State Univ. Ext. Service, Manhattan, Kansas.
Perna, N.T., Plunkett, G., Burland, V., Mau, B., Glasner, J.D., Rose, J.D., Mayhew, G.F., Evans, P.S., Gregor, J., Kirkpatrick, H.A., Posfai, G., Hackett, J., Klink, S., Boutin, A., Shao, Y., Miller, L., Grotbeck, E.J., Davis, N.W., Lim, A., Dimalanta, E.T., Potamousis, K.D., Apodaca, J., Anantharaman, T.S., Lin, J., Yen, G., Schwartz, D.C., Welch, R.A., and Blattner, F.R. 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. Nature 409: 529-533.
Pestka, J.J. 1993. Food, diet and gastrointestinal immune function. Adv. Food Nutr. Res. 37: 1-66.
Pestka, J.J. and Casale, W.L. 1990. Naturally occurring fungal toxins. Adv. Environ. Sci. Technol. 23: 613-638.
Pestka, J.J. and Witt, M.F. 1985. An overview of immune function. Food Technol. 39(2): 83-90.
Pestka, J.J., Abouzied, M.M., and Sutikno, M. 1995. Immunologically-based assays for mycotoxin detection. Food Technol. 49(2): 120-128.
Pflug, I.J. and Gould, G.W. 2000. Heat treatment. Chpt. 3, In "The Microbiological Safety and Quality of Food." Vol. I. ed. B.M. Lund, T.C. Baird-Parker, and G.W. Gould. pp. 36-64. Aspen Publishers Inc., Gaithersburg, Md.
Phebus, R.K., Nutsch, A.L., Schafer, D.E., Wilson, R.C., Riemann, M.J., Leising, J.D., Kastner, C.L., Wolf, J.R., and Prasia, R.K. 1997. Comparison of steam pasteurization and other methods for reduction of pathogens on surfaces of freshly slaughtered beef. J. Food Protect. 59: 751-756.
Phillips, C.A. 1996. Review: Modified atmosphere packaging and its effects on the microbiological quality and safety of produce. Intl. J. Food Sci. Technol. 31: 463-479.
Pitlik, S.D., Fainstein, V., Garza, D., Guarda, L., Bolivar, R., Rios, A., Hopfer, R.L., Mansell, P.A. 1983. Human cryptosporidiosis: Spectrum of disease. Report of six cases and review of the literature. Arch. Intern. Med. 143: 2269-75.
Pitt, J.I. 2000. Toxigenic fungi and mycotoxins. Br. Med. Bull. 56(1): 184-92.
Plumley, F.G. 1997. Marine algal toxins: Biochemistry, genetics, and molecular biology. Limnol. Oceanog. 42(5): 1252-1264.
Porter, J., Edwards, C., and Pickup, R.W. 1995. Rapid assessment of physiological status in *Escherichia coli* using fluorescent probes. J. App. Bacteriol. 79: 399-408.
Potter, M.E. and Tauxe, R.V. 1997. Epidemiology of foodborne diseases: Tools and Applications. World Health Statistics Quarterly 50: 24-29.
Price, S. B., Cheng, C. -M., Kaspar, C.W., Wright, J.C., DeGraves, F., Penfound, T.A., Castanie-Cornet, M.-P., and Foster, J.W. 2000. Role of *rpoS* in acid resistance and fecal shedding of *Escherichia coli* O157: H7. Appl. Environ. Microbiol. 66: 632-637.
Proctor, M.E., Brosch, R., Mellen, J.W., Garrett, L.A., Kaspar, C.W., and Luchansky, J.B. 1995. Use of pulsed field gel electrophoresis to link sporadic cases of invasive listeriosis with recalled chocolate milk. Appl. Environ. Microbiol. 61(8): 3177-3179.
Pruimboom-Brees, I.M., Morgan, T.W., Ackermann, M.R., Nystrom, E.D., Samuel, J.E., Cornick, N.A., and Moon, H.W. 2000. Cattle lack vascular receptors for *Escherichia coli* O157: H7 Shiga toxins. Proc. Natl. Acad. Sci. 97: 10325-10329.

R

Rabsch, W., Hargis, B.M., Tsolis, R.M., Kingsley, R.A., Hinz, K.H., Tschape, H., and Baumler, A.J. 2000. Competitive exclusion of *Salmonella enteritidis* by *Salmonella gallinarum* in poultry. Emerg. Infect. Dis. 6: 443-448.
Rahyaman, S.M., Chira, P., and Koff, R.S. 1994. Idiopathic autoimmune chronic hepatitis triggered by Hepatitis A. Am. J. Gastroenterol. 89(1): 106-8.
Ralston, K., Starket, Y., Brent, P., and Riggins, T. 2000. Awareness of risks changing how hamburgers are cooked. Food Rev. May-Aug. pp. 44-50.
Ramsaran, H., Chen, J., Brunke, B., Hill, A., and Griffiths, M.W. 1998. Survival of bioluminescent *Listeria monocytogenes* and *Escherichia coli* O157: H7 in soft cheeses. J. Dairy Sci. 81: 1810-1817.
Rangarajan, A., Pritts, M., Reiners, S., and Pedersen, L. 2000. Reduce microbial contamination with good agricultural practices. Cornell University, Ithaca, N.Y. (http://www.hort.cornell.edu/veggie/foodsafety/foodsafety.htm.).
Rashid, H.O., Ito, H., and Ishigaki, I. 1992. Distribution of pathogenic vibrios and other bacteria in imported frozen shrimps and their decontamination by gamma-irradiation. World J. Microbiol. Biotechnol. 8: 494-499.
Rasmussen, O.F., Skouboe, P., Dons, L., Rossen, L., and Olsen, J.E. 1995. *Listeria monocytogenes* exists in at least three evolutionary lines: Evidence from flagellin, invasive associated protein, and listeriolysin O genes. Microbiol. 141: 2053-2061.
Ray, B. 1979. Methods to detect stressed microorganisms. J. Food Protect. 42: 346-355.
Ray, B. 1989. "Injured Index and Pathogenic Bacteria: Occurrence and Detection in Foods, Water and Feeds." CRC Press, Inc., Boca Raton, Fla.
Reagan, J.O., Acuff, G.R., Buege, D.R., Buyck, M.J., Dickson, J.S., Kastner, C.L., Marsden, J.L., Morgan, J.B., Nickelson, R., Smith, G.C., and Sofos, J.N. 1996. Trimming and washing of beef carcasses as a method of improving the microbiological quality of meat. J. Food Protect. 59: 751-756.
Reid, S.D., Herbelin, C.J., Bumbaugh, A.C., Selander, R.K., and Whittam, T.S. 2000. Parallel evolution of virulence in pathogenic *Escherichia coli*. Nature. 406(6791): 64-67.
Reilly, P.J.A., Twiddy, D.R., and Fuchs, R.S. 1992. Review of the occurrence of salmonella in cultured tropical shrimp. FAO. Fisheries Cirular No. 851. Food and Agriculture Organization of the United Nations, Rome, Italy.
Reis, A.A., Zaza, S., Langkop, C., Tauxe, R.V., and Blake, P.A. 1990. A multistate outbreak of *Salmonella chester* linked to imported cantaloupe (Abstr.). Proc. of the 3Oth Conference on Antimicrobial Agents and Chemotherapy, Oct. 21-24, Atlanta, Ga. p. 38. American Society for Microbiology, Washington, D.C.
Rice, E.W. and Johnson, C.H. 2000. Short communication: Survival of *Escherichia coli* O157:H7 in dairy cattle drinking water. J. Dairy Sci. 83: 2021-2023.
Richter, H., Timm, M., Lehmann, S., Lochotzke, H.-M., Perlberg, K.-W., Feier, U., and Protz, D. 2000. Influence of different feed rations on E. coli and VTEC in the faeces of cattle. (Abstr. 150). Kyoto, Japan. (http://www.iss.it/laboratori/leb/vtec/abstractsP2.htm).
Robbe-Saule, V., Schaeffer, F., Kowarz, L., and Norel, F. 1997. Relationships between H-NS, ss, SpvR and growth phase in the control of spvR, the regulatory gene of the *Salmonella typhimurium* plasmid virulence operon. Mol. Gen. Genet. 256: 333-347.
Roberts, C.M. and Hoover, D.G. 1996. Sensitivity of *Bacillus coagulans* spores to combinations of high hydrostatic pressure, heat, acidity and nisin. J. App. Bacteriol. 81: 363-368.
Roberts, R.A. 1976. Microbiological guidelines for meat. Proc. 22nd Meeting of European Meat Research Workers, pp. LO: 1 – LO: 4. Malmo, Sweden.
Robertson, R.E. 1999. Fresh Produce: Potential Implications of Country-of-Origin Labeling. Before the Senate Committee on Agriculture, Nutrition, and Forestry. May 26. Testimony GAO/T-RCED-99-200.
Rodrigo, D., Martinez, A., Harte, F., Barbosa-Canovas, G.V., and Rodrigo, M. 2001. Study of inactivation of *Lactobacillus plantarum* in orange-carrot juice by means of pulsed electric fields: Comparison of inactivation kinetics models. J. Food Protect. 64(2): 259-263.
Rouquette, C., Ripio, M.T., Pellegrini, E., Bolla, J.M., Tascon, R.I., Vazquez-Boland, J.A., and Berche, P. 1996. Identification of a ClpC ATPase required for stress tolerance and *in vivo* survival of *Listeria monocytogenes*. Mol. Microbiol. 21: 977-987.
Rowbury, R.J. 1995. An assessment of environmental factors influencing acid tolerance and sensitivity in *Escherichia coli*, *Salmonella* spp. and other enterobacteria. Lett. Appl. Microbiol. 20: 333-337.
Russell, M.W., Lue, C., Van Den Wall Bake, A.W.L., Moldoveanu, Z., and Mestecky, J. 1991. Molecular heterogeneity of human IgA antibodies during an immune response. Clin. Exp. Immunol. 87: 1-6.
Russell, N.J., Evans, R.I., ter Steeg, P.F., Hellemons, J., Verheul, A., and Abee, T. 1995. Membranes as a target for stress adaptation. Intl. J. Food Microbiol. 28: 255-261.

S

Saavedra, J.M., Bauman, N.A., Oung, I., Perman, J.A., and Yolken, R.H. 1994. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet 344: 1046-1049.
Sagliocca, L., Amoroso, P., Stroffolini, T., Adamo, B., Tosti, M.E., Lettieri, G., Esposito, C., Buoocore, S., Pierri, P., and Mele, A. 1999. Efficacy of hepatitis A vaccine in prevention of secondary hepatitis infection: A randomised trial. Lancet 353(9159): 1136-1139.
Salman, M.D., Frank, G.R., MacVean, D.W., Reif, J.S., Collins, J.K., and Jones, R. 1988. Validation of disease diagnoses reported to the National Animal Health Monitoring System from a large Colorado beef feedlot. J. Am. Vet. Med. Assn. 192: 1069-1073.
Sandman, P.M. 1987. Risk communication: Facing public outrage. EPA Journal 13(9): 21-22.
Sandman, P.M. 1997. Persuading the public to take serious food risks seriously and trivial food risks trivially. Presented at "Changing strategies-changing behavior: What food safety communicators need to know." June 12-13, Washington, D.C., U.S. Dept. of Agriculture and Food and Drug Administration, Washington, D.C. (http://www.fda.gov).
Sargeant, J.M., Gillespie, J.R., Oberst, R.D., Phebus, R.K., Hyatt, D.R., Bohra, L.K., and Galland, J.C. 2000. Results of a longitudinal study of the prevalence of *Escherichia coli* O157:H7 on cow-calf farms. Am. J. Vet. Res. 61: 1375-1379.
Savitz, D. 1998. Final Report: Medical evaluation of estuary-exposed persons in North Carolina, November, 1997. Department of Epidemiology, University of North Carolina,. Chapel Hill.
Schuchat, A., Deaver, K.A., Wenger, J.D., Plikaytis, B.D., Mascola, L., Pinner, R.W., Reingold, A.L., Broome, C.V., and the Listeria Study Group. 1992. Role of Foods in Sporadic Listeriosis. J. Am. Med. Assn. 267(15): 2041-2045.
Schwartz, I. 2000. Microbial genomics: From sequence to function. Emerg. Infect. Dis. 6: 493-495.
Seidegard, J. and Ekstrom, G. 1997. The role of huyman glutathione transferases and epoxide hydrolases in the metabolism of xenobiotics. Environ. Health Per-

spect. 105: 791-799.
Shadbolt, C., Ross, T., and McMeekin, T.A. 2001. Differentiation of the effects of lethal pH and water activity: Food safety implications. Lett. Appl. Microbiol. 32: 99-102.
Shane, S.M. 2000. *Campylobacter* infection of commercial poultry. Rev. Sci. Tech. 19: 376-395.
Sharma,V.K. and Carlson, S.A. 2000. Simultaneous detection of *Salmonella* strains and *Escherichia coli* O157: H7 with fluorogenic PCR and single-enrichment-broth culture. Appl. Environ. Microbiol. 66: 5472-5476.
Sharpe, A.N. 1997. Separation and Concentration of Pathogens from Foods. In "Food Microbiological Analysis: New Technologies." ed. M.L. Tortorello and S.M. Gendel. Marcel Dekker. New York, N.Y.
Shearer, A.E.H., Dunne, C.P., Sikes, A., and Hoover, D.G. 2000. Bacterial spore inhibition and inactivation in foods by pressure, chemical preservatives and mild heat. J. Food Protect. 63: 1503-1510.
Shere, J.A., Bartlett, K.J., and Kaspar, C.W. 1998. Longitudinal study of *Escherichia coli* O157:H7 dissemination on four dairy farms in Wisconsin. Appl. Environ. Microbiol. 64: 1390-1399.
Sheridan, J.J. and McDowell, D.A. 1998. Factors affecting the emergence of pathogens on foods. Meat Sci. 49(Suppl. 1): 151-167.
Sheridan, G.E.C., Masters, C.I., Shallcross, J.A., and Mackey, B.M. 1998. Detection of mRNA by reverse transcription PCR as an indicator of viability in *Escherichia coli* cells. Appl. Environ. Microbiol. 64: 1313-1318.
Shoenfeld, Y., George, J., and Peter, J.B. 1996. Guillain-Barré as an autoimmune disease. Intl. Arch. Allergy Immunol. 109: 318-26.
Shumway, S.E. 1989. Toxic algae. World Aquacult. 20: 65-73.
Simonsen, G.S., Haaheim, H., Dahl, K.H., Kruse, H., Lovseth, A., Olsvik, O., Sundsfjord, A. 1998. Transmission of VanA-type vancomycin-resistant enterococci and vanA resistance elements between chicken and humans at avoparcin-exposed farms. Microb. Drug Resist. 4: 313-8.
Simpkins, S.A., Chan, A.B., Hays, J., Popping, B., and Cook, N. 2000. An RNA transcription-based amplification technique (NASBA) for the detection of viable *Salmonella enterica*. Lett. Appl. Microbiol. 30: 75-79.
Siragusa, G.R. 1996. The effectiveness of carcass decontamination systems for controlling the presence of pathogens on the surfaces of meat animal carcasses. In "HACCP: An Integrated Approach to Assuring the Microbiological Safety of Meat and Poultry." ed. J.L. Sheridan, R.L. Buchanan, and T.J. Montville. Food and Nutrition Press, Trumbull, Conn.
Siragusa, G.R., Nawotka, K., Spilman, S.D., Contag, P.R., and Contag, C.H. 1999. Real-time monitoring of *Escherichia coli* O157: H7 adherence to beef carcass surface tissues with a bioluminescent reporter. Appl. Environ. Microbiol. 65: 1738-1745.
Smelt, J.P.P.M., Rijke, A.G.F., and Hayhurst, A. 1994. Possible mechanism of high pressure inactivation of microorganisms. High Pressure Res. 12: 199-203.
Smith, J.A. 1995. Arthritis, guillain-barre' syndrome, and other sequelae of *Campylobacter jejuni* enteritis. J. Food Protect. 58: 1153-1170.
Smith, S.A., Noga, E.J., and Bullis, R.A. 1988. Mortality in *Tilapia aurea* due to a toxic dinoflagellate bloom. (Abstr.) In Proc. of the 3rd Internat. Colloq. Pathol. Marine Aquacul. pp. 167-168. Gloucester Point, Va.
Smith, K.E., Besser, J.M., Hedberg, C.W., Leano, F.T., Bender, J.B., Wicklund, J.H., Johnson, B.P., Moore, K.A., and Osterholm, M.T. 1999. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. Investigation Team. N. Eng. J. Med. 340: 1525-32.
Smoot, L.M. and Pierson, M.D. 1998. Effect of environmental stress on the ability of *Listeria monocytogenes* Scott A to attach to food contact surfaces. J. Food Protect. 61: 1293-1298.
Soderlund, D. et al. 2000. Intl. Conf. Emerg. Infect. Dis. (Abstr. 62) p. 148.
Sorvillo, F.J., Lieb, L.E., Kerndt, P.R., and Ash, L.R. 1994. Epidemiology of cryptosporidiosis among persons with acquired immunodeficiency syndrome in Los Angeles County. Am. J. Trop. Med. Hyg. 51(3): 326-331.
Spencer, J.L. and Garcia, M.M. 1995. Resistance of chicks and poults fed vermicompost to caecal colonization by Salmonella. Avian Pathol. 24: 157-170.
Sperandio, V., Mellies, J.L., Nguyen, W., Shin, S., and Kaper, J.B. 1999. Quorum sensing controls the expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. Proc. Nat. Acad. Sci. 96(26): 15196-15201.
Spratt, B.G. 1999. Multilocus sequence typing: Molecular typing of bacterial pathogens in an era of rapid DNA sequencing and the internet. Curr. Opin. Microbiol. 2(3): 312-316.
St. Louis, M.E., Morse, D.L., Potter, M.E., Demelfig, T.M., Guzewich, J.J., Tauxe, R.V., Blake, P.A., and the *Salmonella enteritidis* Working Group. 1988. The emergence of grade A eggs as a major source of *Salmonella enteritidis* infections: New implications for the control of salmonellosis. J. Am. Med. Assn. 259: 2103-2107.
Stehr-Green, J.K. and Schantz, P.M. 1986. Trichinosis in Southeast Asian refugees in the United States. Am. J. Publ. Health 76: 1238-39.
Steidinger, K.A. and Penta, H.L.M. 1999. "Harmful Microalgae and Associated Public Health Risks in the Gulf of Mexico". Marine Research Institute, St. Petersburg, Fla.
Stewart, G.S.A.B., Aldsworth, T.G., Sharman, R.L., Gibson, P.T., and Dodd, C.E.R. 1997. Bioluminescence: Lux as an enabling tool for the microbiological analysis of food. Chpt. 13 in "Food Microbiological Analysis: New Technologies," ed. M. L. Tortorello and S. M.Gendel, pp. 265–288. Marcel Dekker, New York, N.Y.
Stokes, C.R. 1984. Induction and control of intestinal immune responses. In "Local Immune Responses in the Gut." ed. T.J. Newby and C.R. Stokes. CRC Press, Boca Raton, Fla.
Sulaiman, I.M., Xiao, L., Yang, C., Escalante, L., Moore, A., Beard, C.B., Arrowood, M.J., and Lal, A.A. 1998. Differentiating human from animal isolates of *Cryptosporidium parvum*. Emerg. Infect. Dis. 4: 681-685.
Surette, M.G. and Bassler, B.L. 1998. Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. Proc. Nat. Acad. Sci. 95(12): 7046-7050.
Swaminathan, B. and Feng, P. 1994. Rapid detection of food-borne pathogenic bacteria. Ann. Rev. Microbiol. 48: 401-426.
Swaminathan, B., Barrett. T.J., Hunter, S.B., Tauxe, R.V., and the CDC PulseNet Task Force. 2001. PulseNet: The molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg. Infect. Dis. 7(3): 382-389.
Swift and Company. 1973. Carcass chilling process. U.S. Pat. 1,380,891.

T

Tacket, C.O., Brenner, F., and Blake, P.A. 1984. Clinical features of an epidemiological study of *Vibrio vulnificus* infections. J. Infect. Dis. 149: 558-561.
Tai, J-H. and Pestka, J.J. 1988. Synergistic interaction between the trichothecene T-2 toxin and *Salmonella typhimurium* lipopolysaccharide in C3H/HeN and C3H/HeJ mice. Toxicol. Lett. 44: 191-200.
Takahashi, I. and Kiyono, H. 1999. Gut as the largest immunologic tissue. J. Parenter. Enteral. Nutr. 23(5 Supppl): 7S-12S.
Tao, H., Bausch, C., Richmond, C., Blattner, F.R., and Conway, T. 1999. Functional genomics: Expression analysis of *Escherichia coli* growing on minimal and rich media. J. Bacteriol. 181: 6425-6440.
Taormina, P.J., Beuchat, L.R., and Slutsker, L. 1999. Infections associated with eating seed sprouts: An international concern. Emerg. Infect. Dis. 5: 626-634.
Taunk, J., Roberts, A.I., and Ebert, E.C. 1992. Spontaneous cytotoxicity of human intraepithelial lymphocytes against epithelial cell tumors. Gastroenterol. 102: 69-75.
Tauxe, R.V. 1997. Emerging Foodborne Diseases: An Evolving Public Health Challenge. Emerg. Infect. Dis. 3(4): 425-434.
Tauxe, R., Kruse, H., Hedberg, C., Potter, M., Madden, J., and Wachsmuth, K. 1997. Microbial hazards and emerging issues associated with produce. A preliminary report to the National Advisory Committee On Microbiological Criteria For Foods. J. Food Protect. 60: 1400-1408.
Tenter, A.M., Heckeroth, A.R., and Weiss, L.M. 2000. *Toxoplasma gondi*: From animals to humans. Intl. J. Parasitol. 30(12-13): 1217-58.
Thacker, S.B. and Berkelman, R.L. 1988. Public Health Surveillance in the United States. Epidemiol. Rev. 10(2): 187-203.
Thompson B., Demark-Wahnefried, W., Taylor, G., McClelland, J.W., Stables, G., Havas, S., Feng, Z., Topor, M., Heimendinger, J., Reynolds, K.D., and Cohen, N. 1999. Baseline fruit and vegetable intake among adults in seven 5 a day study centers located in diverse geographic areas. J. Am. Diet. Assn. 99: 1241-1248.
Threlfall, E.J., Frost, J.A., Ward, L.R., and Rowe, B. 1995. Epidemic in cattle of *S. typhimurium* DT104 with chromosomally-integrated multiple drug resistance. Vet. Rec. 134: 577.
Threlfall, E.J., Ward. L.R., and Rowe, B. 1997. Increasing incidence of resistance to trimethoprim and ciprofloxacin in epidemic *Salmonella typhimurium* DT104 in England and Wales. Eurosurveill. 2: 81-84.
Thorne, G.M. 1986. Gastrointestinal infections: Dietary interactions. J. Am. Coll. Nutr. 5: 487-499.
Timbo, B., Headrick, M., Altekruse, S.F., and Klontz, K.C. 1995. Raw shellfish consumption in California: The 1992 California behavioral risk factor survey. Am. J. Prev. Med. 11: 214-6.
Tompkin, R.B. 1983. Indicator organisms in meat and poultry products. Food Technol. 37(6): 107-110.
Tompkins, L.S. 1992. The use of molecular methods in infectious diseases. N. Engl. J. Med. 327(18): 1290-1297.
Toro, A.M. Resto, Gonzalez, N., Dufour, A., and Toranzos, G.A. 1995. Oysters as possible vectors for *Giardia* and *Cryptosporidium*. (Abstr.) p. 405. 95th General Meeting of the American Society of Microbiology, May 21-25, Washington, D.C. American Society for Microbiology, Washington, D.C.

U

Ueno, Y. 1974. Citreoviridin from *Penicillium citreoviride* Biourge. In "Mycotoxins." ed. I.F.H. Purchase. pp. 283-302. Elsevier, New York, N.Y.
Uljas, H.E., Schaffner, D.W., Duffy, S., Zhao, L.H., and Ingham, S.C. 2001. Modeling of combined processing steps for reducing *Escherichia coli* O157: H7 populations in apple cider. Appl. Environ. Microbiol. 67(1): 133-141.
USDA/AMS. 2000. National Organic Program; Final Rule. 56FR 80548. U.S. Dept. of Agriculture/Agricultural Marketing Service, Washington, D.C.
USDA/FSIS. 1996. Nationwide federal plant ground

beef microbiological survey. U.S. Dept. of Agriculture/ Food Safety and Inspection Service. Washington, D.C.
USDA/FSIS. 1998a. *Salmonella* Enteritidis risk assessment: Shell eggs and egg products. Final Report. Prepared for the Food Safety and Inspection Service by the *Salmonella* Enteritidis Risk Assessment Team. U.S. Dept. Agriculture/Food Safety and Inspection Service, Washington, D.C. (http://www.fsis.usda.gov/OPHS/risk/index.htm).
USDA/FSIS. 1998b. Risk assessment for *E. coli* O157:H7 in beef and ground beef. U.S. Dept. of Agriculture/Food Safety and Inspection Service, Washington, D.C.
USDA/FSIS. 2000. Progress report on *Salmonella* testing of raw meat and poultry products, 1998-2000. U.S. Dept. of Agriculture/Food Safety and Inspection Service, Washington, D.C. (http://www.fsis.usda.gov).
USDA/FSIS. 2001. FSIS Guidance for Water, Ice, and Solution Reuse. U.S. Dept. of Agriculture/Food Safety and Inspection Service. Washington, D.C. (http://www.fsis.usda.gov/oppde/rdad/frpubs/san-update1.pdf).
USDA/FSIS. 2002. Microbiological results of raw ground beef products analyzed for *Escherichia coli* O157:H7: Calendar year 2001. U.S. Dept. of Agriculture/Food Safety and Inspection Service. Washington, D.C. (http://www.fsis.usda.gov/OPHS/ecoltest/ecpositives2001.htm).
USDA/NASS. 2000. Meat animals production, disposition, and income, 1999 summary. U.S. Dept. of Agriculture/ National Agriculture Statistics Service. Washington, D.C.
USDA/NASS. 2001. Fruit and vegetable agricultural practices –1999, June. U.S. Department of Agriculture/National Agricultural Statistics Service, Washington, D.C. (http://www.usda.gov/nass/pubs/rpts106.htm).
USPSTF. 1997. Guide to Clinical Preventive Services, 2nd Ed. pp. 625-642. U.S. Preventive Services Task Force. International Medical Publishing. Washington, D.C.
U.S. Senate Agriculture Committee. 1998. Animal Waste Pollution in America: An Emerging National Problem. U.S. Congress, Democratic Staff, Washington, D.C.

V

Van Beneden, C.A, Keene, W.E., Strang, R.A., Werker, D.H., King, A.S., Mahon, B., Hedberg, K., Bell, A., Kelly, M.T., Balan, V.K., MacKenzie, W.R., and Fleming, D. 1999. Multinational outbreak of Salmonella enterica serotype. Newport infections due to contaminated alfalfa sprouts. J. Am. Med. Assn. 281(2): 158-162.
Van den Braak, N., van Belkum, A., van Keulen, M., Vliegenthart, J., Verbrugh, H.A., and Endtz, H.P. 1998. Molecular characterization of vancomycin-resistant enterococci from hospitalized patients and poultry products in The Netherlands. J. Clin. Microbiol. 36: 1927-32.
Van der Poel, W.H.M., Vinje, J., van der Heide, R., Herrere, M.-I., Vivo, A., and Koopmans, M.P.G. 2000. Norwalk-like calicivirus genes in farm animals. Emerg. Infect. Dis. 6: 36-41.
Van Embden, J.D., Cave, M.D., Crawford, J.T., Dale, J.W., Eisenach, K.D., Gicquel, B., Hermans, P., Martin, C., McAdam, R., Shinnick, T.M., and Small, P.M. 1993. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: Recommendations for a standardized methodology. J. Clin. Microbiol. 31(2): 406-409.
Versalovic, J., Woods, C.R., Jr., Georghiou, P.R., Hamill, R.J., and Lupski, J.R. 1993. DNA based identification and epidemiologic typing of bacterial pathogens. Arch. Pathol. Lab Med. 117(11): 1088-1098.

W

Waldor, M.K. and Mekalanos, J.J. 1996. Lysogenic conversion by a filamentous phage encoding cholera toxin. Science 272: 1910-1914.
Walker, W.A. 1987. Pathophysiology of intestinal uptake and absorption of antigens in food allergy. Annals Allergy 59: 7-16.
Wall, P.G., Morgan, D., Lamden, K., Ryan, M., Griffin, M., Threlfall, E.J., and Ward, L.R. 1994. A case control study of infection with an epidemic strain of multiresistant *Salmonella* Typhimurium DT104 in England and Wales. CDR Rev. 4: R130-R135.
Wallace, B.J., Guzewich, J.J., Cambridge, M., Altekrause, S., and Morse, D.L. 1999. Seafood-associated disease outbreaks in New York, 1980-1994. Am. J. Prev. Med. 17: 48-54.
Wang, Q.P. and Kaguni, J.M. 1989. A novel sigma factor is involved in expression of the *rpoH* gene of *Escherichia coli*. J. Bacteriol. 171: 4248-4253.
Weijer, C. 2000. The future of research into rotavirus vaccine: Benefits of vaccine may outweigh risks for children in developing countries. Brit. Med. J. 321(7260): 525-526.
Wells, C.L., Maddaus, M.A., Simmons, R.L. 1988. Proposed mechanisms for the translocation of intestinal bacteria. Rev. Infect. Dis. 10: 958-979.
WHO. 1999. Food safety issues associated with products from aquaculture. Report of a Joint FAO/NACA/WHO Study Group. WHO Technical Report Series 883. World Health Organization, Geneva.
WHO. 2002. Use of antimicrobials outside human medicine. World Health Organization, Geneva. Accessed Jan. 29,.2002. (http://www.who.int/emc/diseases/zoo/antimicrobial.html).
Wiedmann, M., Bruce, J.L., Keating, C., Johnson, A.E., McDonough, P.L., and Batt, C.A. 1997. Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. Infect. Immun. 65: 2707-2716.
Williamson, D.M. 1991. Home food preparation practice: Results of a national consumer survey. M.S. thesis. Cornell Univ., Ithaca, N.Y.
Witte, W. 1998. Medical consequences of antibiotic use in agriculture. Science 279: 996-997.
Woese, C.R., Kandler, O., and Wheelis, M.L. 1990. Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. Proc. Natl. Acad. Sci. 87(12): 4576-4579.
Wong, A.C. 1998. Biofilms in food processing environments. J. Dairy Sci. 81: 2765-2770.
Wood, R.C., Hedberg, C., White, K., MacDonald, K., Osterholm, M. 1991. A multi-state outbreak of *Salmonella javiana* infections associated with raw tomatoes. (Abstr.) Proc. of the 40th Annual Conference of the Epidemic Intelligence Service, p. 69. April 8-12. Centers for Disease Control and Prevention, Washington, D.C.
Wright, A.C., Simpson, L.M., and Oliver, J.D. 1981. Role of iron in the pathogenesis of *Vibrio vulnificus* infections. Infect. Immun. 34: 503-507.
Wu, S., de Lancastre, H., and Tomasz, A. 1996. Sigma-B, a putative operon encoding alternative sigma factor of *Staphylococcus aureus* RNA polymerase: Molecular cloning and DNA sequencing. J. Bacteriol. 178: 6036-6042.

X

Xu, H.S., Robert, N., Singleton, F.L., Attwell, R.W., Grimes, D.J. and Colwell, R.R. 1982. Survival and viability of non-culturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. Microb. Ecol. 8: 313-323.

Y

Yang, S., Leff, M.G., McTague, D., Horvath, K.A., Jackson-Thompson, J., Murayi, T. Boeselager, G.K., Melnik, T.A., Gildemaster, M.C., Ridings, D.L., Altekruse, S.F., and Angulo, F.J. 1998. Multistate surveillance for food-handling, preparation, and consumption behaviors associated with foodborne diseases: 1995 and 1996 BRFSS food-safety questions. CDC Surveill. Summ. 47(SS-4): 33-54.
Yang, S., Angulo, F.J., and Altekruse, S.F. 2000. Evaluation of safe food-handling instructions on raw meat and poultry products. J. Food Protect. 63: 1321-1325.

Z

Zagaevskii, I.S. 1973. Beef contamination by *Clostridium perfringens*. Veterina. 1: 101-103.
Zagory, D. 1995. Principles and practice of modified atmosphere packaging of horticultural commodities. In "Principles of Modified Atmosphere and Sous Vide Product Packaging." ed. J. M. Farber and K. L Dodds. pp. 175-204.Technomic Publishing Co. Inc., Lancaster, Pa.
Zambrano, M. M. and Kolter, R. 1996. GASPing for life in stationary phase. Cell 86: 181-184.
Zhou, H-R., Harkema,J.R., Yan, D., and Pestka, J.J. 1999. Amplified proinflammatory cytokine expression and toxicity in mice coexposed to lipopolysaccharide and the trichothecene vomitoxin (deoxynivalenol). J. Toxicol. Environ. Health 56: 115-136.
Zhou, H-R., Harkema, J.R., Hotchkiss, J.A., Yan, D., Roth, R.A, and Pestka J.J. 2000. Lipopolysaccharide and the trichothecene vomitoxin (deoxynivalenol) synergistically induce apoptosis in murine lymphoid organs. Toxicol. Sci. 53(2): 253-263.
Zook, C.D., Parish, M.E., Braddock, R.J., and Balaban, M.O. 1999. High pressure inactivation kinetics of *Saccharomyces cerevisiae* ascospores in orange and apple juice. J. Food Sci. 64(3): 533-535.
Zottola, E.A. 1994. Microbial attachment and biofilm formation: A new problem for the food industry? A Scientific Status Summary published by the Institute of Food Technologists' Expert Panel on Food Safety and Nutrition, Chicago, Ill. Food Technol. 48: 107-114.
Zottola, E.A. 1997. Special techniques for studying microbial biofilms in food systems. Chpt. 16 In "Food Microbiological Analysis: New Technologies," ed. M. L. Tortorello and S. M.Gendel, pp. 315-346. Marcel Dekker, New York, N.Y.