EMERGING INFECTIOUS DISEASES

Tracking trends and analyzing new and reemerging infectious disease issues around the world

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Conference on Emerging Foodborne Pathogens

Pathogen Emergence

Host Susceptibility

Chronic Sequelae

Risk Assessment

Consumer Concern

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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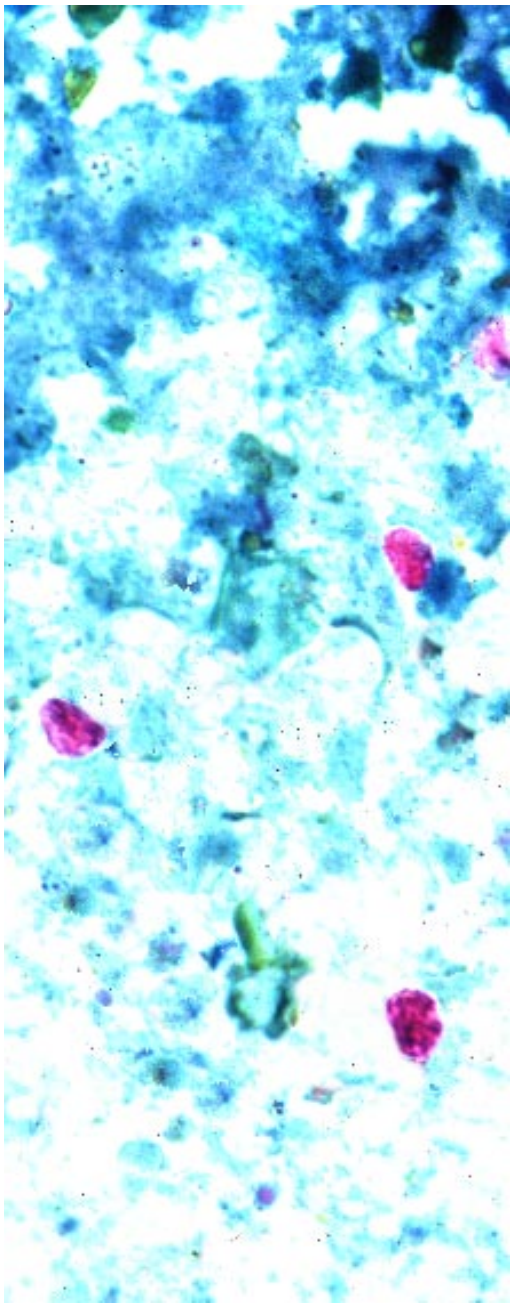

Cyclospora oocysts in human stool; Dr. Mark Eberhard, Centers for Disease Control and Prevention, Atlanta, GA, USA.

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Electron micrograph of *Escherichia coli* O157:H7; Elizabeth White, Centers for Disease Control and Prevention, Atlanta, GA, USA.

Foods from around the world; Dr. Michael Osterholm, Minnesota Department of Health, Minneapolis, MN, USA.

About the National Conference on Emerging Foodborne Pathogens: Implications and Control*

Infectious diseases transmitted by foods have become a major public health concern in recent years. Response by both the food industry and public health and food safety regulatory agencies to new microbiologic health threats and reemerging pathogens in foods has been primarily reactive. The multiplicity of factors and complex interactions involved in the emergence and reemergence of microbial foodborne hazards and the need for a multifaceted and integrated approach to protecting the population prompted a national Conference on Emerging Foodborne Pathogens: Implications and Control (March 24-26, 1997, Alexandria, Virginia).

The conference, attended by more than 400 scientists in basic and applied research, epidemiology, and public health, was organized to elucidate programs and initiatives that could be used to identify and respond appropriately and proactively to emerging and reemerging foodborne disease threats.

Representing the conference organizers were Dr. Alex Malaspina, president of the International Life Sciences Institute; Dr. David Satcher, director of the Centers for Disease Control and Prevention; Mr. Thomas Billy, administrator of the Food Safety and Inspection Service of the U.S. Department of Agriculture; Dr. Fred Shank, director of the Center for Food Safety and Applied Nutrition in the U.S. Food and Drug Administration; and Sir George Alleyne, director of the Pan American Health Organization. Their opening remarks reflected a strong commitment to collaboration among different sectors, development of integrated approaches to food safety, implementation of President Clinton's food safety initiative, and international cooperation in the fight against foodborne disease.

Nobel laureate Dr. Joshua Lederberg delivered the keynote address, in which he called for a global public health approach to the threats posed by microbial foodborne illness. Dr. Richard Hall's closing address summarized the key points of the conference presentations, emphasized the need for concerted control efforts by the public and private sectors, and suggested prioritizing foodborne disease risks according to their probable impact.

Conference organizers hope that the publication of conference presentations and discussions in this journal will stimulate initiatives to improve the safety of food and draw much needed attention to foodborne microbial hazards.

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*The conference was sponsored by the International Life Sciences Institute (ILSI), ILSI North America Technical Committee on Food Microbiology, the Centers for Disease Control and Prevention, the U.S. Department of Agriculture, and the U.S. Food and Drug Administration, in cooperation with the Food and Agriculture Organization of the United Nations and the Pan American Health Organization/World Health Organization. Conference grant support was also provided by the National Institute of Diabetes and Digestive and Kidney Diseases. Additional support was provided through unrestricted education grants from the American Meat Institute, American Society for Microbiology, American Veterinary Medical Association, Animal Health Institute, Association of American Veterinary Medical Colleges, Frito-Lay, Inc., Land O'Lakes, Inc., McDonald's Corporation, National Food Processors Association, The Quaker Oats Company, Roquette America, Inc., and Ross Products Division, Abbott Laboratories. The members of the Technical Committee on Food Microbiology are Campbell Soup Company, The Coca-Cola Company, General Mills, Gerber Products Company, H.J. Heinz Company, Kraft Foods, Inc., Lipton, M&M/Mars, Nabisco, Inc., Nestlé USA, Inc., PepsiCo, Inc., The Pillsbury Company, and The Procter & Gamble Company.

Infectious Disease as an Evolutionary Paradigm

Joshua Lederberg
Sackler Foundation Scholar, Rockefeller University,
New York, New York, USA

The basic principles of genetics and evolution apply equally to human hosts and to emerging infections, in which foodborne outbreaks play an important and growing role. However, we are dealing with a very complicated coevolutionary process in which infectious agent outcomes range from mutual annihilation to mutual integration and resynthesis of a new species. In our race against microbial evolution, new molecular biology tools will help us study the past; education and a global public health perspective will help us deal better with the future.

Life expectancy in the United States from 1900 to the present (Figure 1) shows an overall steady rise, reflecting improved health conditions in general, the result of advances in medical science, hygiene, personal care, health technologies, and public health administrations. The rise decelerates asymptotically to a near plateau from the 1950s to the 1970s, reflecting an epidemic of coronary disease, which we do not yet fully understand. Improvements in medical care, attention to life style, or indiscriminate use of aspirin may all be responsible for the subsequent decrease in deaths from coronary disease. Up to the 1940s, the rising curve is jagged, reflecting sporadic infectious disease outbreaks, especially the Spanish influenza outbreak of 1918. Whether the life expectancy curve continues to rise smoothly or whether it has some jagged declines depends on what we do about transmission of infectious disease, including foodborne disease. When plotted another way (Figure 2), both the absolute number of deaths from infectious disease and the proportion of total deaths attributable to infectious disease also show steady amelioration from 1900 almost to the present.

The 1918 Spanish influenza pandemic may be a prototype for future emerging infections.

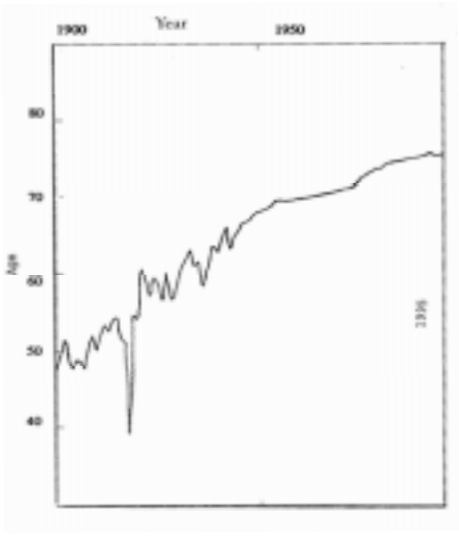

Figure 1. Life expectancy in the United States, at birth, 20th century.

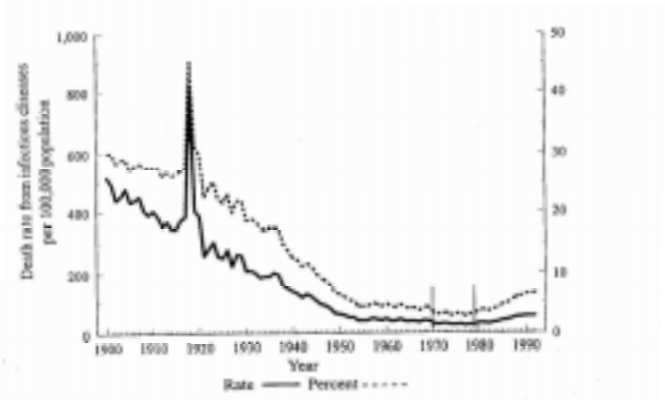

Figure 2. Trends in infectious diseases mortality, 1900–1992. Source: CDC, unpub. data.

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Although minimized as not much more than a bad cold, influenza took a terrible toll in 1918, especially on young people (Figure 3). Somewhat older persons may have been protected by immunity from prior exposure to related strains of influenza. The disease, with rapid onset of fulminating pneumonic symptoms, killed 20 to 25 million persons worldwide. The infectious agent was not available for study at that time. However, very recently the Armed Forces Institute of Pathology recovered with PCR technology genetic fragments of the 1918 influenza virus (1). Less than 10% of the entire genome has been recovered to date, but recovery of complete sequences is likely. Although the target genes have not yet provided a clue as to why the 1918 influenza was so devastating, they demonstrate the enormous potential of today's molecular biology tools.

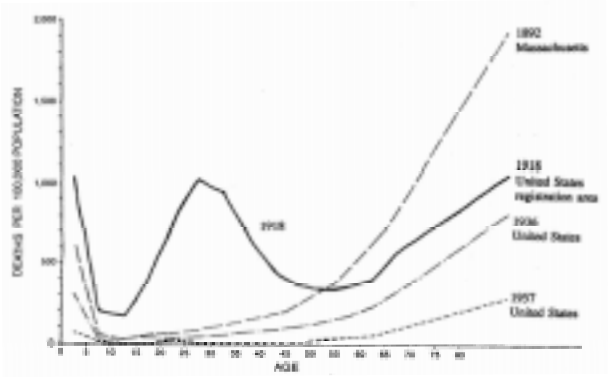

Figure 3. Pneumonia and influenza mortality, by age, in certain epidemic years. (Reprinted with permission of W. Paul Glezen and Epidemiologic Reviews. Emerging Infections: Pandemic Influenza. Epi Rev 1996;18:66).

These tools will enable us to better study paleovirology and paleomicrobiology. We are accustomed to stereotyping historical disease outbreaks as if we really knew what they were, but we really know very little detail about their genetic features. For example, we talk about the great historic plagues as if they indeed were *Yersinia* or cholera or malaria. We should look forward to finding out about the 14th century black death, if it was indeed *Yersinia pestis.* Although clinically unmistakable, that is not to say it was caused by the identical genotype of present *Yersinia* strains.

We need to look ahead as well as back. In this century, emerging and reemerging infections have stimulated flurries of interest, but in general we have been complacent about infectious diseases ever since the introduction of antibiotics. The effect of antibiotics on acute infections and tuberculosis as well as the effect of polio vaccination led to a national, almost worldwide, redirection of attention to chronic and constitutional diseases. However, the HIV pandemic in the early 1980s caught us off guard, reminding us that there are many more infectious agents in the world. It is fortuitous that retroviruses had already been studied from the perspective of cancer etiology; otherwise, we would have had no scientific platform whatsoever for coping with HIV and AIDS.

The Committee on International Science Engineering and Technology provided an interagency review setting out a policy framework for the United States' global response to infectious disease (Table 1). The policy provides a worldwide mantle for surveillance and monitoring, remedial measures, development of new drugs, vaccines, and treatment modalities. The global outlook is necessary, even if for purely selfish reasons, because to infectious agents the world is indivisible, with no national boundaries. Our thinking has been impoverished in terms of budget allocations for dealing with health on an international basis.

We are engaged in a type of race, enmeshing our ecologic circumstances with evolutionary changes in our predatory competitors. To our advantage, we have wonderful new technology; we have rising life expectancy curves. To our disadvantage, we have crowding; we have social, political, economic, and hygienic stratification. We have crowded together a hotbed of opportunity for infectious agents to spread over a significant part of the population. Affluent and mobile people are ready, willing, and able to carry afflictions all over the world within 24 hours' notice. This condensation, stratification, and mobility is unique, defining us as a very different species from what we were 100 years ago. We are enabled by a different set of technologies. But despite many potential defenses—vaccines, antibiotics, diagnostic tools—we are intrinsically more vulnerable than before, at least in terms of pandemic and communicable diseases.

We could imaginably adapt in a Darwinian fashion, but the odds are stacked against us. We cannot compete with microorganisms whose populations are measured in exponents of 10^{12},

Table 1. Examples of pathogenic microbes and infectious diseases recognized since 1973 (2)

Year	Microbe	Type	Disease
1973	Rotavirus	Virus	Major cause of infantile diarrhea worldwide
1975	Parvovirus B19	Virus	Aplastic crisis in chronic hemolytic anemia
1976	*Cryptosporidium*	Parasite	Acute and chronic diarrhea parvum
1977	Ebola virus	Virus	Ebola hemorrhagic fever
1977	*Legionella*	Bacteria	Legionnaires' disease pneumophila
1977	Hantaan virus	Virus	Hemorrhagic fever with renal syndrome (HRFS)
1977	*Campylobacter jejuni*	Bacteria	Enteric pathogens distributed globally
1980	Human T-lymphotropic virus I (HTLV-1)	Virus	T-cell lymphoma-leukemia
1981	Toxic producing strains of *Staphylococcus aureus*	Bacteria	Toxic shock syndrome(tampon use)
1982	*Escherichia coli* O157:H7	Bacteria	Hemorrhagic colitis; hemolytic uremic syndrome
1982	HTLV-II	Virus	Hairy cell leukemia
1982	*Borrelia burgdorferi*	Bacteria	Lyme disease
1983	Human immunodeficiency virus(HIV)	Virus	Acquired immunodeficiency syndrome (AIDS)
1983	*Helicobacter pylori*	Bacteria	Peptic ulcer disease
1985	*Enterocytozoon bieneusi*	Parasite	Persistent diarrhea
1986	*Cyclospora cayetanensis*	Parasite	Persistent diarrhea
1988	Human herpes-virus-6 (HHV-6)	Virus	Roseola subitum
1988	Hepatitis E	Virus	Enterically transmitted non-A, non-B hepatitis
1989	*Ehrlichia chafeensis*	Bacteria	Human ehrlichiosis
1989	Hepatitis C	Virus	Parenterally transmitted non-A, non-B liver infection
1991	Guanarito virus	Virus	Venezuelan hemorrhagic fever
1991	*Encephalitozoon hellem*	Parasite	Conjunctivitis, disseminated disease
1991	New species of *Babesia*	Parasite	Atypical babesiosis
1992	*Vibrio cholerae* O139	Bacteria	New strain associated with epidemic cholera
1992	*Bartonella henselae*	Bacteria	Cat-scratch disease; bacillary angiomatosis
1993	Sin Nombre virus	Virus	Adult respiratory distress syndrome
1993	*Encephalitozoon cuniculi*	Parasite	Disseminated disease
1994	Sabia virus	Virus	Brazilian hemorrhagic fever
1995	HHV-8	Virus	Associated with Kaposi sarcoma in AIDS patients

10^{14}, 10^{16} over periods of days. Darwinian natural selection has led to the evolution of our species but at a terrible cost. If we were to rely strictly on biologic selection to respond to the selective factors of infectious disease, the population would fluctuate from billions down to perhaps millions before slowly rising again. Therefore, our evolutionary capability may be dismissed as almost totally inconsequential. In the race against microbial genes, our best weapon is our wits, not natural selection on our genes.

New mechanisms of genetic plasticity of one microbe species or another are uncovered almost daily. Spontaneous mutation is just the beginning. We are also dealing with very large populations, living in a sea of mutagenic influences (e.g., sunlight). Haploid microbes can immediately express their genetic variations. They have a wide range of repair mechanisms, themselves subject to genetic control. Some strains are highly mutable by not repairing their DNA; others are relatively more stable. They are extraordinarily flexible in responding to environmental stresses (e.g., pathogens' responses to antibodies, saprophytes' responses to new environments). Mechanisms proliferate whereby bacteria and viruses exchange genetic material quite promiscuously. Plasmids now spread throughout the microbial world (3). They can cross the boundaries of yeast and bacteria. Lateral transfer is very important in the evolution of microorganisms. Their pathogenicity, their toxicity, their antibiotic resistance do not rely exclusively on evolution within a single clonal proliferation.

We have a very powerful theoretical basis whereby the application of selective pressure (e.g., antibiotics in food animals) will result in drug resistance carried by plasmids, or pathogens attacking humans. It is not easy to get direct

and immediate epidemiologic evidence, but the foundations for these phenomena exist and must be taken into account in the development of policies. We have barely begun to study the responses of microorganisms under stress, although we have examples where root mechanisms of adaptive mutability are themselves responses to stress. In recent experiments, bacterial restriction systems are more permissive of the introduction of foreign DNA, possibly letting down their guard in response to "mutate or die" circumstances. This does not reflect bacterial intelligence—that they know exactly what mutations they should undergo in response to environmental situations. Their intrinsic mutability and capacity to exchange genetic information without knowing what it is going to be is not a constant; it is certainly under genetic control and in some circumstances varies with the stress under which the microbes are placed.

Evolution is more or less proportionate to the degree of genetic divergence among the different branches of the three-tiered tree of life, with the archaeal branch, the eubacterial branch, and the eukaryotes (Figure 4). The tree illustrates the small territory occupied by humans in the overall world of biodiversity. It shows mitochondria right next to *Escherichia coli*. Bacterial invasion of a primitive eukaryote 2-1/2 to 3 billion years ago, synchronized with the development of primitive green oxygen-generating plants, conferred a selective advantage to complexes that could use oxygen in respiration. Our ancestors were once invaded by an oxidative-capable bacterium that we now call a mitochondrium and that is present in every cell of every body and almost every species of eukaryote. We did not evolve in a monotonous treelike development; we are also the resynthesis of components of genetic development that diverged as far as the bacteria and were reincorporated into the mitochondrial part of our overall genome. Another example of lateral transfer is the symbiosis that resulted from chloroplast invasion of green plants.

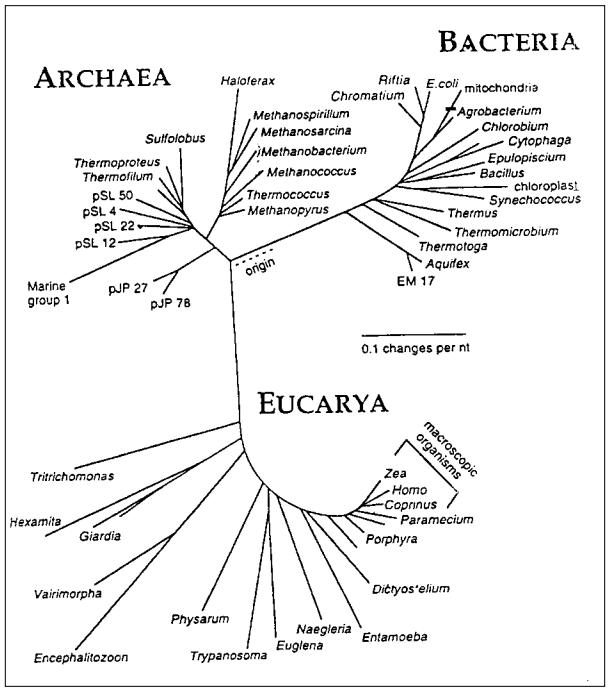

Figure 4. The three-domain tree of life based on small-subunit rRNA sequences. Reprinted with permission of Norman R. Pace and ASM News. ASM News 1996;62(9):464.

The outcome of encounters between mutually antagonistic organisms is intrinsically unpredictable. The 1918 influenza outbreak killed half percent of the human population; but because the consequences were to either kill the host or leave the host immune, the virus died out totally, leaving no trace in our genomes, as far as we know. Historic serology on survivors has found memory cells and antibodies against H1N1, the serotype of the resurrected 1918 virus. Unlike the influenza virus, which left no known genetic imprint, 400 to 500 retroviruses are integrated into our human genome. The full phylogeny of these encounters is unknown, but many of these viruses may precede the separation of homo sapiens from the rest of the hominid line.

Infectious agent outcomes range from mutual annihilation to mutual integration and resynthesis of a new species. Much has been made of the fact that zoonoses are often more lethal to humans than to their original host, but this phenomenon cannot necessarily be generalized. Most zoonoses do not affect humans adversely. Some are equally capable in a new host. We tend to pay most attention, however, to those, such as yellow fever, for which we have not genetically or serologically adapted and which cause severe disease.

Canine distemper provides an example of a quasihereditary adaptation. In the Serengeti, the disease migrated from village dogs to jackals, which shared prey and had contact with lions. About one-fourth of the preserve's 4,000 lions died of canine distemper (4) but the survivors are immune and will pass immunoglobulin, to their offspring. The cubs' maternal immunity will

likely mitigate infection and permit a new equilibrium, not because of genetic adaptation but because of the preimmunized host. This is also the most plausible explanation for how savage the polio virus has been as a paralytic infection of young people. It may also apply to hepatitis, where cleaner is not always better if it means we do not have the "street smarts" to respond to new infectious challenges. These nongenetic adaptations between parasite and host complicate our outcome expectations.

Short-term shifts in equilibrium can give ferocious but temporary advantages to a virus. Long-term outcomes are most stable when they involve some degree of mutual accommodation, with both surviving longer. New short-term deviants, however, can disrupt this equilibrium. The final outcome of the HIV pandemic cannot be predicted. More strains with longer latency may be taking over, mitigating the disease. However, deviant strains could counteract this effect by overcoming immunity and rapidly proliferating, with earlier and more lethal consequences.

We should also consider somatic evolution, a Darwinian process that occurs with every infection. In the clonal selection model of immunogenesis (5), an apparently random production of immunoglobulin variants, both by reassortment of parts and by localized mutagenesis, gives rise to candidate antibodies, which then proliferate in response to matching epitopes. We do not understand the details of how a given epitope enhances stepwise improvements in affinity and productivity of antibodies at various stages. The process may be more complicated than we realize; so may Darwinian evolution.

Despite the prior arguments against relying on host or genotype evolution as a response to infection, historically we have done so and now have "scars of experience." A notable example is malaria, wherein the Duffy mutation against *Plasmodium vivax* is the only host defense with no deleterious consequences. The thalassemias, G6PD deficiency, and hemoglobin S are all hemopoietic modifications that thwart the plasmodia; but in homozygotes, they themselves cause disease. In the evolution of our species, for every child spared an early death because a hemoglobin S mutation impeded *Plasmodium* development, another will succumb to sickle cell disease unless we can intervene. Specific remedies do not exist. Although somatic gene therapy is an interesting possibility, one that will probably progress in the next 20 years, it is paradoxical that we know more about hemoglobin S than any other molecular disease. The entire concept of genetic determination of protein structure has been based on these early observations, yet we are still searching with limited success for ways to put it to therapeutic use.

Biotechnology may enable other forms of genetic intervention through which homo sapiens could conceivably bypass natural selection and random variation. In the absence of alternatives, we might speculate about these kinds of "aversive therapies" as a last resort to save our species.

The ultimate origin of life is still the subject of many theories, as is the origin of viruses (Table 2). Each virus is different. We know nothing of virus phylogenies and cannot even substantiate the distinctions of the several hundred categories. We do not know their origin, only that they interact with host genomes in many ways. Particles could come out of any genome, become free-living (i.e., independent, autonomously replicating units in host cells), reenter a host genome as retroviruses and possibly others do, and repeat the cycle dozens of times. But no one can give a single example or claim to have significant knowledge of how any particular virus evolved, thus presenting a scientific challenge for the next 20 or 30 years.

We are dealing with more than just predation and competition. We are dealing with a very

Table 2. The origin of viruses

Viruses are genomic fragments that can replicate only in the context of an intact living cell. They cannot therefore be primitive antecedents of cells.

Within a given species, viruses may have emerged as genetic fragments or reduced versions from chromosomes, plasmids, or RNA of

1) the host or related species
2) distant species
3) larger parasites of the same or different hosts
4) further evolution and genetic interchange among existing viruses

Once established, they may then cycle back into the genome of the host as an integrated episome; there they may have genetic functions or in principle might reemerge as new viruses.

These cycles have some substantiation in the world of bacterial viruses; but we have no clear data on the provenience of plant or animal viruses.

complicated coevolutionary process, involving merger, union, bifurcation, and reemergence of new species (Table 3). Divergent phenomena can occur in any binary association, with unpredictable outcomes. We have hundreds of retroviruses in our genome and no knowledge of how they got there. As to HIV, we have no evidence as yet that it has ever entered anyone's germ line genome: we really do not know whether it ever enters germ cells. The outcomes of even that interaction could be much more complicated than the purely parasite/host relationships we are accustomed to.

Innovative technologies for dealing with microbial threats have the potential for fascinating therapeutic opportunities (Table 4). Some, like bacteriophage, have been set aside as laboratory curiosities. Nothing is more exciting than unraveling the details of pathogenesis. Having the full genomes of half a dozen parasitic organisms opens up new opportunities for therapeutic invention in ways that we could not have dreamed of even 5 years ago, which will lead to many more technologies. In food microbiology, we should keep in mind the probiotic as well as the adversarial and pathogenetic opportunities in our alimentary tracts.

The Committee on International Science Engineering and Technology report (2) provides some recommendations (Table 5). We need a global perspective. We need to invest in public health, especially food microbiology, not just medical care, in dealing with disease. It is important to prevent foodborne disease through sensible monitoring, standards of cleanliness, and consumer and food-handler education and not just care for its victims.

Today we emphasize individual rights over community needs more than we did 50 to 75 years ago. Restraining the rights and freedoms of individuals is a far greater sin than allowing the infection of others. The restraints placed on Typhoid Mary might not be acceptable today, when some would prefer to give her unlimited rein to infect others, with litigation their only recourse. In the triumph of individual rights, the public health perspective has had an uphill struggle in recent pandemics.

Education, however, is a universally accepted countermeasure, especially important in foodborne diseases. Food safety programs should more specifically target food handlers, examining their hands to determine if they are carriers, to ensure they are complying with basic sanitation.

Table 3. Genetic evolution

- Microbes (bacteria, viruses, fungi, protozoa)
  - Rapid and incessant
  - Huge population sizes 10^{14}+ and generation times in minutes vs. years
- Intraclonal process
  - DNA replication—may be error-prone—in sea of mutagens sunlight; unshielded chemicals, incl. natural products
  - RNA replication—intrinsically unedited, $>10^{-3}$ swarm species
  - Haploid: immediate manifestation, but partial recessives not accumulated contra multicopy plasmids
  - Amplification
  - Site-directed inversions and transpositions: phase variation
  - ?? Other specifically evolved mechanisms: genome quadrant duplication; silencing
- Interclonal process
  - Promiscuous recombination—not all mechanisms are known
  - Conjugation—dozens of species
  - Viral transduction and lysogenic integration: universal
    - Classical: phage-borne toxins in *C. diphtheriae*
  - Plasmid interchange (by any of above) and integration
    - Toxins of *B. anthracis*
    - Pasteur: heat attenuation: plasmid loss; chemically induced
  - RNA viral reassortment; ?? and recombination?
  - Transgressive—across all boundaries
    - Artificial gene splicing
    - Bacteria and viruses have picked up host genes (antigenic masking?)
    - Interkingdom: *P. tumefaciens* and plants, *E. coli* and yeast
    - Vegetable and mineral! oligonucleotides and yeast.
- Host-parasite coevolution
  - Coadaptation to mutualism or accentuation of virulence?
  - Jury is still out (May and Anderson). Many zoonotic convergences.
  - Probably divergent phenomena, with short-term flareups and Pyrrhic victories, atop long-term trend to coadaptation.

Table 4. Technologies to address microbial threats

Antibacterial chemotherapy
- Potentially unlimited capability; bacterial metabolism and genetic structure notably different from human genome sequencing pointing to bacterial vulnerabilities
- Economic-structural factors—public expectation for unachievable bargains in safety assurance, cost of development, and ultimate pricing
- Dilemmas of regulation of (ab)use
- Resurgent interest in bacteriophage and other biologically oriented approaches

Antiviral chemotherapy
- Much more difficult program, inherently
- Gross underinvestment
- New approaches: antisense, ribozymes, targeted D/RNA cleavers
- Problematics of sequence-selective targets

Vaccines
- Gross underinvestment; other structural problems as above
- Liability/indemnification
- Vaccination as service to the herd
- New approaches: hot biotechnology is coming along especially live attenuated: but testing dilemmas
- Safety issues about use of human cells lines; adjuvants

Immunoglobulins and their progeny
- Phage display and diversification: biosynthetic antibody
- Passive immunization for therapy

Biologic response modifiers
- New world of interleukins, cell growth factors so far just scratching surface
- Interaction with pathogenesis
- Intersection with somatic gene therapy

Technologies for diagnosis and monitoring
- Etiologic agents and control
- Host polymorphisms and sensitivities

Homely technologies needed
- Simple, effective face-masks
- Palatable water-disinfectants
- Home-use diagnostics of contamination

Table 5. CISET* recommendations for addressing global infectious disease threats

1. Concerted global and domestic surveillance and diagnosis of disease outbreaks and endemic occurrence. This must entail the installation of sophisticated laboratory capabilities at many centers now lacking them.
2. Vector management and monitoring and enforcement of safe water and food supplies; and personal hygiene (e.g., Operation Clean Hands).
3. Public and professional education.
4. Scientific research on causes of disease, pathogenic mechanisms, bodily defenses, vaccines, and antibiotics.
5. Cultivation of the technical fruits of such research, with the full involvement of the pharmaceutical industry and a public understanding of the regulatory and incentive structures needed to optimize the outcomes.

*Committee on International Science, Engineering and Technology Policy of the National Science and Technology Council.

We typically do this only after an outbreak. Perhaps we should have further debate on the social context for constraints and persuasion to contain the spread of infectious agents.

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Emerging Foodborne Diseases: An Evolving Public Health Challenge

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The epidemiology of foodborne disease is changing. New pathogens have emerged, and some have spread worldwide. Many, including *Salmonella*, *Escherichia coli* O157:H7, *Campylobacter*, and *Yersinia enterocolitica*, have reservoirs in healthy food animals, from which they spread to an increasing variety of foods. These pathogens cause millions of cases of sporadic illness and chronic complications, as well as large and challenging outbreaks over many states and nations. Improved surveillance that combines rapid subtyping methods, cluster identification, and collaborative epidemiologic investigation can identify and halt large, dispersed outbreaks. Outbreak investigations and case-control studies of sporadic cases can identify sources of infection and guide the development of specific prevention strategies. Better understanding of how pathogens persist in animal reservoirs is also critical to successful long-term prevention. In the past, the central challenge of foodborne disease lay in preventing the contamination of human food with sewage or animal manure. In the future, prevention of foodborne disease will increasingly depend on controlling contamination of feed and water consumed by the animals themselves.

Every year, in the United States foodborne infections cause millions of illnesses and thousands of deaths; most infections go undiagnosed and unreported. As the epidemiology of foodborne infections evolves, old scenarios and solutions need to be updated. This article reviews main trends in the evolution of foodborne disease epidemiology and their effect on surveillance and prevention activities.

Preventing foodborne disease is a multifaceted process, without simple and universal solutions. For most foodborne pathogens, no vaccines are available. Consumer education about basic principles of food safety, an important component of prevention, by itself is insufficient. Food reaches the consumer through long chains of industrial production, in which many opportunities for contamination exist. The general strategy of prevention is to understand the mechanisms by which contamination and disease transmission can occur well enough to interrupt them. An outbreak investigation or epidemiologic study should go beyond identifying a suspected food and pulling it from the shelf to defining the chain of events that allowed contamination with an organism in large enough numbers to cause illness. We learn from the investigation what went wrong, in order to devise strategies to prevent similar events in the future. Although outbreaks make the news, most foodborne infections occur as individual or sporadic cases. Therefore, the sources of sporadic cases must also be investigated and understood.

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Emerging Foodborne Pathogens

Substantial progress has been made in preventing foodborne diseases. For example, typhoid fever, extremely common at the beginning of the 20th century, is now almost forgotten in the United States. It was conquered in the preantibiotic era by disinfection of drinking water, sewage treatment, milk sanitation and pasteurization, and shellfish bed sanitation (Figure 1). Similarly, cholera, bovine tuberculosis, and trichinosis have also been controlled in the United States. However, new foodborne pathogens have emerged. Among the first of these were infections caused by nontyphoid strains of *Salmonella*, which have increased decade by decade since World War II (Figure 1).

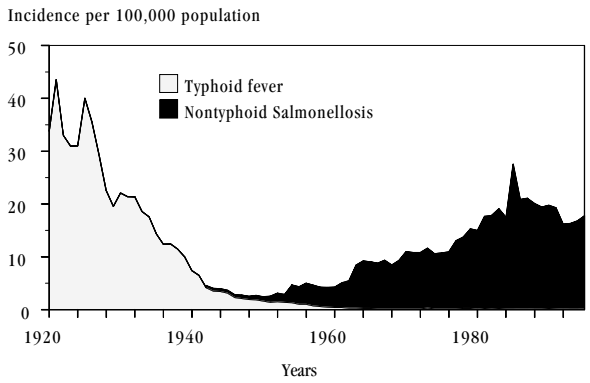

Figure 1. Reported incidence of typhoid fever and nontyphoidal salmonellosis in the United States, 1920–1995.

Table 1. New pathogens that are foodborne and pathogens newly recognized as predominantly foodborne in the United States in the last 20 years

Campylobacter jejuni
Campylobacter fetus ssp. *fetus*
Cryptosporidium cayetanensis
Escherichia coli O157:H7 and related *E. coli* (e.g., O111:NM, O104:H21)
Listeria monocytogenes
Norwalk-like viruses
Nitzschia pungens (cause of amnesic shellfish poisoning)
Salmonella Enteritidis
Salmonella Typhimurium DT 104
Vibrio cholerae O1
Vibrio vulnificus
Vibrio parahaemolyticus
Yersinia enterocolitica

In the last 20 years, other infectious agents have been either newly described or newly associated with foodborne transmission (Table 1). *Vibrio vulnificus*, *Escherichia coli* O157:H7, and *Cyclospora cayetanensis* are examples of newly described pathogens that often are foodborne. *V. vulnificus* was identified in the bloodstream of persons with underlying liver disease who had fulminant infections after eating raw oysters or being exposed to seawater; this organism lives in the sea and can be a natural summertime commensal organism in shellfish (1). *E. coli* O157:H7 was first identified as a pathogen in 1982 in an outbreak of bloody diarrhea traced to hamburgers from a fast-food chain (2); it was subsequently shown to have a reservoir in healthy cattle (3). *Cyclospora,* known previously as a cyanobacterialike organism, received its current taxonomic designation in 1992 and emerged as a foodborne pathogen in outbreaks traced to imported Guatemalan raspberries in 1996 (4,5). The similarity of *Cyclospora* to *Eimeria* coccidian pathogens of birds suggests an avian reservoir (4,5).

Some known pathogens have only recently been shown to be predominantly foodborne. For example, *Listeria monocytogenes* was long known as a cause of meningitis and other invasive infections in immunocompromised hosts. How these hosts became infected remained unknown until a series of investigations identified food as the most common source (6). Similarly, *Campylobacter jejuni* was known as a rare opportunistic bloodstream infection until veterinary diagnostic methods used on specimens from humans showed it was a common cause of diarrheal illness (7). Subsequent epidemiologic investigations implicated poultry and raw milk as the most common sources of sporadic cases and outbreaks, respectively (8). *Yersinia enterocolitica,* rare in the United States but a common cause of diarrheal illness and pseudoappendicitis in northern Europe and elsewhere, is now known to be most frequently associated with undercooked pork (9).

These foodborne pathogens share a number of characteristics. Virtually all have an animal reservoir from which they spread to humans; that is, they are foodborne zoonoses. In marked contrast to many established zoonoses, these new zoonoses do not often cause illness in the infected host animal. The chicken with lifelong ovarian infection with *Salmonella* serotype Enteritidis, the calf carrying *E. coli* O157:H7, and the oyster carrying Norwalk virus or *V. vulnificus* appear healthy; therefore, public health concerns must now include apparently healthy animals. Limited existing research on how animals acquire and transmit emerging pathogens among themselves often implicates contaminated fodder and water; therefore, public health concerns must now include the safety of what food animals themselves eat and drink.

For reasons that remain unclear, these pathogens can rapidly spread globally. For example, *Y. enterocolitica* spread globally among pigs in the 1970s (10); *Salmonella* serotype Enteritidis appeared simultaneously around the world in the 1980s (11); and *Salmonella* Typhimurium Definitive Type (DT) 104 is now appearing in North America, Europe, and

perhaps elsewhere (12); therefore, public health concerns must now include events happening around the world, as harbingers of what may appear here.

Many emerging zoonotic pathogens are becoming increasingly resistant to antimicrobial agents, largely because of the widespread use of antibiotics in the animal reservoir. For example, *Campylobacter* isolated from human patients in Europe is now increasingly resistant to fluoroquinolones, after these agents were introduced for use in animals (13). Salmonellae have become increasing resistant to a variety of antimicrobial agents in the United States (14); therefore, public health concerns must include the patterns of antimicrobial use in agriculture as well as in human medicine.

The foods contaminated with emerging pathogens usually look, smell, and taste normal, and the pathogen often survives traditional preparation techniques: *E. coli* O157:H7 in meat can survive the gentle heating that a rare hamburger gets (15); *Salmonella* Enteritidis in eggs survives in an omelette (16); and Norwalk virus in oysters survives gentle steaming (17). Following standard and traditional recipes can cause illness and outbreaks. Contamination with the new foodborne zoonoses eludes traditional food inspection, which relies on visual identification of foodborne hazards. These pathogens demand new control strategies, which would minimize the likelihood of contamination in the first place. The rate at which new pathogens have been identified suggests that many more remain to be discovered. Many of the foodborne infections of the future are likely to arise from the animal reservoirs from which we draw our food supply.

Once a new foodborne disease is identified, a number of critical questions need to be answered to develop a rational approach to prevention: What is the nature of the disease? What is the nature of the pathogen? What are simple ways to easily identify the pathogen and diagnose the disease? What is the incidence of the infection? How can the disease be treated? Which foods transmit the infection? How does the pathogen get into the food, and how well does it persist there? Is there is an animal reservoir? How do the animals themselves become infected? How can the disease be prevented? Does the prevention strategy work?

The answers to these questions do not come rapidly. Knowledge accumulates gradually, as a result of detailed scientific investigations, often conducted during outbreaks (18). After 15 years of research, we know a great deal about infections with *E. coli* O157:H7, but we still do not know how best to treat the infection, nor how the cattle (the principal source of infection for humans) themselves become infected. Better slaughter procedures and pasteurization of milk are useful control strategies for this pathogen in meat and milk, as irradiation of meat may be in the future. More needs to be learned: for example, it remains unclear how best to prevent this organism from contaminating lettuce or apple juice. For more recently identified agents, even less is known.

New Food Vehicles of Transmission

Along with new pathogens, an array of new food vehicles of transmission have been implicated in recent years. Traditionally, the food implicated in a foodborne outbreak was undercooked meat, poultry or seafood, or unpasteurized milk. Now, additional foods previously thought safe are considered hazardous. For example, for centuries, the internal contents of an egg were presumed safe to eat raw. However, epidemic *Salmonella* Enteritidis infection among egg-laying flocks indicates that intact eggs may have internal contamination with this *Salmonella* serotype. Many outbreaks are caused by contaminated shell eggs, including eggs used in such traditional recipes as eggnog and Caesar salad, lightly cooked eggs in omelettes and French toast, and even foods one would presume thoroughly cooked, such as lasagna and meringue pie (19,20). *E. coli* O157:H7 has caused illness through an ever-broadening spectrum of foods, beyond the beef and raw milk that are directly related to the bovine reservoir. In 1992, an outbreak caused by apple cider showed that this organism could be transmitted through a food with a pH level of less than 4.0, possibly after contact of fresh produce with manure (21). A recent outbreak traced to venison jerky suggests a wild deer reservoir, so both cattle and feral deer manure are of concern (22). Imported raspberries contaminated with *Cyclospora* caused an epidemic in the United States in 1996, possibly because contaminated surface water was used to spray the berries with fungicide before harvest (5). Norwalk-like viruses, which appear to have a human reservoir, have contaminated oysters harvested from pristine waters by oyster catchers who did not use toilets with holding tanks on

their boats and were themselves the likely source of the virus (23).

The new food vehicles of disease share several features. Contamination typically occurs early in the production process, rather than just before consumption. Because of consumer demand and the global food market, ingredients from many countries may be combined in a single dish, which makes the specific source of contamination difficult to trace. These foods have fewer barriers to microbial growth, such as salt, sugar, or preservatives; therefore, simple transgressions can make the food unsafe. Because the food has a short shelf life, it may often be gone by the time the outbreak is recognized; therefore, efforts to prevent contamination at the source are very important.

An increasing, though still limited, proportion of reported foodborne outbreaks are being traced to fresh produce (24). A series of outbreaks recently investigated by the Centers for Disease Control and Prevention (CDC) has linked a variety of pathogens to fresh fruits and vegetables harvested in the United States and elsewhere (Table 2). The investigations have often been triggered by detection of more cases than expected of a rare serotype of *Salmonella* or *Shigella* or by diagnosis of a rare infection like cyclosporiasis. Outbreaks caused by common serotypes are more likely to be missed. Various possible points of contamination have been identified during these investigations, including contamination during production and harvest, initial processing and packing, distribution, and final processing (Table 3). For example, fresh or inadequately composted manure is used sometimes, although *E. coli* O157:H7 has been shown to survive for up to 70 days in bovine feces (25). Untreated or contaminated water seems to be a particularly likely source of contamination. Water used for spraying, washing, and maintaining the appearance of produce must be microbiologically safe. After two large outbreaks of salmonellosis were traced to imported cantaloupe, the melon industry considered a "Melon Safety Plan," focusing particularly on the chlorination of water used to wash melons and to make ice for shipping them. Although the extent to which the plan was implemented is unknown, no further large outbreaks have occurred. After two large outbreaks of salmonellosis were traced to a single tomato packer in the Southeast, an automated chlorination system was developed for the packing plant wash tank. Because tomatoes absorb water (and associated bacteria) if washed in water colder than they are, particular attention was also focused on the temperature of the water bath (26,27). No further outbreaks have been linked to southeastern tomatoes. Similar attention is warranted for water used to rinse lettuce heads in packing sheds and to crisp them in grocery stores as well as for water used in processing other fresh produce.

Table 2. Foodborne outbreaks traced to fresh produce, 1990–1996

Yr.	Pathogen	Vehicle	Cases (No.)	States (No.)	Source
'90	*S.* Chester	Cantaloupe	245	30	C.A.[a]
'90	*S.* Javiana	Tomatoes	174	4	U.S.[b]
'90	Hepatitis A	Strawberries	18	2	U.S.
'91	*S.* Poona	Cantaloupe	>400	23	U.S./ C.A.
'93	*E. coli* O157:H7	Apple cider	23	1	U.S.
'93	*S.* Montevideo	Tomatoes	84	3	U.S.
'94	*Shigella flexneri*	Scallions	72	2	C.A.
'95	*S.* Stanley	Alfalfa sprouts	242	17	N.K.[c]
'95	*S.* Hartford	Orange juice	63	21	U.S.
'95	*E. coli* O157:H7	Leaf lettuce	70	1	U.S.
'96	*E. coli* O157:H7	Leaf lettuce	49	2	U.S.
'96	Cyclospora	Raspberries	978	20	C.A.
'96	*E. coli* O157:H7	Apple juice	71	3	U.S.

[a]Central America
[b]United States
[c]Source not known

Table 3. Events and potential contamination sources during produce processing

Event	Contamination sources
Production and harvest	
Growing, picking, bundling	Irrigation water, manure, lack of field sanitation
Initial processing	
Washing, waxing, sorting, boxing	Wash water, handling
Distribution	
Trucking	Ice, dirty trucks
Final processing	
Slicing, squeezing, shredding, peeling	Wash water, handling, cross-contamination

A New Outbreak Scenario

Because of changes in the way food is produced and distributed, a new kind of outbreak has appeared. The traditional foodborne outbreak scenario often follows a church supper, family picnic, wedding reception, or other social

event. This scenario involves an acute and highly local outbreak, with a high inoculum dose and a high attack rate. The outbreak is typically immediately apparent to those in the local group, who promptly involve medical and public health authorities. The investigation identifies a food-handling error in a small kitchen that occurs shortly before consumption. The solution is also local. Such outbreaks still occur, and handling them remains an important function of a local health department.

However, diffuse and widespread outbreaks, involving many counties, states, and even nations (28), are identified more frequently and follow an entirely different scenario. The new scenario is the result of low-level contamination of a widely distributed commercial food product. In most jurisdictions, the increase in cases may be inapparent against the background illness. The outbreak is detected only because of a fortuitous concentration of cases in one location, because the pathogen causing the outbreak is unusual, or because laboratory-based subtyping of strains collected over a wide area identifies a diffuse surge in one subtype. In such outbreaks, investigation can require coordinated efforts of a large team to clarify the extent of the outbreak, implicate a specific food, and determine the source of contamination. Often, no obvious terminal food-handling error is found. Instead, contamination is the result of an event in the industrial chain of food production. Investigating, controlling, and preventing such outbreaks can have industrywide implications.

These diffuse outbreaks can be caused by a variety of foods. Because fresh produce is usually widely distributed, most of the produce-related outbreaks listed in Table 2 were multistate events. Some of the largest outbreaks affected most states at once. For example, a recent outbreak of *Salmonella* Enteritidis infections caused by a nationally distributed brand of ice cream affected the entire nation (29). Although it caused an estimated 250,000 illnesses, it was detected only when vigorous routine surveillance identified a surge in reported infections with *S.* Enteritidis in one area of southern Minnesota. The consumers affected did not make food-handling errors with their ice cream, so food safety instruction could not have prevented this outbreak. The ice cream premix was transported after pasteurization to the ice cream factory in tanker trucks that had been used to haul raw eggs. The huge epidemic was the result of a basic failure on an industrial scale to separate the raw from the cooked.

S. Enteritidis infections also illustrate why surveillance and investigation of sporadic cases are needed. A diffuse increase in sporadic cases can occur well before a local or large outbreak focuses attention on the emergence of a pathogen. The isolation rate for *S.* Enteritidis began to increase sharply in the New England region in 1978 (Figure 2); all cases were sporadic. In 1982, an outbreak in a New England nursing home was traced to eggs from a local supplier. However, the egg connection was not really appreciated until 1986, when a large multistate outbreak of *S.* Enteritidis infections was traced to stuffed pasta made with raw eggs and labeled "fully cooked." This outbreak, affecting an estimated 3,000 persons in seven states, led to the documentation that *S.* Enteritidis was present on egg-laying farms and to the subsequent demonstration that both outbreaks and sporadic cases of infections were associated with shell eggs (19,30). Since then, Enteritidis has become the most common serotype of *Salmonella* isolated in the United States, accounting for 25% of all *Salmonella* reported in the country and causing outbreaks coast to coast. Eggs remain the dominant source of these infections, causing large outbreaks when they are pooled and undercooked and individual sporadic cases among consumers who eat individual eggs (20,31). Perhaps focused investigation and control measures taken when the localized increase in sporadic *Salmonella* cases was just beginning might have prevented the subsequent spread.

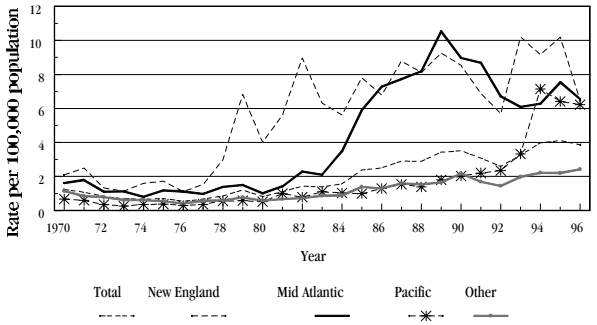

Figure 2. *Salmonella* Enteritidis isolation rates from humans by region, United States, 1970–1996.

Changing Surveillance Strategies

In the United States, surveillance for diseases of major public health importance has been conducted for many years. The legal framework for surveillance resides in the state public health epidemiology offices, which share data with CDC. The first surveillance systems depended on physician or coroner notification of specific diseases and conditions, with reports going first to the local health department, then to state and federal offices. Now electronic, this form of surveillance is still used for many specific conditions (32). In 1962, a second channel was developed specifically for *Salmonella*, to take advantage of the added public health information provided by subtyping the strains of bacteria (33). Clinical laboratories that isolated *Salmonella* from humans were requested or required to send the strains to the state public health laboratory for serotyping. Although knowing the serotype is usually of little benefit to the individual patient, it has been critical to protecting and improving the health of the public at large. Serotyping allows cases that might otherwise appear unrelated to be included in an investigation because they are of the same serotype. Moreover, infections that are close in time and space to an outbreak but are caused by nonoutbreak serotypes and are probably unrelated can be discounted. Results of serotyping are now sent electronically from public health laboratories and can be rapidly analyzed and summarized. *Salmonella* serotyping was the first subtype-based surveillance system and is a model for similar systems (34). Yet another source of surveillance data involves summary reports of foodborne disease outbreak investigations from local and state health departments (35). About 400 such outbreaks are reported annually, by a system that remains paper-based, labor-intensive, and slow.

Existing surveillance systems provide a limited and relatively inexpensive net for tracing large-scale trends in foodborne diseases under surveillance and for detecting outbreaks of established pathogens in the United States. However, they are less sensitive to diffuse outbreaks of common pathogens, provide little detail on sporadic cases, and are not easy to extend to emerging pathogens. In the future, changes in health delivery may impinge on the way that diagnoses are made and reported, leading to artifactual changes in reported disease incidence.

Therefore, CDC, in collaboration with state health departments and federal food regulatory agencies, is enhancing national surveillance for foodborne diseases in several ways. First, the role of subtyping in public health laboratories is being expanded to encompass new molecular subtyping methods. Beginning in 1997, a national subtyping network for *E. coli* O157:H7 of participating state public health department laboratories and CDC will use a single standardized laboratory protocol to subtype strains of this important pathogen. The standard method, pulsed-field gel electrophoresis, can be easily adapted to other bacterial pathogens. In this network, each participating laboratory will be able to routinely compare the genetic gel patterns of strains of *E. coli* O157:H7 with the patterns in a national pattern bank. This will enable rapid detection of clusters of related cases within the state and will focus investigative resources on the cases most likely to be linked. It will also enable related cases scattered across several states to be linked so that a common source can be sought.

Another surveillance strategy, now implemented, is active surveillance in sentinel populations. Since January 1996, at five U.S. sentinel sites, additional surveillance resources make it possible to contact laboratories directly for regular reporting of bacterial infections likely to be foodborne (36; Figure 3). In addition, surveys of the population, physicians, and laboratories measure the proportion of diarrheal diseases that are undiagnosed and unreported so that the true disease incidence can be estimated. This surveillance, known as FoodNet, is the platform on which more detailed investigations, including case-control studies of sporadic cases of common foodborne infections, are being conducted.

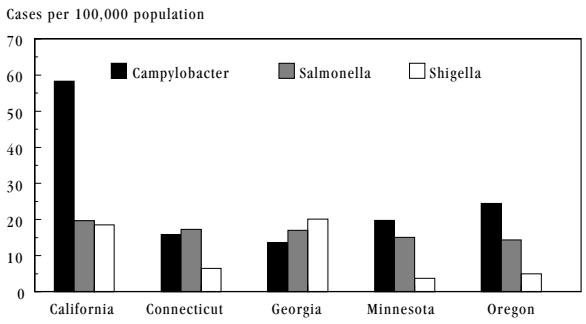

Figure 3. Incidence of three infections in FoodNet surveillance areas, 1996.

Yet another new surveillance initiative is the routine monitoring of antimicrobial resistance among a sample of *Salmonella* and *E. coli* O157:H7 bacteria isolated from humans (37). A new cluster detection algorithm is being applied routinely to surveillance data for *Salmonella* at the national level, making it possible to detect and flag possible outbreaks as soon as the data are reported (38). Implementation of such algorithms for other infections and at the state level will further increase the usefulness of routine surveillance.

Further enhancements are possible as active surveillance through FoodNet is extended to a wider spectrum of infections, including foodborne parasitic and viral infections. In 1997, active surveillance for *Cyclospora* began in FoodNet, which quickly resulted in the detection of a diffuse outbreak among persons who had been on a Caribbean cruise ship that made stops in Mexico and Central America (CDC, unpub. data). Application of standardized molecular subtyping methods to other foodborne pathogens will provide a more sensitive warning system for diffuse outbreaks of a variety of pathogens. To handle outbreaks in areas not covered by FoodNet, standard surveillance and investigative capacities in state health department epidemiology offices and laboratories should be strengthened. In addition, enhanced international consultation will be critical to better detect and investigate international or global outbreaks (28).

Implications of the New Outbreak Scenario for Public Health Activities

Our public health infrastructure is tiered, both in surveillance responsibilities and in response to emergency situations (39). At the local level, the county or city health department, first developed in response to epidemic cholera and other challenges in the 19th century, is responsible for most basic surveillance, investigation, and prevention activities. At the state level, epidemiologists, public health laboratorians, sanitarians, and educators conduct statewide surveillance and prevention activities and consult with and support local authorities. At the national level, CDC is the primary risk-assessment agency for public health hazards and conducts the primary national surveillance as well as epidemic response in support of state health departments. The Food and Drug Administration, Department of Agriculture, and Environmental Protection Agency are the primary regulatory agencies, charged with specific responsibilities regarding the nation's food and water supplies that interlock and are not always predictable. The Food and Drug Administration regulates low-acid canned foods, imported foods, pasteurized milk, many seafoods, rabbits raised for meat, and food and water provided on aircraft and trains. The Department of Agriculture regulates meat and poultry, including primary slaughter and further processing, and pasteurized eggs; investigates animal and plant diseases; and maintains the county extension outreach program. Shell eggs do not have a clear regulatory home, as the Department of Agriculture regulates the grading of shell eggs for quality, but the Food and Drug Administration, since 1995, has responsibility for the microbiologic safety of shell eggs.

The new outbreak scenario has several implications for the practice of public health, starting at the local level. One is that when diffuse outbreaks are detected, a local health department may need to investigate a few cases that are part of a larger outbreak despite their apparently small local impact. Second, an apparently local outbreak may herald the first recognized manifestation of a national or even international event.

When a diffuse outbreak of a potentially foodborne pathogen is detected, rapid investigation is needed to determine whether the outbreak is foodborne, and if possible, identify a specific food vehicle. These investigations, which typically include case-control studies, may need to be conducted in several locations at once. While all cases or all affected states may not need to be included in such an investigation, combining cases from several locations in one investigation and repeating the investigation in more than one location can be helpful. For example, in a recent international outbreak of *Salmonella* Stanley infections traced to alfalfa sprouts, concentrations of cases in Arizona, Michigan, and Finland led to case-control studies in each location, each of which linked illness to eating sprouts grown from the same batch of alfalfa seeds. This proved that the seeds were contaminated at the source (40). Parallel investigations can also lead to new twists. In the large West Coast outbreak of *E. coli* O157:H7 infections in 1993, a parallel investigation conducted in Nevada identified a type of hamburger other than the one implicated in the

initial case-control investigation in Washington, leading to a broader recall and a more complete investigation of the circumstances of contamination (15,41). Because well-conducted investigations may lead to major product recalls, industrial review, and overhaul, and even international embargoes, it is essential that they be of the highest scientific quality.

Foodborne outbreaks are investigated for two main reasons. The first is to identify and control an ongoing source by emergency action: product recall, restaurant closure, or other temporary but definitive solutions. The second reason is to learn how to prevent future similar outbreaks from occurring. In the long run this second purpose will have an even greater impact on public health than simply identifying and halting the outbreaks. Because all the answers are not available and existing regulations may not be sufficient to prevent outbreaks, the scientific investigation often requires a careful evaluation of the chain of production. This traceback is an integral part of the outbreak investigation. It is not a search for regulatory violations, but rather an effort to determine where and how contamination occurred. Often, the contamination scenario reveals that a critical point has been lost. Therefore, epidemiologists must participate in traceback investigations.

Intervention during outbreaks often depends on having enough good epidemiologic data to act with confidence, without waiting for a definitive laboratory test, particularly if potentially lethal illnesses are involved. For example, if five persons with classic clinical botulism ate at the same restaurant the preceding day (but have nothing else apparent in common), prudence dictates closing the restaurant quickly while the outbreak is sorted out—that is, before a specific food is identified or confirmatory cultures are made, which may take several days or even weeks. Good epidemiologic data, including evidence of a clear statistical association with a specific exposure, biologic plausibility of the illness syndrome, the potential hazard of that food, and the logical consistency of distribution of the suspect food and cases are essential.

The role of the regulatory agency laboratory is also affected by the new scenario. Because of the short shelf life and broad distribution of many of the new foods responsible for infection, by the time the outbreak is recognized and investigated the relevant food may no longer be available for culture. Because contamination may be restricted to a single production lot, blind sampling of similar foods that does not include the implicated lot can give a false sense of security. Good epidemiologic information pointing to contamination of a specific food or production lot should guide the microbiologic sampling and the interpretation of the results. Available methods may be insufficient to detect low-level contamination, even of well-established pathogens.

New Approaches to the Prevention of Foodborne Disease

Meeting the complex challenge of foodborne disease prevention will require the collaboration of regulatory agencies and industry to make food safely and keep it safe throughout the industrial chain of production. Prevention can be "built in" to the industry by identifying and controlling the key points—from field, farm, or fishing ground to the dinner table—at which contamination can either occur or be eliminated. The general strategy known as Hazard Analysis and Critical Control Points (HACCP) replaces the strategy of final product inspection. Some simple control strategies are self-evident, once the reality of microbial contamination is recognized. For example, shipping fruit from Central America with clean ice or in closed refrigerator trucks, rather than with ice made from untreated river water, is common sense. Similarly, requiring oyster harvesters to use toilets with holding tanks on their oyster boats is an obvious way to reduce fecal contamination of shallow oyster beds. Pasteurization provides the extra barrier that will prevent *E. coli* O157:H7 and other pathogens from contaminating a large batch of freshly squeezed juice.

For many foodborne diseases, multiple choices for prevention are available, and the best answer may be to apply several steps simultaneously. For *E. coli* O157:H7 infections related to the cattle reservoir, pasteurizing milk and cooking meat thoroughly provide an important measure of protection but are insufficient by themselves. Options for better control include continued improvements in slaughter plant hygiene and control measures under HACCP, developing additives to cattle feed that alter the microbial growth either in the feed or in the bovine rumen to make cows less hospitable hosts for *E. coli* O157, immunizing or otherwise protecting the cows so that they do not become

infected in the first place, and irradiating beef after slaughter. For *C. jejuni* infections related to the poultry reservoir, future control options may include modification of the slaughter process to reduce contamination of chicken carcasses by bile or by water baths, freezing chicken carcasses to reduce *Campylobacter* counts, chlorinating the water that chickens drink to prevent them from getting infected, vaccinating chickens, and irradiating poultry carcasses after slaughter.

Outbreaks are often fertile sources of new research questions. Translating these questions into research agendas is an important part of the overall prevention effort. Applied research is needed to improve strategies of subtyping and surveillance. Veterinary and agricultural research on the farm is needed to answer the questions about whether and how a pathogen such as *E. coli* O157:H7 persists in the bovine reservoir, to establish the size and dynamics of a reservoir for this organism in wild deer, and to look at potential routes of contamination connecting animal manure and lettuce fields. More research is needed regarding foods defined as sources in large outbreaks to develop better control strategies and better barriers to contamination and microbial growth and to understand the behavior of new pathogens in specific foods. Research is also needed to improve the diagnosis, clinical management, and treatment of severe foodborne infections and to improve our understanding of the pathogenesis of new and emerging pathogens. To assess and evaluate potential prevention strategies, applied research is needed into the costs and potential benefits of each or of combinations.

To prepare for the 21st century, we will enhance our public health food safety infrastructure by adding new surveillance and subtyping strategies and strengthening the ability of public health practitioners to investigate and respond quickly. We need to encourage the prudent use of antibiotics in animal and human medicine to limit antimicrobial resistance. We need to continue basic and applied research into the microbes that cause foodborne disease and into the mechanisms by which they contaminate our foods and cause outbreaks and sporadic cases. Better understanding of foodborne pathogens is the foundation for new approaches to disease prevention and control.

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Emergence of New Pathogens as a Function of Changes in Host Susceptibility

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A pathogen may emerge as an important public health problem because of changes in itself or its transmission pathways. Alternatively, a microorganism may emerge as a pathogen or acquire new public health importance because of changes in host susceptibility to infection. Factors influencing host susceptibility within the population as a whole include increases in the number of immunocompromised patients; increased use of immunosuppressive agents, particularly among persons receiving cancer chemotherapy or undergoing organ transplantation; aging of the population; and malnutrition. In considering the emergence of foodborne pathogens and designing interventions to limit their spread, the susceptibility of these population subgroups to specific infections should be taken into account.

Occurrence of disease is a function of several major variables: the virulence of the microorganism (i.e., its possession of factors that allow it to cause illness), its mode of transmission (i.e., how it gets to the host), and host susceptibility (i.e., how well the host can defend itself against the microorganism). Increased susceptibility to infection may be measured in terms of infectious dose (the number of microorganisms it takes to cause illness) and of the ability of the host to limit spread of the microorganism (e.g., the ability to limit spread of microorganisms from the intestinal tract to the bloodstream). These same variables apply to emerging pathogens. Microorganisms may emerge as pathogens because they have developed new virulence genes or resistance to standard therapeutic methods. Emergence may be related to changes in transmission pathways, which permit a known pathogen to move into new, previously unexposed populations. Finally, increases in the number of persons susceptible to a specific microorganism may result in its emergence as an important public health problem; at the same time, when attention is focused on populations with increased susceptibility to infection, organisms that might not otherwise be recognized as pathogens may be identified.

Many factors influence the susceptibility of populations to infection, including increases in diseases that cause immunosuppression, increased use of immunosuppressive agents, aging of the population, and malnutrition. In considering these categories, it should be recognized that "host susceptibility" is not a single entity. Changes in host susceptibility may be due to various mechanisms, with each mechanism having a greater or lesser impact on the ability of the host to defend itself against infection with specific pathogens or classes of pathogens. These are very complex biologic systems; nonetheless, the general categories outlined below may be of value in identifying groups or populations for further study.

Increases in Diseases That Cause Immunosuppression

Hereditary diseases associated with immunosuppression are present in a small but relatively constant proportion of the population. The most common of these diseases, a selective immunoglobulin A deficiency, has been found in as many as 0.3% of some blood donor populations (1) and may be associated with recurrent diarrhea; infections with giardia, in particular,

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have been noted to be more common among such immunocompromised patients.

In contrast to patients with hereditary immunodeficiencies, the population of patients with acquired immunodeficiencies is rapidly increasing. As of June 1996 in the United States, an estimated 223,000 persons ≥ 13 years of age had AIDS (Figure 1); this represents increases of 10% and 65% since mid-1995 and January 1993, respectively. During 1995, human immunodeficiency virus (HIV) infection remained the leading cause of death among persons 25 to 44 years of age (Figure 2), accounting for 19% of deaths from all causes in this age group (2).

Persons with AIDS show a clear increase in susceptibility to infection with *Salmonella* species. Data suggest that risk for nontyphi *Salmonella* infections is increased 20- to 100-fold among AIDS patients (3-7). Among persons infected with *Salmonella*, AIDS results in a severalfold increase in the risk for septicemia (3,5,6); AIDS also results in increases in infections at other extraintestinal sites, compatible with an overall increase in risk for dissemination of the organism. This increase in risk is reflected in increases in the percentage of total *Salmonella* isolated from blood. For example, for persons ages 25 to 49 years in states with high AIDS incidence, the percentage of *Salmonella* isolates from blood increased from 2.3% in 1978 to 17.8% in 1987 among men and from 3.1% to 8.1% among women; in contrast, no changes in blood-isolate percentages occurred for either sex in states with low AIDS incidence (8). These latter studies further suggest that serotype is an important risk determinant, with increases in bacteremia in states with high AIDS incidence associated primarily with infections due to *Salmonella* Enteritidis and *Salmonella* Typhimurium. While these data are for nontyphoidal *Salmonella*, studies outside the United States suggest that AIDS patients have a similar increase in risk for infection with *Salmonella typhi* in areas endemic for typhoid fever. For example, in Lima, Peru, the risk for typhoid increased 25-fold in HIV-infected persons 15 to 35 years of age (9); HIV infection also appeared to influence the clinical presentation of typhoid fever, with severe diarrhea and gastrointestinal symptoms seen more often than expected.

While the above data suggest a continuing climb in salmonellosis and, in particular, *Salmonella* bacteremia in conjunction with an increasing AIDS prevalence, anecdotal data from AIDS clinicians do not support this view. The standard of care for AIDS patients now includes routine prophylactic therapy with trimethoprim/sulfamethoxazole to prevent *Pneumocystis carinii* pneumonia. Trimethoprim/sulfamethoxazole is also one of the first-line therapies for salmonellosis; widespread prophylactic use of this drug in the AIDS population may have reduced the

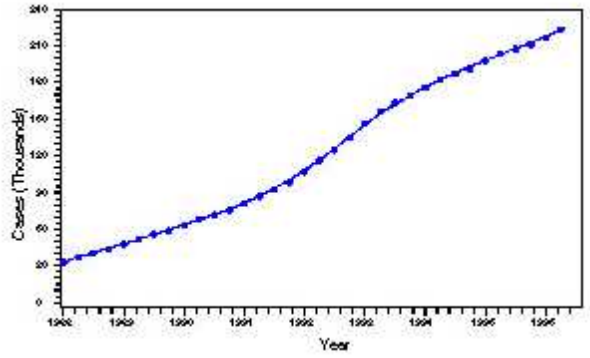

Figure 1. Cases among persons aged ≥ 13 years, adjusted for delays in reporting, by quarter year, United States, 1988–June 1996. (Points represent quarterly prevalence; the line represents "smoothed" prevalence. Estimates are not adjusted for incomplete reporting of diagnosed AIDS cases or AIDS deaths. From the Centers for Disease Control and Prevention. Update: Trends in AIDS incidence, deaths, and prevalence—United States, 1996. MMWR Morb Mortal Wkly Rep 1997;46:165-73).

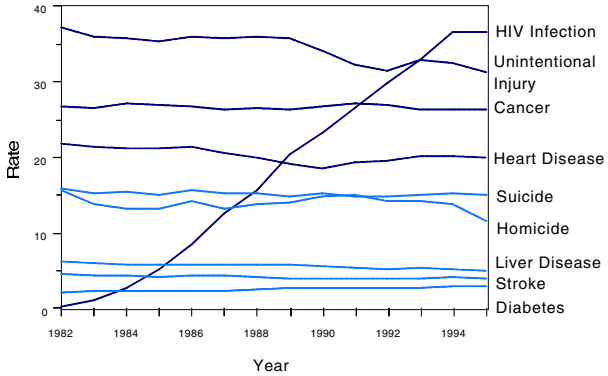

Figure 2. Death rates per 100,000 population for leading causes of death among persons ages 25 to 44 years, by year, United States, 1982–1995. (Based on underlying causes of death reported on death certificates, using final data for 1982–1994 and preliminary data for 1995. From the Centers for Disease Control and Prevention. Update: Trends in AIDS incidence, deaths, and prevalence, United States, 1996. MMWR Morb Mortal Wkly Rep 1997;46:165-73).

incidence of serious *Salmonella* infections, although this protective effect could be diminished in the face of increasing resistance to this antimicrobial agent among clinical isolates of *Salmonella* (10).

Fewer data are available on susceptibility of AIDS patients to other acute bacterial foodborne infections. While the initial impression was that the risk for *Campylobacter jejuni* infections was not higher among AIDS patients, a 35-fold increase in the *Campylobacter* case rate among persons with AIDS was noted in one study from Los Angeles (11). Other data indicate that HIV-positive patients can contract persistent *C. jejuni* infections, with chronic diarrhea, fever, and fecal leukocytes (12). In studies in the San Francisco area, AIDS patients were estimated to have a 280-fold increase in incidence of listeriosis, as compared with the general population (13). Of 98 nonpregnant adults with invasive *Listeria* infection identified between November 1988 and December 1990 in selected counties in California, Tennessee, Georgia, and Oklahoma, 20% were HIV-positive (13).

Before AIDS, *Toxoplasma gondii* was of concern primarily because of the risk for congenital infection in infants of mothers who had acute illness during pregnancy. *T. gondii* is now the leading cause of space-occupying cranial lesions in persons with AIDS (14,15); data from the 1980s suggest that 5% to 10% of AIDS patients get toxoplasmic encephalitis (16). In an estimated 50% of cases, *Toxoplasma* is transmitted by food (17). In this context, *Toxoplasma* must be regarded as an important emerging pathogen in this patient population.

The AIDS epidemic has also drawn attention to microorganisms not previously recognized as pathogens. Perhaps the most important of these is *Cryptosporidium.* In early investigations of AIDS-associated diarrhea, it rapidly became apparent that most patients were not infected with traditional enteric pathogens. Many of these patients were infected with *Cryptosporidium*; an estimated 10% to 20% of cases of AIDS-associated diarrhea are due to this microorganism (18). In subsequent years, *Cryptosporidium* was also recognized as the cause of intestinal infections in healthy hosts (19); and, most recently, it has been recognized as a major waterborne pathogen (20). *Isospora belli* has also been implicated as a cause of diarrhea in AIDS patients, as have the microsporidia (18). More recently, enteroaggregative *Escherichia coli* and nonpathogenic bioserovars of *Yersinia enterocolitica* were associated with diarrheal disease in AIDS cases (21,22). Further work will determine if these latter agents are indeed emerging pathogens in this patient population.

Increased Use of Immunosuppressive Agents

Advances in medical treatment have resulted in increasing numbers of immunosuppressed patients (including patients undergoing organ transplantation or cancer chemotherapy) and patients with serious underlying chronic diseases; these patients, too, may be at increased risk for infection with microorganisms that might otherwise not be recognized or associated with serious illness. The number of new cancer cases has steadily increased over the past 20 years. For white males, cancers at all sites have increased from an estimated 364 new cases/100,000 population in 1973 to 462 new cases/100,000 population in 1994; white females show an increase from 295 new cases/100,000 population in 1973 to 347 new cases/100,000 population in 1994 (23). Patients are also surviving longer. For white males, the 5-year relative cancer survival rate was 56.6% in 1986 to 1993, compared with 42% in 1974 to 1976; for white females, the 1986 to 1993 rate was 62.3%, compared with 57.6% in 1974 to 1976. While specific data are lacking, these increases appear to have been accompanied by increased use of chemotherapeutic regimens and regimens that may have more toxicity than those used in the past. The number of solid organ transplants has also increased substantively (Figure 3). In particular, more complex procedures such as liver, heart, and lung transplants have increased. This, in turn, has resulted in larger numbers of chronically immunocompromised persons in the general population.

Aside from the direct immunosuppressive effect of the agents administered to these patients, other associated factors may contribute to susceptibility to infection. Many, if not most, patients receiving chemotherapy or immunosuppressive agents are also treated with antimicrobial drugs, which can have profound effects on the bacterial flora of the intestinal tract. These disturbances of gut microbial ecology may predispose to colonization and infection with other microorganisms, some of which may have increased virulence. Many chemotherapeutic

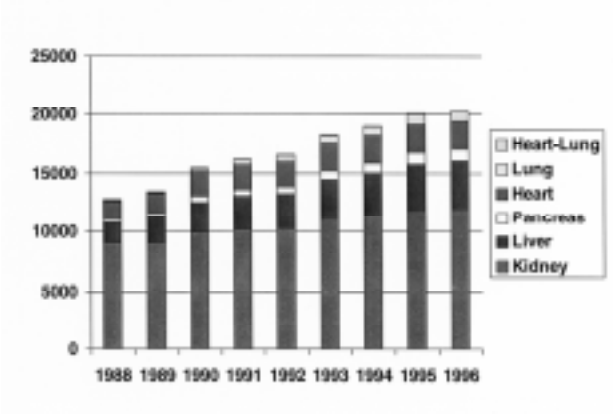

Figure 3. Number of organ transplants, by year and by site, 1988–1996 (data obtained from the United Network for Organ Sharing).
Note: Heart-lung transplants too few to be visible.

agents have direct toxicity for the gut mucosa; the resulting mucositis increases the susceptibility of these patients to bloodstream infections with whatever microorganisms are present in gut. The concentration of these highly susceptible patients on certain wards or units in a hospital may also increase the risk for nosocomial transfer of specific microorganisms. For example, vancomycin-resistant enterococci (VRE) (which, at least in Europe, have been associated with food [24]) have emerged as a substantive problem in cancer centers and transplant units (25-27). Persons who are immunosuppressed or have serious underlying illness are much more susceptible to colonization and infection with the organism. In some oncology and transplant units, more than 10% of patients are colonized or infected with VRE (26,27), providing well-documented opportunities for transfer of the organism to other immunosuppressed hosts in the same unit.

Cancer patients who have just undergone chemotherapy are often profoundly neutropenic. In this setting, especially in conjunction with the mucositis mentioned above, virtually any microorganism in the intestinal tract can enter the bloodstream and cause potentially fatal illness. For example, it has been found that raw produce in salads may be an important route by which patients acquire *Pseudomonas* (28); as a result, severely neutropenic patients are generally restricted to cooked food. *Salmonella* infections are reported among cancer patients, although the relative risk for infection in this population is not well characterized. Patients with neoplastic disease do appear to have a substantively increased risk for *Salmonella* septicemia, with 35% patients in one series having septicemia (29), versus fewer than 1% in healthy hosts. Cancer patients appear to be at increased risk for invasive *Listeria* infections (13). Toxoplasmosis tends to be of particular concern among transplant patients (30,31).

Aging of the Population

The absolute number of the elderly in the United States is rapidly increasing, as is the proportion of the U.S. population they comprise. In 1950, 3.8 million persons (2.6% of the population) were over the age of 74, as opposed to 14.7 million (5.6% of the population) in 1995 (23; Figures 4 and 5). The elderly appear to be at a clearly increased risk for death from foodborne and diarrheal disease. Between 1979 and 1987, 28,538 persons in the United State had diarrhea as an immediate or underlying cause of death; 51% of these persons were more than 74 years of age, 27% were adults age 55 to 74, and 11% were children under the age of 5 (32).

The increased susceptibility of the elderly to infection and death may be due to a number of factors. Aging results in senescence of the gut-associated lymphoid tissue, increasing susceptibility to infection. Aging may also result in a decrease in gastric acid secretion: in one study, stimulated acid output was reduced approximately 30% in persons aged 65 to 98, with a 40% reduction in pepsin output (33). As a low pH of the

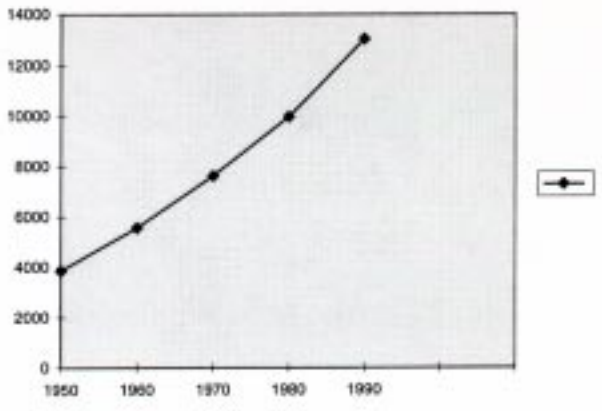

Figure 4. Number of persons >74 years of age, U.S. population, for selected years, 1950–1990. From the National Center for Health Statistics. Health, United States, 1996–97 and Injury Chartbook. Hyattsville, Maryland, 1997.

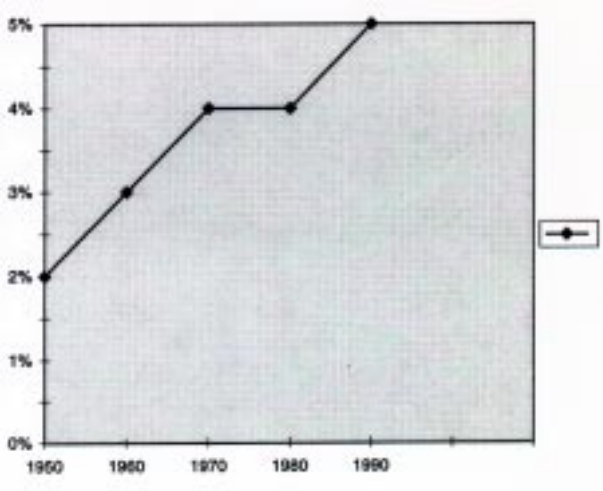

Figure 5. Percentage of U.S. population >74 years of age, for selected years, 1950–1990. From National Center for Health Statistics. Health, United States, 1996–97 and Injury Chartbook. Hyattsville, Maryland, 1997.

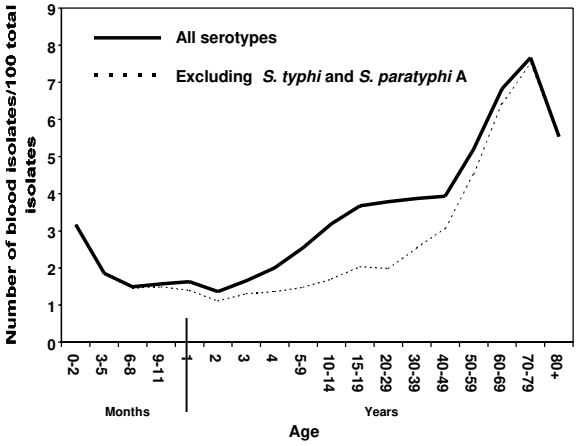

Figure 6. Ratio of blood isolates to total isolates of *Salmonella* by age group of the person from whom the isolate was obtained as reported to the Centers for Disease Control, Atlanta, GA, in 1968–79. From Blaser MJ, Feldman RA. Salmonella bacteremia: reports to the Centers for Disease Control and Prevention, 1968–1979. J Infect Dis 1981;143:743-6.

stomach is a major barrier to entry of enteric pathogens, reductions in gastric acidity can clearly increase the susceptibility to infection. Social factors may also influence susceptibility. For the elderly, residence in a long-term care facility was a major independent risk factor for diarrheal death (32). While a number of factors contribute to the increased risk, the communal living environment, combined with problems of fecal incontinence, create an environment in which enteric and foodborne pathogens are easily spread (32,34).

Incidence of salmonellosis and *Campylobacter* diarrhea appears to be higher among the elderly (35,36). More striking, however, is the increase in frequency of *Salmonella* bacteremia as compared with isolations from other sites: *Salmonella* infections in the elderly are more likely to cause bacteremia (37; Figure 6), which, in turn, substantively increases the risk for death. For example, in a recent nursing home outbreak in Maryland, 50 (35%) of 141 residents became ill, seven had bacteremia, nine were hospitalized (with a median length of hospitalization of 22 days), and four died (38). *E. coli* O157:H7 is also a common pathogen in nursing homes and among the elderly. In one reported nursing home outbreak, 55 of 169 residents were affected; overall, 19 (35%) of the affected residents died (39).

Malnutrition

The above discussions have focused on issues most relevant to the United States and other industrialized countries. However, on a global scale, probably the leading cause of increased host susceptibility to infection is malnutrition. While accurate data on the prevalence of malnutrition are difficult to obtain, problems are accentuated in developing countries, in areas of political unrest, and among marginalized populations in the United States and other affluent nations. In Mexico, according to a probabilistic survey in 1990, 42.3% of children under 5 years of age had some degree of malnutrition (40).

Malnutrition increases host susceptibility through a number of mechanisms. It weakens epithelial integrity and may have a profound effect on cell-mediated immunity, with functional deficiencies in immunoglobulins and defects in phagocytosis. Malnutrition also may initiate a "vicious cycle" of infection predisposing to malnutrition and growth faltering, which in turn may lead to an increased risk for further infection (40,41). In studies in Bangladesh, malnourished and well-nourished children had the same number of infections with diarrheal pathogens such as enterotoxigenic *E. coli*; however, diarrhea in malnourished children was of longer duration and had greater potential long-term nutritional consequences (42). Overall, malnutrition appears

to result in a 30-fold increase in the risk for diarrhea-associated death (40).

Conclusions

Host susceptibility (and changes in the susceptibility to infection of groups within the general population) is a critical variable in assessing the public health effects and understanding the emergence and spread of pathogenic microorganisms. Surveillance within populations with increased susceptibility to infection may allow identification of new pathogens before they are recognized within the general population. Studies designed to identify the reasons for the increased susceptibility of a specific population to a specific agent may reveal how a microorganism is able (or not able) to breach normal host defense mechanisms. Finally, from a public health standpoint, risk management strategies for emergent foodborne pathogens must clearly identify and focus on populations with increased susceptibility to infection.

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Chronic Sequelae of Foodborne Disease

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In the past decade, the complexity of foodborne pathogens, as well as their adaptability and ability to cause acute illness, and in some cases chronic (secondary) complications, have been newly appreciated. This overview examines long-term consequences of foodborne infections and intoxications to emphasize the need for more research and education.

The term foodborne disease encompasses a variety of clinical and etiologic conditions and describes a subset of enteric disease (1-4), which in the United States ranks second in prevalence to respiratory disease (2). In foodborne disease, the food may act as a vehicle for the transmission of actively growing microorganisms or products of metabolism (toxins), or it may have a passive role as a vehicle for the transmission of nonreplicating bacteria, viruses or protozoa, or stable biologic toxins. In most cases, the clinical conditions usually associated with foodborne disease are acute: diarrhea, vomiting, or other gastrointestinal manifestations such as dysentery. However, other pathophysiologic responses may occur independently or accompany acute-phase responses (1-4). A number of chronic sequelae may result from foodborne infections, including ankylosing spondylitis, arthropathies, renal disease, cardiac and neurologic disorders, and nutritional and other malabsorptive disorders (incapacitating diarrhea). The evidence that microorganisms or their products are either directly or indirectly associated with these long-term sequelae ranges from convincing to circumstantial (1-4). The reason for this disparity is that, except in rare circumstances, chronic complications are unlikely to be identified or epidemiologically linked to a foodborne cause because these data are not systematically collected. Moreover, host symptoms induced by a specific pathogen or product of a pathogen are often wide-ranging and overlapping and therefore difficult to link temporally to a specific incident. These impediments manifest themselves because the problems associated with chronic disease can result from an infection without overt illness. Alternatively, the chronic sequelae may be unrelated to the acute illness and may occur even if the immune system successfully eliminates the primary infection; therefore, activation of the immune system may initiate the chronic condition as a result of an autoimmune response (2-4). The variability of the human response—from overt illness to chronic carrier status—is perhaps the most confounding issue.

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Cost of Chronic Sequelae

As the incidence of foodborne disease increases, the incidence of chronic sequelae may also rise. Several authors have estimated that chronic sequelae may occur in 2% to 3% of foodborne disease cases and suggest that the long-term consequences to human health and the economy may be more detrimental than the acute disease. An estimated 80 million cases of foodborne disease occur annually in the United States, which suggests significant morbidity figures and costs to society in the billions of dollars per year (2,4).

Infection: The Microbe/Toxin versus the Host

Several microbial pathogens are highly adapted to parasitization, exhibiting environmentally responsive and adaptive traits that allow attachment, invasion, and replication in the host (2,4). Microbial pathogenicity can be viewed solely from the perspective of the microbe; however, this would be not only unidimensional, but also wrong (2,4). A major selective force that regulates the phenotype of an infecting microbial pathogen population is the host's immune system, which is also highly adaptive, especially in discriminating

self and nonself antigens (2,4). When the host-parasite relationship is examined holistically, mechanisms that successful pathogens have apparently evolved to elude the immune system include antigenic heterogeneity or variation; sequestration, either intracellularly or in certain specific host sites; molecular mimicry, through either imitation (cross-reaction) or adsorption of host protein; and direct immune stimulation and/or suppression (2-5).

Rheumatoid Disease

Several bacteria, including salmonellae, induce septic arthritis by hematogenous spread to the synovial space, causing inflammation. Viable organisms are recoverable from synovial fluid, and treatment usually involves antibiotic therapy. Prognosis depends on host factors and virulence of the organism; either complete resolution or permanent joint damage can occur (1-4,6,7).

Yersinia enterocolitica, Y. pseudotuberculosis, Shigella flexneri, Sh. dysenteriae, Salmonella spp., *Campylobacter jejuni,* and *Escherichia coli* initiate aseptic or reactive arthritis, an acute, nonpurulent joint inflammation following infection elsewhere in the body, for example the bowel. *Klebsiella pneumoniae* has been implicated, although it appears now that the bacterium is connected with fecal carriage by ankylosing spondylitis probands (4). Although a distinct clinical disease, reactive arthritis also occurs in the Reiter syndrome triad with conjunctivitis and uveitis. A subset of patients with symptoms of reactive arthritis and Reiter syndrome get ankylosing spondylitis, a rheumatoid inflammation of synovial joints and entheses within and distal to the spine (8).

The relative risk of developing these seronegative spondyloarthropathies after a gram-negative enterobacterial infection is high for persons positive for the major histocompatibility class (MHC) antigen B27 and the cross-reacting MHC B7 group. Indeed, persons who are human leukocyte antigen (HLA)-B27 positive have an 18-fold greater risk for reactive arthritis, a 37-fold greater risk for Reiter syndrome, and up to a 126-fold greater risk for ankylosing spondylitis than persons who are HLA-B27 negative and have the same enteric infections. Other genes that may be related or act in concert appear to determine which disease is acquired (2,5,7,9). These chronic complications are related to a genetically determined host risk factor in combination with an environmental trigger. No cause-and-effect relationship of enteric pathogens in ankylosing spondylitis has been established (4); however, a low but consistent incidence (0.2% to 2.4%) of reactive arthritis occurs after outbreaks of *S.* Typhimurium, *Sh. flexneri,* and *C. jejuni*. Biotypes and phage types of *Y. enterocolitica* O:3 and O:9, endemic to Scandinavia, are either highly arthritogenic or affect a more genetically predisposed population with persistent and debilitating symptoms that may last for years (2,3).

The sharing of antigenic determinants by a microbe and its host is a frequent natural occurrence, and bacterial antigens from the pathogens that directly cross-react with MHC B27 have been demonstrated (6,9). Additionally, the plasmid-mediated synthesis of bacterial B27 "modifying factor," a protein that binds to and subsequently alters the conformation of B27, has been reported (10). In both of these models, immune recognition of the foreign antigen leads to an autoimmune anti-B27 response. Alternatively, B27 may act nonimmunologically because dissemination of bacterial antigens to infected joints stimulates a local T-cell inflammatory response. Here, B27 may act as a receptor for bacteria or antigens thereof, facilitating invasiveness from mucosal surfaces in the gut (9). Indeed, transfected B27 on the surface of mouse L cells reportedly can alter bacterial invasion capability (11).

Despite the strong familial association related to the MHC B27 gene, B27-negative persons are known to become ill, albeit less often, but with apparently equal severity, as shown by an epidemiologic investigation of rheumatoid arthritis following the 1985 *S.* Typhimurium gastroenteritis outbreak due to contaminated milk (4).

Autoimmune Thyroid Disease

Graves disease is an autoimmune disease mediated by autoantibodies to the thyrotropin receptor (12,13). The first indication that the disease may be linked to infection was finding antibody titers to *Y. enterocolitica* serotype O:3 suggestive of molecular mimicry in a majority of patients with Graves disease. Several studies have shown that two low molecular weight envelope proteins of *Yersinia* contain epitopes cross-reactive with the thyrotropin receptor. These proteins are chromosomally encoded, exposed to the surface of the bacterium, and produced by both virulent and avirulent strains of *Yersinia* (*Y. pestis, Y. pseudotuberculosis, Y. enterocolitica*

VW+ and WV-). In addition to autoantibody, a suppressor cell dysfunction may be involved in Graves disease (12,13). Severe hypothyroidism may also result from chronic intestinal giardiasis due to infection by *Giardia lamblia*; treatment with metronidazole can result in complete elimination of the parasite and recovery of regular intestinal thyroid hormone absorption (14).

Inflammatory Bowel Disease

Inflammatory bowel disease is the collective term for Crohn disease and ulcerative colitis. While both infections are chronic inflammatory diseases with histologic infiltrates of macrophages and lymphocytes and a prolonged clinical course, the primary clinical and pathologic effects are gastrointestinal. The infections can be difficult to differentiate because the symptoms are often similar (15). The acute clinical characteristics are diarrhea, abdominal pain, fever, and weight loss; and the acute pathologic features include a constant flux of neutrophils into inflamed mucosa, eventually penetrating the epithelium into the intestinal lumen. The chronic spontaneously relapsing disorder exhibits many of the symptoms of the acute state; however, this phase has an average symptom duration of 3.2 years before correct diagnosis. Abdominal abscesses are a common and dangerous complication of Crohn disease, while in ulcerative colitis, abdominal perforations may lead to peritonitis. Crohn disease involves the ileum or colon (anaerobes are important), while ulcerative colitis appears restricted to the colon (aerobes are important). Nationality and familial associations suggest a genetic predisposition for the disease (4,15).

Although the cause of inflammatory bowel disease and the mechanism(s) for spontaneous exacerbations and remissions are unknown, much research has focused on transmissible agents, including foodborne pathogens. An association between bacterial L-forms and inflammatory bowel disease has been sporadically reported, with isolation of *Pseudomonas, Mycobacterium, Enterococcus fecalis,* and *E. coli* from affected tissue but not from appropriate controls. There is considerable debate as to whether L-forms are pathogenic in humans or persist in affected tissue.

Mycobacterium paratuberculosis, the causative agent of Johne disease in ruminants, may be associated with Crohn disease through the production of L-forms of the bacterium. Subclinically infected cows shed *M. paratuberculosis*, and the organism has been identified in pasteurized milk by polymerase chain reaction specific for the *M. paratuberculosis* insertion sequence IS900. The pathogen model suggests that a susceptible human neonate first contracts the organism after ingesting commercial dairy products. This invokes an antigen-poor (lacking a cell wall) L-form that grows slowly and persists in the lamina propria, stimulating a chronic low-grade inflammation. The immune response increases in severity over years without bacterial replication, ultimately producing the pathologic features of Crohn disease (15,16). Another model proposes an autoimmune phenomenon mediated by alterations in inflammatory cytokine profiles, possibly as a result of infection (4).

Recent immunocytochemical techniques demonstrated antigens to *Listeria monocytogenes, E. coli,* and *Streptococcus* spp. in Crohn disease tissues. Macrophages and giant cells immunolabelled for antigen specific to these organisms were found beneath ulcers, around abscesses, along fissures, within the lamina propria, in granulomas, and in germinal centers of mesenteric lymph nodes (17).

Superantigens and Autoimmunity

In contrast to conventional antigens, superantigens interact with the variable side of the Vß chain of the T-cell receptor by recognizing elements shared by a subset of T cells. Depending on the type of interaction, recognition can have different consequences, including proliferation and expansion, suppression (clonal deletion), or, alternatively, induction of prolonged unresponsiveness (anergy) or cell death (apoptosis) (18-21). Superantigens from several foodborne bacteria (e.g., *Staphylococcus*, *Streptococcus*, *Yersinia,* and *Clostridium*) have been isolated and characterized. Many are thought to be associated with several autoimmune disorders, for example, rheumatic heart disease, rheumatoid arthritis, multiple sclerosis, Graves disease, Sjogren syndrome, autoimmune thyroiditis, psoriasis, Kawasaki disease, Crohn disease, and insulin-dependent diabetes mellitus (6,18-24).

Although it is accepted that superantigens have a role in autoimmune disorders, the acceptance is based on extensive animal model studies (6,18-24) but limited human clinical studies. In human diseases where superantigens have been clearly demonstrated as the cause, for example, toxic shock syndrome, initial T-cell

proliferation and T-cell receptor–mRNA up-regulation have been observed, but the long-term sequelae in terms of T-cell function are unknown (22). Recent studies suggest that superantigens may also cause an acute flare of a disease within patients in remission from a preexisting autoimmune disorder.

Renal Disease

After colitis caused by *E. coli* O157:H7 and other enterohemorrhagic strains of *E. coli*, hemolytic uremic syndrome develops in some patients (1,2,25). The syndrome is characterized by a triad of symptoms: acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia. Acute renal failure is the leading cause of death in children, and thrombocytopenia is the leading cause of death in adults. Hemolytic uremic syndrome is a worldwide problem that mirrors the distribution of *E. coli* O157:H7 and other Shiga and Shiga-like toxin–producing microorganisms. Outbreaks of hemorrhagic colitis and subsequent cases of hemolytic uremic syndrome have developed as a result of various food vehicles. Besides O157:H7, other Shiga-like toxin–producing *E. coli, Citrobacter, Campylobacter, Shigella, Salmonella,* and *Yersinia* have been linked to the disorder (1,2,25,26).

The toxin-mediated damage to the kidneys may not be limited to the glomerular endothelial cells as once thought but may include the tubular epithelial cells (26-28). Binding studies showed the toxins to be specific for the glycosphingolipid globotriaosylceramide (Gb3), which is present on renal but not umbilical endothelial cells. This may account for the differential sensitivity of renal cells to toxin-induced damage, since Gb3 was present in the glomeruli of infants under 2 years of age but not in the glomeruli of adults. Thus, the presence of Gb3 in the pediatric renal glomerulus may be a risk factor for development of hemolytic uremic syndrome (28). Characterization of the Shiga toxin receptor has led to a potential preventive treatment (4).

Neural and Neuromuscular Disorders

Guillain-Barré syndrome is a subacute, acquired, inflammatory demyelinating polyradiculoneuropathy that frequently occurs after acute gastrointestinal infection. The disease is characterized by alexia, motor paralysis with mild sensory disturbances, and an acellular increase in the total protein content in the cerebrospinal fluid. The disease occurs worldwide and is the most common cause of neuromuscular paralysis. Cases have three dominant characteristics: the predilection to nerve roots, mononuclear infiltration of peripheral nerves, and eventual demyelination (primary axonal degeneration) (29). Severe cases tend to occur in the summer and have been linked to previous infection with *C. jejuni*, although other enteric pathogens may trigger the syndrome.

Some controversy exists regarding whether Guillain-Barré syndrome is an autoimmune disease. Although adequate data exist to classify the syndrome as an autoimmune disease (four major Rose-Witebsky criteria are almost completely met), the immunologic mechanisms at work in Guillain-Barré syndrome triggered by *C. jejuni* are likely to be complex (29-31). Studies of the relationship between Guillain-Barré syndrome and *C. jejuni* support the hypothesis of molecular mimicry, since peripheral nerves may share epitopes with surface antigens of *C. jejuni* (32). This has been supported by studies in which anti-GM1 IgG antibodies recognized surface epitopes on intact *C. jejuni*, and the reaction was strain-specific for certain Penner serotypes. There are inconclusive data with regard to Guillain-Barré syndrome and HLA, although some studies have shown a predilection for the HLA-B35 haplotype (29-31). Cytokines may be responsible for inducing the inflammatory process and probably play a role in the response leading to nerve demyelination. Complement has a role in the process leading to nerve damage, possibly through the production of activation products, which lead to an increase in the permeability of the blood nerve barrier, which perpetuates the inflammation. Although Guillain-Barré syndrome might be considered an autoimmune response, it also serves as an example of a disease with an infectious origin, a disease that entails the integrated actions of both humoral and cellular immunity.

Ciguatera poisoning is the most common foodborne disease related to the consumption of fin fish; this distinctive clinical syndrome is characterized by a plethora of gastrointestinal, neurologic, and sometimes cardiovascular features (33,34). Two toxins are involved in toxicosis. Ciguatoxin-1 (cig-1), the principal toxin, is a heat stable, lipid-soluble polyether that is not inactivated by heat, cold, or gastric juices, nor

eliminated by drying, salting, smoking, or marinating. Cig-1 induces membrane depolarization in nerve and muscle tissue by opening voltage-dependent sodium channels. A second toxin, maitotoxin, is water-soluble and opens calcium channels. The role of this second toxin in the pathophysiology of the disease is less well understood. The acute symptoms of the toxicosis are varied and include paresthesia of the extremities, circumoral paresthesia, reversal of hot and cold sensations, dental pain, myalgias, arthralgias, generalized pruritus, cranial nerve dysfunction, and dysuria. Severe acute symptoms require urgent care with parenteral atropine for bradyarrhythmias. Mannitol is often administered to counter the effect of the toxin on the sodium channels; however, the mechanism of action is unknown, and the therapy is useless after 24 hours. Many of these symptoms may remain chronic and are often misdiagnosed as chronic fatigue syndrome, brain tumors, or multiple sclerosis. The management of the chronic symptoms is frustrating for the patient and clinician. Interventions include amitriptyline, tocaidine, or mexilitine to modulate sodium channels in conjunction with calcium channel blockers such as nifedipine. Antidepressants such as Prozac also appear to be useful. Patients with chronic symptoms frequently report waxing and waning of symptoms. Activities such as sexual intercourse and drinking alcohol significantly exacerbate expression. Some women with chronic symptoms report worsening during menses. Mood levels, weather conditions, and dietary constituents often exacerbate symptoms. Some clinicians advocate a strict diet that avoids all seafood, fish byproducts, nuts, and alcohol, and in some cases, patients are asked to abstain from sex. One distinctive feature in this toxicosis is that one episode of ciguatera poisoning does not confer immunity. In fact, it is likely to sensitize the patient to otherwise subthreshold doses of toxin (33,34).

Amnesic shellfish poisoning is caused by domic acid, a conjugate of kainic and glutamic acid (35). In small quantities domic acid has an excitatory effect, but in large amounts it is neurotoxic. The toxicosis is first characterized by gastrointestinal symptoms followed by neurologic dysfunction. Severe cases may be prolonged and chronic; sequelae include confusion with disorientation, paucity of speech, lack of response to deep pain due to blocking of receptors in the spinal cord, autonomic nervous system dysfunction, seizures, abnormal ocular movements, grimacing posture, myoclonus, loss of reflexes, and coma. Other prominent chronic sequelae include loss of visual-spacial recall and mononeuropathies without dementia, mimicking Alzheimer's disease. The toxicosis is particularly serious in the elderly, and any deaths usually occur within this population. Valium, calcium channel blockers, phenobarbital, diazapam, thiobarbiturates, and hypothermia are treatments for patients with severe and chronic cases.

General Immunity, Organ Impairment, and Neurologic Disorders

Toxoplasmosis due to *Toxoplasma gondii* is a chronic latent parasitic infection (36,37). In humans the parasite exists in two forms: the tachyzoite, the rapidly multiplying stage that actively invades host cells and represents the principal form of the acute phase of the disease; and the bradyzoite, the form that multiplies very slowly in host cells, resulting in the formation of cysts that persist in tissues. Toxoplasma infection in humans is usually asymptomatic because of effective immunity involving antibodies, T cells, and cytokines. Activated macrophages, CD4 and CD8 lymphocytes, and the cytokines IFN-γ, TNF-α, and IL-6 play a major role in control of both the acute infection and maintenance or prevention of the chronic stage (37,38). Indeed, treatment with IFN-γ is used to control passage into the chronic stage, and treatment with anti-IFN-γ reactivates chronic infection (39). The production of nitric oxide may have opposing effects. Nitric oxide production protects against *T. gondii* and at the same time limits the immune response, probably contributing to the establishment of the chronic state (40).

The incidence of congenital toxoplasmosis is uncertain but may be as high as 9,500 cases a year (1). The percentage attributable to food is uncertain; however, consumption or contact with contaminated meat is more important as a cause than is contact with cats (1). Congenital impairments associated with maternal toxoplasmosis infection passed to the fetus include hearing loss, visual impairment (retinal lesions, strabismus), and slight to severe mental retardation. These impairments are still present in 80% of persons who reach the age of 20 years (1). Chronic toxoplasmic encephalitis may occur when a person's immune system is impaired. Indeed, toxoplasmic encephalitis marked by

dementia and seizures has become the most commonly recognized cause of central nervous system opportunistic infections in AIDS patients. Additionally, it appears that certain cancer treatments weaken the immune system, and old infections in the muscles can become reactivated, causing severe complications or death (1,41,42).

Helminth parasites can cause serious disease in infected persons (42). The impact of helminth infections is due less to the severity of the diseases they cause, than to the vast number of persons infected. For example, more than one billion people are infected with the largest intestinal nematode, *Ascaris lumbricoides*. Although there is usually no overt clinical sign of infection, disease can arise from an overwhelming infection or an inappropriate immune response. Additionally, infected persons frequently harbor more than one parasite for years. Most intestinal helminth parasites have direct life cycles, with no intermediate host or vector, and are transferred by contaminated food. Some species, such as *Trichuris* (whipworm) and *Enterobius* (pinworm), are restricted to the gut, but others, such as *Ascaris,* have tissue-migrating phases. All, however, induce a dramatic expansion of the Th2 lymphocyte subset. It remains unclear whether these Th2-derived responses (induction of interleukin-4 [IL-4] and down-regulation of IFN-γ), resulting in stimulation of IgG1 and IgE isotypes, eosinophilia, and mastocytosis are responsible for the immune-mediated pathologic response. Immunologic lesions may occur where early infection is associated with a strong T-cell proliferative response that becomes down-regulated in established chronic disease (evidence of a Th1 defect in the chronic disease). In ascariasis, an allergic response generated by the lung migratory phase (chronic immune sensitization) can cause pneumonia and, in animal models, spontaneous development of idiopathic bronchial asthma. A formative influence on the response of the immune system is the antigenic environment during pregnancy. Children born to infected mothers may have significantly higher susceptibility to the same infection later in life (42).

Viral agents induce autoimmune disorders, and one potential mechanism of induction is molecular mimicry (43,44). Hepatitis A virus infection is a well-recognized cause of acute hepatitis with jaundice in adults. In most affected persons, the course is usually relatively short-lived and benign, and symptoms are usually resolved within weeks. Occasionally, relapses occur after initial recovery, or recovery is marked by severe or prolonged cholestasis. However, even in these cases recovery is usual. Chronic sequelae of hepatitis A virus infections are rare and poorly defined; however, several recent studies suggest that hepatitis A virus infection triggers the onset of (idiopathic) autoimmune chronic active hepatitis within a genetically predisposed subgroup. Apparently, the chronic disorder may develop despite normal serologic response to hepatitis A virus infection. The triggering factors and mechanism of action remain ill-defined; however, in the most recent study, the authors concluded that hepatitis A virus infection may be the precipitating event in the pathogenesis of this disorder (45).

Metabolic activation and detoxification play a crucial role in determining the toxic response of humans to mycotoxin exposure. These highly toxic secondary metabolites are produced by a wide variety of molds including *Aspergillus, Fusarium,* and *Penicillium*. Mycotoxins exhibit properties of acute, subacute, and chronic toxicities with some molecules being carcinogenic, mutagenic, and teratogenic. Because mycotoxins are resistant to food processing and do not degrade at high temperatures, they enter the human food supply (46-49).

In many cases, the relationship between mycotoxins as the causative agent of disease in humans is difficult to determine. Acute effects of gastroenteritis may be easily identified; however, chronic effects often result from ingestion of low to moderate levels and can be difficult to recognize (46-50). The most threatening effects of ochratoxin A are its nephrotoxicity and carcinogenicity. Ochratoxin A is increasingly involved in an endemic nephropathy, a human chronic interstitial neuropathy that is usually associated with urinary tract tumors. Aflatoxins have been implicated in both acute and chronic liver disease in humans; however, other organs (kidney, spleen, pancreas) may also be affected (51). The best studied chronic effects are those induced by the fumonisins, zearalenone, and trichothecene mycotoxins produced by *Fusarium* sp. (49). Fumonisin levels in corn-based foods have been statistically associated with an increased risk of human esophageal cancer. Zearalenone is an estrogenic mycotoxin. Ingestion by animals, especially swine, causes hyperestrogenism with symptoms of enlargement and prolapse of the

uterus, atrophy of ovaries and testicles, enlargement of mammary glands, and infertility. This mycotoxin might add to the estrogen load of humans. Human consumption of trichothecene-contaminated foods causes acute symptoms of headaches, chills, severe nausea, vomiting, and visual disturbances, which may last 7 to 10 days. Since trichothecenes modulate immune function, over time mycotoxicosis could reduce immune resistance to infectious diseases, facilitate tumor growth through reduced immune function, and cause autoimmune disease (48).

Heart and Vascular Diseases

Several foodborne pathogens have been either directly or indirectly associated with endocarditis and myocarditis, and any heart damage appears to be permanent (2,52). Persons with ankylosing spondylitis linked to enteric pathogens as the trigger show a high incidence of cardiac conduction abnormalities, which may be sequelae to other seronegative arthropathies. A possible connection between foodborne gram-negative bacteria and atherosclerosis has been proposed, suggesting that the bacteria gain access to the lymphoid and general circulation with relative frequency. Endotoxin from degrading bacteria in macrophages may act in concert with the inflammatory factors (cytokines) induced by endotoxin from endothelium and smooth muscle cells. Although the process of atherogenesis is complex and involves many factors, the hypothesis is attractive and provides a model system for further study. Oxidative stress responses by *E. coli* and *S.* Typhimurium and the induction of the peroxide stimulon and the superoxide stimulon have also been recently implicated in atherosclerosis, rheumatoid arthritis, and inflammatory bowel disease (53).

Nutritional and Gastrointestinal Disturbances

Enteric pathogen-induced diarrhea may lead to a variety of conditions including loss of fluids, anorexia, and malabsorption of nutrients, all forms of malnutrition. The enteric pathogens that cause malabsorption and nutrient loss vary and include *Enterobacteriaceae*, Rotavirus, *Amoeba, Cryptosporidium,* and *Giardia* (2,54-58). Unless treated with antimicrobial drugs, many diarrheal episodes become chronic; however, the stress of even short periods of diarrhea may result in subtle changes in immunologic status. The extent of the diseases depends mostly on the immune status of the person and may last for several years or for life. Death due to diarrheal illness in the immunosuppressed and in persons with AIDS is nearly 80%. No effective treatment is available, although treatment with several antibiotics in combination shows promising results. However, AIDS patients may also develop further sequelae. *Cryptosporidium* are host-adapted, which may lead to pulmonary or tracheal cryptosporidiosis accompanied by coughing and frequent low-grade fever. In these cases there is no effective treatment. Similarly, in cyclosporidiosis, AIDS patients' enteric infection is chronic. Long-term prophylaxis with trimoxazole is required, and discontinuation of the treatment causes severe relapse (2,54-58).

Severe cases of diarrhea lasting months or years and characterized by dysentery, with foul-smelling, mucous bloody stools accompanied by flatulence and abdominal distention, may result from *C. jejuni*, *Citrobacter, Enterobacter,* or *Klebsiella* enteric infections. These infections always require extensive antibiotic therapy and usually result in failure to thrive. Enteric infections may alter bowel permeability which allows absorption of otherwise excluded food components. Proteins that can modulate the immune system can be absorbed possibly with deleterious consequences such as the induction of autoimmunity and atopy. Several studies of both human and porcine models indicate that significant quantities of unwanted proteins can be absorbed by damaged gut tissue and that maximum expression of diarrhea corresponds with peak protein uptake (2).

Helicobacter pylori is the undisputed cause of chronic gastritis. Environmental sources indicate that *H. pylori* can survive in water, chilled foods, milk, and fresh vegetables for several days because of fecal contamination. The species has never been isolated from these sources; however, infectious, viable but nonculturable (nonspiral coccoid) bodies may survive in fresh water for more than a year. *H. pylori* can be found in human feces and can be transmitted directly from person to person by the fecal-oral or oral-oral route. *H. pylori* can be found in several animal reservoirs; however, the possibility of animal-to-animal or zoonotic transmission is unknown (59,60). Ingestion of *H. pylori* leads to acute gastritis, and colonization of the stomach is virtually always accompanied by chronic inflammation that

disappears within 6 to 12 months after eradication of the infection. Infections are generally acquired during childhood or adolescence and result in chronic gastritis lasting for decades or life. On the basis of histologic and serologic follow-up studies, this chronic gastritis has been suggested as an important risk factor (odds ratio 9.0; p = 0.001) or first stage in a multistep process leading to gastric mucosal atrophy, intestinal metaplasia, and eventually gastric cancer (61,62).

Chronic Sequelae and Personality Changes

One area that has received scant interest is the effect of a chronic infection on human personality factors. Personality changes might be predicted: continual pain from arthritis, an irritable bowel, or chronic diarrhea would be enough to make anyone temperamental, moody, or depressed. Studies using Cattell's 16 Personality Factor questionnaire showed highly significant correlations (p < 0.01 - 0.002) between chronic toxoplasmosis and several personality factors (63,64). Men and women showed distinct differences in behavioral states. For men, low superego strength, protension, guilt-proneness, and group dependency were positively associated, whereas in women the related factors were affectothymia, alexia, untroubled adequacy, and self-sufficiency. A correlation of the intensity of the personality factor-shifts with the duration of the infection suggested that the infection per se induced the shift in personality, not vice versa.

An exploratory study using the 16 Personality Factor questionnaire and the Holmes and Rahe Social Readjustment Rating Scale, which measures stressful life events, was made of patients with rheumatoid arthritis (65). As a group, patients with rheumatoid arthritis exhibited higher stress at disease onset (p < 0.01); a large high-stress-at-onset subgroup of rheumatoid arthritis patients had a worse prognosis. Although there were important personality changes in the high-stress-at-onset-rheumatoid arthritis patients, the study concluded that the interaction between the variables that determined personality changes were very complex and could not simply be referred to as the "rheumatoid arthritis personality" complex.

Conclusion

Foodborne diseases are for the most part preventable; however, there is an inherent risk associated with the consumption of certain types of uncooked foods. Recognition by the public health community and the public that many foodborne illnesses may have serious chronic sequelae would help eliminate many illnesses and reduce health-care cost. Public health authorities could make a substantial impact by reducing poor or unhygienic food production or food-handling practices and by educating the public about how harmful microorganisms enter the food chain and how they can be avoided.

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Public, Animal, and Environmental Health Implications of Aquaculture

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Aquaculture is important to the United States and the world's fishery system. Both import and export markets for aquaculture products will expand and increase as research begins to remove physiologic and other animal husbandry barriers. Overfishing of wild stock will necessitate supplementation and replenishment through aquaculture. The aquaculture industry must have a better understanding of the impact of the "shrouded" public and animal health issues: technology ignorance, abuse, and neglect. Cross-pollination and cross-training of public health and aquaculture personnel in the effect of public health, animal health, and environmental health on aquaculture are also needed. Future aquaculture development programs require an integrated Gestalt public health approach to ensure that aquaculture does not cause unacceptable risks to public or environmental health and negate the potential economic and nutritional benefits of aquaculture.

U.S. Fisheries System

Coastal estuaries serve as a breeding ground and provide habitats for more than 75% of commercial landings and 80% to 90% of the recreational catch of fish and shellfish. From these habitats, hundreds of species of seafood are produced. Aquacultured species now contribute up to 15% of the U.S. supply (1,2). Wild species are harvested by 17,000,000 recreational anglers and nearly 300,000 commercial harvesters. Commercial harvesters deploy 93,000 vessels, while recreational fishermen have millions of recreational fishing boats. Nearly 5,000 domestic plants are located in every state throughout the United States, not just in the coastal areas (3). Current per capita consumption of commercially harvested species averages 15 pounds; it is estimated that per capita consumption of recreationally harvested seafood approaches an additional 3 to 4 pounds per person (4).

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The seafood business community—in the United States and in other industrialized countries—cannot rely solely on domestically produced stock. For a number of years, more than half of U.S. seafood consumption has relied on imported stock. Currently, the United States imports more than 50% of the consumed seafood, which originates in 172 countries around the world (3). This trend toward economic reliance on imported stock has steadily increased over the past 10 years so that now the United States is the world's second largest importer of seafood. The principal seafood imports are tuna, shrimp, salmon, lobster, and groundfish (3).

It is difficult to determine where imported fish was harvested. For example, the United States imports salmon from Switzerland and Panama, although neither Switzerland nor Panama is noted for vast salmon resources. U.S. participation in the international seafood trade is very complex, since in addition to being the world's second largest importer, the United States is also the world's second largest exporter of seafood (3). This dichotomy requires that U.S. marketing and import/export food control

inspection strategies be carefully planned. For example, the United States exports seafood to 162 countries, which has come about with the full development of northwest and Alaska fisheries and improved efficiency in processing techniques. Major U.S. exports are salmon, crab, surimi, fish blocks, groundfish, flatfish, shrimp, and lobster (3).

Current Aquaculture Status

In 1996, U.S. aquaculture production of nearly 227,000 metric tons consisted of baitfish, catfish, salmon, trout, clams, crawfish, mussels, oysters, fresh and saltwater shrimp, and miscellaneous species such as ornamental fish, alligators, algae, aquatic plants, tilapia, and hybrid striped bass. The United States exported principally rainbow trout, Atlantic salmon, tilapia, catfish, freshwater crawfish, and live mussels to 19 countries in Europe, North and South America, and Asia. Freshwater crawfish led the export seafood market at slightly over $8 million, with the other species accounting for less than $1 million each (3). The United States also imports large volumes of aquacultured products, approximately $2.5 billion in cultured products, primarily shrimp and salmon. Imported cultured seafood accounts for most of the current U.S. trade deficit for edible fishery products, which was approximately $3.5 billion in 1995.

The Safety of Seafood

Most seafood is safe; however, like all foods, it carries some risk. The food safety issues for seafood are highly focused, well-defined, and limited to a very few species. For seafood-borne illnesses (in which the cause was known) reported to the Centers for Disease Control and Prevention, more than 90% of the outbreaks and 75% of the individual cases were associated with ciguatoxin (from a few reef fish species) and scombrotoxin (from tuna, mackerel, bluefish, and a few other species) and the consumption of mollusks (mostly raw) (5-12).

Hazards associated with the consumption of all food (including seafood) can be categorized into three areas: product safety; food hygiene (clean vs. dirty plants, wholesome vs. unwholesome products); and mislabeling or economic fraud. Traditionally, the food safety risks of seafood products (aquacultured and wild-caught) have been subcategorized by environment, process, distribution, and consumer-induced risk; the environmental risk category is further subdivided into natural hazards (e.g., biotoxins) and anthropogenic contaminants (e.g., polychlorinated biphenyls) (13).

"Shrouded" Aquaculture Hazards

The future of aquaculture is bright; aquaculture products are as safe and wholesome as wild-caught species. However, in addition to the consumer hazards listed above, there are some less obvious "shrouded" public health hazards associated with ignorance, abuse, and neglect of aquaculture technology.

Technology Ignorance

A common practice in many developing countries is the creation of numerous small fish pond impoundments. However, this approach may have a greater adverse effect on human health than the creation of a single large impoundment (14). Small impoundments greatly increase the overall aggregate shoreline of ponds, causing higher densities of mosquito larvae and cercaria, which can increase the incidence and prevalence of diseases such as lymphatic filariasis and schistosomiasis, respectively. Centralized planning approaches for new freshwater and marine aquaculture sites should include discussions of the potential effect of large or small impoundments on such issues as disease transmission, water supply, irrigation, and power generation (14).

Ignorance of the microbial profile of aquaculture products can also affect human health as evidenced by the recent transmission of streptococcal infections from tilapia to humans, which resulted in several meningitis cases in Canadian fish processors (15). A change in marketing strategies to sell live fish in small containers, instead of ice-packs, resulted in human Vibrio infections from live tilapia in Israel in 1996. Such bacteria can be present in other aquacultured and wild-caught species in addition to tilapia.

Ignorance of the hazards associated with the use of untreated animal or human waste in aquaculture ponds to increase production also has tremendous human health implications (16). For centuries, food growers have cultured species in waste-water–fed ponds and grown secondary vegetable crops in waste water and sediment material in integrated aquaculture operations. However, the potential for transmission of human pathogens to cultured species and

secondary vegetable crops is rarely considered by fishery aquaculturists. For example, of more than 250 presentations at the 1997 World Aquaculture Society meeting held in Seattle, Washington, few referred to the potential human health implications of aquaculture (17).

The potential transmission of animal pathogens from exotic aquacultured species to wild-stock species also affects animal health. Recent outbreaks of taura, yellow spot, and white head viruses have occurred in aquaculture shrimp in South Carolina and Texas. Recent studies indicate that native wild white shrimp may also be susceptible to these exotic viruses (18).

Technology Abuse

Technology abuse includes the willful misuse of therapeutic drugs, chemicals, fertilizers, and natural fishery habitat areas. The widespread use and misuse of antibiotics to control diseases in aquaculture species is worldwide and will probably increase as aquaculturists move towards more intensive animal husbandry–rearing techniques and stocking densities. For example, the illegal use of chloramphenicol in shrimp culture to control diseases may result in violative levels in the harvested product. Similarly, the improper or illegal use of chemicals (e.g., tributyl tin) to control pond pests such as snails can also result in human health hazards. The abuse and misuse of raw chicken manure as pond fertilizer may result in the transmission of *Salmonella* from manure to the cultured product (16).

The destruction of mangrove areas to build aquaculture ponds can have a drastic impact on the survival of wild aquatic species through the degradation of essential fish habitats and nurseries. In Brazil, destruction of mangrove areas for shrimp ponds affected climatic changes to such an extent that the aquaculture operations have been terminated because consequent reduced rainfall resulted in excessive pond salinity (19).

Technology Neglect

The final "shrouded" hazard associated with aquaculture involves technology neglect, which includes such events as the abandonment of small aquaculture ponds in tropical countries, leading to increased mosquito habitats and concomitant increases in malaria (14). Facility management can be responsible for technology neglect if employees are not trained in the proper use and application of therapeutics and chemicals, for example. Finally, from an animal health perspective, ignorance or willful neglect of the International Council for Explorations of the Sea/European Inland Fisheries Advisory Commission Code of Practice for the Introduction and Transfer of Marine Organisms can result in the escape of exotic species and animal pathogens into the environment with a potential tragic impact on native aquatic species (20).

Health Control Considerations

Human Health

Procedures to help protect humans from aquaculture-associated risks include better education and training of aquaculture personnel on the proper use and storage of therapeutics and chemical compounds. Additional research on new more effective and, we hope, safer, antibiotic and vaccine treatment of aquaculture species is under way. Likewise, certain extralabel use applications for selected antibiotics are under consideration. Streamlined enforcement efforts are being developed to ensure compliance with new food safety regulations and new regulatory control procedures such as Hazard Analysis and Critical Control Points (HACCP) and the application of HACCP principles to animal and environmental control procedures (21,22).

The Food and Agriculture Organization and World Health Organization recommend that the HACCP concept be applied to fresh water aquaculture programs to control foodborne digenetic trematode infections in humans. Experiments are being carried out in Asia by a multidisciplinary team of experts in public health, parasitology, aquaculture, fisheries extension, and fish inspection (22). In one study in Vietnam, experimental activities were conducted in two side-by-side fish ponds. In the experimental ponds, fish were cultured in conjunction with HACCP principles, and control pond fish were cultured according to conventional local aquaculture practices (22). Water supply, fish fry, fish feed, and pond conditions in the experimental pond were identified as critical control points. The HACCP principles of hazard analysis, preventative measures, critical limits, monitoring, recordkeeping, and verification procedures relating to the critical control points were applied; study results showed *Clonorchis*

sinensis eggs and fish infected with the parasite metacercaria and the first intermediate host (*Melanoides tuberculata*) in the experimental ponds (22). Forty-five percent of control pond fish were infected with *C. sinensis* metacercaria, while white fish from the experimental pond monitored according to HACCP principles were completely free of trematode infection (22). Preliminary results indicate that application of HACCP-based principles to silver carp culture in North Vietnam is an effective way to prevent and control *C. sinensis*. Similarly, the application of these principles to fresh water aquaculture ponds in Thailand and Laos to control *Opisthorchis viverrini* infections has also been successful. Additional studies are recommended to confirm these preliminary results (22).

Animal Health

Procedures to safeguard animal health are set out in the International Council for Explorations of the Sea and the European Inland Fisheries Advisory Commission Codes of Practice, which describe how to prevent the adverse effects of introducing new and exotic species and emerging animal pathogens. Education and on-site training programs for aquaculture employees will help them understand the detrimental impact of introduction of exotic species and animal pathogens, misuse and abuse of therapeutics and chemicals, and willful habitat destruction. High priority issues also include implementation of biosecurity procedures in aquaculture operations to prevent the escape and spread of exotic species and pathogens into the facility and surrounding natural environment and the use of the HACCP principles to help control the spread of exotic pathogens to wild aquatic populations (17,23).

The application of HACCP principles to control transmission of exotic shrimp viruses from cultured to wild shrimp was proposed at a shrimp pathogen workshop held in June 1996 in New Orleans, Louisiana (21). Natural resource regulatory agencies are concerned about the possible transmission of exotic shrimp pathogenic viruses, recently found in shrimp aquaculture ponds in Texas and South Carolina, to wild native shrimp populations. The principles of HACCP, in conjunction with International Council for Explorations of the Sea and the European Inland Fisheries Advisory Commission Codes of Practice, were proposed to control the spread of exotic animal viruses into the environment. Shrimp aquaculture has the following proposed critical control points: pond site selection; water supply quality; pond management techniques; and transportation, especially as it relates to the live transport of aquaculture shrimp species (21).

Approximately 600 million pounds of shrimp are also imported for further processing into the United States on a yearly basis, half of which are aquacultured species (3). Natural resource managers, particularly at the state level, are concerned about the possible transmission of exotic shrimp pathogens into the environment from shrimp processing plant wastewater discharge and solid waste material landfill leakage. Proposed HACCP shrimp processing plant critical control points include unload/receive; de-ice/wash; thaw; dehead/peel/devein; wash; re-ice; de-ice/wash; re-ice; and dip/glaze (21).

Application of HACCP principles at aquaculture site and processing plant locations has the potential to control transmission of exotic human and animal pathogens. However, to our knowledge, except for the application of HACCP principles to control of human pathogens in Asia (17), no research has been conducted on this issue.

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Produce Handling and Processing Practices

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In the past decade, outbreaks of human illness associated with the consumption of raw vegetables and fruits (or unpasteurized products produced from them) have increased in the United States. Changes in agronomic, harvesting, distribution, processing, and consumption patterns and practices have undoubtedly contributed to this increase. Pathogens such as *Listeria monocytogenes, Clostridium botulinum,* and *Bacillus cereus* are naturally present in some soil, and their presence on fresh produce is not rare. *Salmonella, Escherichia coli* O157:H7, *Campylobacter jejuni, Vibrio cholerae*, parasites, and viruses are more likely to contaminate fresh produce through vehicles such as raw or improperly composted manure, irrigation water containing untreated sewage, or contaminated wash water. Contact with mammals, reptiles, fowl, insects, and unpasteurized products of animal origin offers another avenue through which pathogens can access produce. Surfaces, including human hands, which come in contact with whole or cut produce represent potential points of contamination throughout the total system of growing, harvesting, packing, processing, shipping, and preparing produce for consumption. Treatment of produce with chlorinated water reduces populations of pathogenic and other microorganisms on fresh produce but cannot eliminate them. Reduction of risk for human illness associated with raw produce can be better achieved through controlling points of potential contamination in the field; during harvesting; during processing or distribution; or in retail markets, food-service facilities, or the home.

Advances in agronomic, processing, preservation, packaging, shipping, and marketing technologies on a global scale have enabled the fresh fruit and vegetable industry to supply consumers with a wide range of high-quality produce year round. Some of the same technologies and practices have also introduced an increased risk for human illness associated with pathogenic bacteria, mycotoxigenic molds, viruses, and parasites. The use of manure rather than chemical fertilizer, as well as the use of untreated sewage or irrigation water containing pathogens, viruses, or parasites, undoubtedly contributes to this increased risk. Changes in the produce industry, social demographics, food consumption patterns, and awareness of fresh fruits and vegetables as potential vehicles of infection may also be contributing to an increase in documented produce-associated outbreaks of human illness.

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Changing factors that contribute to the epidemiology of diseases that may be associated with fresh fruits and vegetables were discussed by Hedberg et al. (1). Increases in foodborne illness during the summer are not fully understood, although fresh produce is likely to play a role since it is consumed in higher quantities during the summer. The per capita consumption of fresh produce has increased in the United States in recent years (Figure 1), not only in the summer but also in other seasons, partly because of increased importation. Knowledge of the presence and numbers of specific pathogens on produce imported to the United States from countries that may have lower sanitation standards is minimal. However, produce from a single grower, packinghouse, or shipper, whether located outside or within the United States, may be routinely distributed throughout the country, thus facilitating widespread dissemination of potential pathogens. The epidemiology of foodborne diseases is greatly influenced by these global changes. Control or

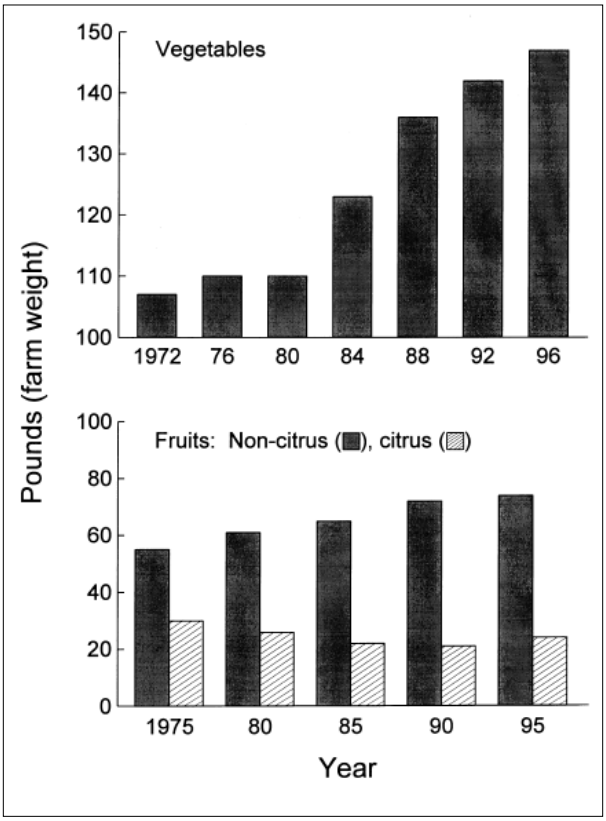

Figure 1. Per capita consumption of fresh fruits and vegetables in the United States. From USDA, Economic Research Service (2).

elimination of pathogenic microorganisms from fresh fruits and vegetables can be achieved only by addressing the entire system, from the field, orchard, or vineyard to the point of consumption.

We reviewed some of the practices (particularly preharvest practices) used by the fresh fruit and vegetable industry that may promote contamination of produce with pathogenic microorganisms.

Sources of Contamination

The presence of pathogenic bacteria, viruses, and parasites on fresh fruits and vegetables has been extensively documented (3). Contamination of produce can occur in the field or orchard; during harvesting, postharvest handling, processing, shipping, or marketing; or in the home (Table).

Preharvest Sources

Spores of *Clostridium* species, including *C. botulinum* and *C. perfringens,* as well as spores of enterotoxigenic *Bacillus cereus*, are commonly found in soil, so their occasional presence on fruits and vegetables should not be unexpected.

Table. Sources of pathogenic microorganisms on fresh fruits and vegetables*

Preharvest
Feces
Soil
Irrigation water
Water used to apply fungicides, insecticides
Green or inadequately composted manure
Air (dust)
Wild and domestic animals (including fowl and reptiles)
Insects
Human handling
Postharvest
Feces
Human handling (workers, consumers)
Harvesting equipment
Transport containers (field to packing shed)
Wild and domestic animals (including fowl and reptiles)
Insects
Air (dust)
Wash and rinse water
Sorting, packing, cutting, and further processing equipment
Ice
Transport vehicles
Improper storage (temperature, physical environment)
Improper packaging (includes new packaging technologies)
Cross-contamination (other foods in storage, preparation, and display areas)
Improper display temperature
Improper handling after wholesale or retail purchase

*Adapted from Beuchat (3)

Numbers of clostridial spores on some types of vegetables appear to increase during the summer (4). Perhaps the most prevalent disease-causing microorganism in soil is *Listeria monocytogenes* (5,6). Twenty-seven strains were isolated from soil and vegetation taken from 19 sites in the Netherlands (7). Plant materials from which the organism was isolated included dead and decayed corn and soybean plants and wild grasses, indicating its preference to exist in nature as a saprophyte. A study of soil and domestic animal feces has shown that *Listeria* is more often present during July to September than other months (8). *L. monocytogenes* and *L. innocua* were predominant in feces, whereas *L. ivanovi* and *L. seeligeri* were most common in soil.

Vegetation in a rural area in Virginia where clinical listeriosis is rare was analyzed

for *L. monocytogenes* (9). Dead soybean plant material and stalks, leaves, and tassels of corn were collected in April following the previous planting year. Eight of twelve sampling sites yielded plant materials positive for *L. monocytogenes*. Only 25% of the strains were pathogenic for mice, a low frequency compared with the percentage of pathogenic strains isolated from *Listeria*-positive humans and animals in Virginia and the United States as a whole. These observations suggest that the predominance of certain serotypes of *L. monocytogenes* may be influenced by the environment and that some strains indigenous to decaying plant vegetation are incapable of causing human illness.

Weiss and Seeliger (10) isolated 154 strains of *L. monocytogenes* in Germany from soil and plants, 16 from feces of deer and stag, nine from moldy fodder and wildlife feeding grounds, and eight from birds. Corn, wheat, oats, barley, and potato plants and soils from the fields in which they were growing were among the materials analyzed. Nearly 10% of the corn plants and 13% of the grain plants were infected with *L. monocytogenes*. Plants from cultivated fields had a lower incidence (12.5%) than plants from uncultivated fields (44%). Twenty-three percent of samples collected from wildlife feeding grounds were positive for *L. monocytogenes*. It was suggested that *L. monocytogenes* is a saprophyte that lives in a plant-soil environment and could therefore be contracted by humans and animals through many possible routes from many sources. Birds and animals are unlikely to be the only sources responsible for the distribution of *L. monocytogenes* in nature and its presence on fruits and vegetables.

The presence of other pathogenic bacteria, viruses, and parasites in soil likely results largely from application of feces or untreated sewage, either by chance or design. Whatever the case, soil on the surface of fruits and vegetables may harbor pathogenic microorganisms that remain viable through subsequent handling to the point of consumption unless effective sanitizing procedures are administered.

Irrigation and surface run-off waters can be sources of pathogenic microorganisms that contaminate fruits and vegetables in the field. Irrigation water containing raw sewage or improperly treated effluents from sewage treatment plants may contain hepatitis A, Norwalk viruses, or enteroviruses (poliomyelitis, echoviruses, and Coxsackie viruses) (11). Rotaviruses are known to retain viability on the surface of vegetables held at 4°C for up to 30 days (12).

Listeria and other potentially pathogenic bacteria have been reported in sewage. Watkins and Sleath (13) analyzed 52 sewage, river water, and industrial effluents for pathogens. Effluents were from abattoirs, cattle markets, and poultry packing plants. *L. monocytogenes* was isolated from all samples. In many instances, populations of *L. monocytogenes* were higher than those for salmonellae and, in some instances, *L. monocytogenes* was isolated when no salmonellae were detected. Application of sludge containing *L. monocytogenes* and salmonellae to soil showed that *L. monocytogenes* could survive longer. Populations of *L. monocytogenes* in soil remained essentially unchanged during 7 weeks after application.

Treatment of sewage does not always yield a sewage sludge cake or a final discharge free of *Listeria* (14). The use of sewage as a fertilizer could contaminate vegetation destined for human consumption. MacGowan et al. (8) examined sewage at 2-month intervals in 1991 to 1992 and found 84% to 100% contained *L. monocytogenes* or *L. innocua*.

Application of sewage sludge or irrigation water to soil is one avenue through which parasites can contaminate fruits and vegetables. *Ascaris* ova sprayed onto tomatoes and lettuce remain viable for up to 1 month, while *Endamoeba histolytica* could not be recovered 1 week after spraying (15,16). If sewage irrigation or night soil application is stopped 1 month before harvest, the produce would not likely be vectors for transmission of diseases caused by these parasites.

Wang and Dunlop (17,18) recovered *Salmonella, Ascaris* ova, and *Endamoeba coli* cysts from more than half of irrigation water samples contaminated with either raw sewage or primary-treated, chlorinated effluents. Only one of 97 samples of vegetables irrigated with this water yielded *Salmonella*, but *Ascaris* ova were recovered from two of 34 vegetable samples. Barbier et al. (19) concluded that application of sewage sludge containing *Taenia saginata* eggs offers a serious risk for cattle even after a 3-week no-grazing period.

Feces have been suspected as sources of pathogens on contaminated fruits, vegetables, or minimally processed produce that have subsequently been associated or confirmed as

causes of human disease outbreaks (3). Among the more recent outbreaks are those linking unpasteurized apple juice to *Escherichia coli* O157:H7 infections. This pathogen can remain viable in bovine feces for up to 70 days, depending on inoculum level and temperature (17). *Cryptosporidium* infection linked to consumption of unpasteurized apple juice was hypothesized to have been caused by contamination of apples by calf feces (20). Contact of fruits and vegetables by pickers and handlers at the time of harvest also offers a mechanism by which pathogens in feces can contaminate raw produce.

Wild birds are known to disseminate *Campylobacter* (21,22), *Salmonella* (22,23), *Vibrio cholerae* (24), and *Listeria* species (25). More recently, *E. coli* O157:H7 has been isolated from wild bird feces. In a survey of wild birds (mainly gulls), 0.9% of the bacterial isolates from fecal samples at an urban landfill and 2.9% of bacterial isolates from fecal samples on intertidal sediments were Vero cytotoxin-producing *E. coli* O157:H7 (26). Pathogenic bacteria are apparently picked up as a result of birds feeding on garbage, sewage, fish, or lands that are grazed with cattle or have had applications of fresh manure. Control of preharvest contamination of fruits and vegetables with pathogenic bacteria by wild birds would be exceptionally difficult.

Postharvest Sources

Some of the possible preharvest sources of pathogenic microorganisms may also be postharvest sources (Table). The fecal-oral route of transmission of pathogens broadens to include workers handling fruits and vegetables from the point of removal from the plant through all stages of handling, including preparation at the retail and food service levels and in the home. Changes in eating habits, particularly the increased consumption of meals away from home, must be considered when attempting to provide reasons for increased frequency of outbreaks associated with fresh produce. Proper training of food-service workers in hygienic practices is essential. One cannot assume that newly hired personnel have even rudimentary knowledge of food microbiology. This is particularly critical among teenagers who, partly because they and their parents are eating more meals away from home, have had minimal or no exposure to proper food-handling practices. Instruction in elementary principles of food hygiene at the high school or middle school levels has diminished greatly in the past two decades.

Traditionally recognized postharvest control points for access of pathogens to whole or cut produce include transport containers and vehicles and sorting, packing, cutting, and further processing equipment. The development of new processing equipment and technologies should include a team of experts in food microbiology as well as engineering. Too often, aspects of sanitizing equipment are not considered or are an afterthought and can increase the risk for contaminated end products. Temperature control is absolutely critical at every stage of postharvest handling if any success is to be achieved in minimizing the growth of pathogens.

Removal of Pathogens

Sanitizers that can be used to wash or to assist in lye peeling of fruits and vegetables are regulated by the U.S. Food and Drug Administration in accordance with the Federal Food, Drug and Cosmetic Act as outlined in the Code of Federal Regulations, Title 21, Ch. 1, Section 173.315. As noted by Barmore (27), no chlorine substitute effective for washing fruits and vegetables is available. Numerous alternatives for sanitizing equipment (28) can be used in a total sanitation program, but none has as broad a spectrum of activity as chlorine.

Chlorine is routinely used as a sanitizer in wash, spray, and flume waters used in the fresh fruit and vegetable industry. Antimicrobial activity depends on the amount of free available chlorine (as hypochlorous acid) in water that comes in contact with microbial cells. The efficacy of chlorine in killing pathogenic microorganisms has been extensively studied. Possible uses in packinghouses and during washing, cooling, and transport to control postharvest diseases of whole produce have been reviewed by Eckert and Ogawa (29). The effect of chlorine concentration on aerobic microorganisms and fecal coliforms on leafy salad greens was studied by Mazollier (30). Total counts were markedly reduced with increased concentrations of chlorine up to 50 ppm, but a further increase in concentration up to 200 ppm did not have an additional substantial effect. A standard procedure for washing lettuce leaves in tap water was reported to remove 92.4% of the microflora (31). Including 100 ppm available free chlorine in wash water reduced the count by 97.8%. Adjusting the pH from 9 to 4.5 to

5.0 with inorganic and organic acids resulted in a 1.5- to 4.0-fold increase in microbicidal effect. Increasing the washing time in hypochlorite solution from 5 to 30 minutes did not decrease microbial levels further, whereas extended washing in tap water produced a reduction comparable to hypochlorite. The addition of 100 ppm of a surfactant (Tween 80) to a hypochlorite washing solution enhanced lethality but adversely affected sensory qualities of lettuce.

Dipping Brussels sprouts into chlorine solution (200 ppm) for 10 seconds decreased the number of viable *L. monocytogenes* cells by about 2 $\log_{10}$ CFU/g (32). The maximum $\log_{10}$ reduction of *L. monocytogenes* on shredded lettuce and cabbage treated with 200 ppm chlorine for 10 minutes was 1.3 to 1.7 $\log_{10}$ CFU/g and 0.9 to 1.2 $\log_{10}$ CFU/g, respectively (12). Numbers decreased only marginally with increased exposure time from 1 to 10 minutes, which agrees with observations made by Brackett (32) that the action of chlorine against *L. monocytogenes* occurs primarily during the first 30 seconds of exposure. Nguyen-the and Carlin (33) concluded that the elimination of *L. monocytogenes* from the surface of vegetables by chlorine is unpredictable and limited.

Populations of *Salmonella* Montevideo on the surface and in the stem core tissue of tomatoes can be substantially reduced by dipping fruits 2 minutes in a solution containing 60 or 110 ppm chlorine, respectively; however, treatment in a solution containing 320 ppm chlorine does not result in complete inactivation (34). The ineffectiveness of 100 ppm chlorine against *S.* Montevideo injected into cracks in the skin of mature green tomatoes was demonstrated by Wei et al. (35). Treatment of alfalfa seeds injected with *Salmonella* Stanley (10^2 to 10^3 CFU/g) in 100 ppm chlorine solution for 10 minutes has been reported to cause a substantial reduction in population, and treatment in 290 ppm chlorine solution resulted in a substantial reduction compared with treatment with 100 ppm chlorine (36). Initial free chlorine concentrations up to 1,000 ppm, however, did not result in further reductions. Treatment of seeds containing 10^1 to 10^2 CFU/g of *S.* Stanley for 5 minutes in a solution containing 2,040 ppm chlorine reduced the population to less than 1 CFU/g.

We have studied the efficacy of chlorine, hydrogen peroxide, and ethanol in removing *Salmonella* from injected alfalfa sprouts. Sprouts were dipped in solutions containing 200, 500, or 2,000 ppm chlorine for 2 minutes. The pathogen was reduced by about 2 $\log_{10}$ CFU/g after treatment with 500 ppm chlorine, compared with the control, and to an undetectable level (<1 CFU/g) after treatment with 2,000 ppm chlorine (Figure 2). Chlorine treatment (2,000 ppm) of cantaloupe cubes injected with the same five-serotype cocktail of *Salmonella* resulted in less than 1 $\log_{10}$ reduction in viable cells (Figure 2). The very high level of organic matter in the juice released from cut cantaloupe tissue apparently neutralizes the chlorine before its lethality can be manifested.

As noted by Lund (37), the inaccessibility of chlorine to microbial cells in crevices, creases, pockets, and natural openings in the skin also undoubtedly contributes to the overall lack of effectiveness of chlorine in killing pathogens. The hydrophobic nature of the waxy cuticle on tissue surfaces protects surface contaminants from exposure to chlorine and other produce sanitizers that do not penetrate or dissolve these waxes. Surface-active agents lessen the hydrophobicity of fruit and vegetable skins as well as the surfaces

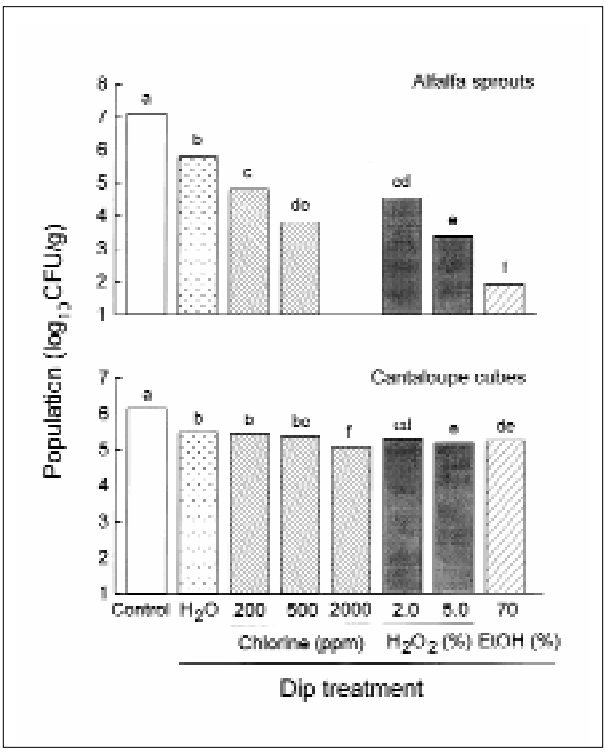

Figure 2. Efficacy of chlorine, hydrogen peroxide, and ethanol in killing *Salmonella* on alfalfa sprouts and cantaloupe cubes. Bars not noted by the same letter are significantly different ($p \leq 0.05$).

of edible leaves, stems, and flowers, but they may also cause deterioration of sensory qualities (31,38). Sanitizers that contain a solvent that would remove the waxy cuticle layer, and with it enmeshed contaminants, without adversely affecting sensory characteristics would hold greater potential than chlorinated water in reducing microbial populations on whole raw produce. Such sanitizers may be limited to use on produce that will be further processed into juice or cut products, or on whole fruits, vegetables, or plant parts destined for immediate consumption, since their application could adversely affect visual appearance. Clearly, chlorine, at concentrations currently permitted for use by the industry to wash fresh fruits and vegetables, cannot be relied upon to eliminate pathogens.

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The Impact of Consumer Demands and Trends on Food Processing

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In the United States, consumer demand for new foods and changes in eating habits and food safety risks are affecting the food processing industry. The population is becoming older on average; moreover, consumers want fresh and minimally processed foods without synthetic chemical preservatives. To address the need for safer food and compete for consumer acceptance, manufacturers are exploring new food processing and preservation methods.

Fresh, Preservative-Free Foods That Promote Health

Food industry marketers perceive that consumers want foods that are convenient; fresh (less-processed and less-packaged); all natural—with no preservatives (a so-called "clean label"); without a perceived negative (i.e., foods without high fat, high salt, and high sugar); and healthy. The industry perception is that consumers want foods that not only cause no harm but also remedy ailments from heart disease, osteoporosis, and fatigue to memory loss. Categories of foods that promote health are fortified foods, performance-enhancing food additives, probiotics, and prebiotics.

Food fortification is an old process. Milk (with vitamins A and D), bread (with iron and niacin), or salt (with iodine) have long been fortified to replace nutrients thought to be lost during processing. Newer foods fortified with nutrients needed by the body to stave off the progression of diseases associated with aging or enhance physical performance attract the consumer's attention and sell well in today's marketplace. For example, marketers are promoting all sorts of foods fortified with calcium to women concerned about osteoporosis. Performance-enhancing foods are popular. Such foods range from beverages to replace electrolytes and prolong physical endurance to amino acids and fatty acids to improve alertness and memory. Probiotics and prebiotics are two paths to the same result. Research studies suggest that a desirable intestinal microflora causes the host to be less susceptible to intestinal pathogens. Probiotics create this desirable state by incorporating the microorganism directly into the food, either as a stable culture or as part of food fermentation. This process is costly, and the microorganisms often do not survive well in the food. Thus, manufacturers must add 10 to 100 times the needed number of microorganisms to account for a loss of viability during the product's normal shelf life. Prebiotics overcomes the limitations of probiotics by adding specific nutrients, usually a particular carbohydrate, to the food. When ingested as part of the diet, these specific nutrients "select" for a beneficial microflora in the intestinal tract.

Food Processing and Food Product Development

The consumer's quest for health is having a great impact on the food processor. Compared with the marketplace of 25 years ago, today's marketplace has more perishable products, including fruits and vegetables, and more innovative packaging. In addition, consumer aversion to traditional chemical preservatives has left food processors with less flexibility in choosing preservation methods. To find a technologic edge in the marketplace, food processors are exploring new processing and preservation technologies. Some of these technologies include ohmic heating, high-pressure, pulsed electric field, bright light, and aseptic processing. Ohmic heating involves passing an electric current through the food to create heat due to electrical resistance within the food. With

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ohmic heating, food particles heat at the same rate as the carrier medium or sauce. Ohmic heating can enhance food quality by limiting heat damage to the sauce and food particles. High-pressure processing uses very high pressure, often thousands of atmospheres, to pasteurize foods without heat. This technology is ideal for heat-sensitive foods, but some enzymes are difficult to inactivate with high-pressure processing. Pulsed electric field processing uses a very strong pulsed electric current to disrupt microbial cells and pasteurize foods with little or no heating. Bright light processing uses an intense white light to kill bacteria on the surface of foods; this light does not penetrate deeply into foods and can only be used for surface pasteurization.

Aseptic processing dates back to at least the mid-1940s but has yet to realize its full potential. The most widely used of these new technologies, aseptic processing involves sterilizing a food product in a continuous process through a heat exchanger and then filling that food in an aseptic filler. The aseptic filler is a highly specialized piece of equipment designed to sterilize the packaging material, fill the sterile product into its container in a sterile environment, and then seal the package.

Food processors have also explored novel food preservation systems. An ideal food preservative would come from a natural source and preserve food without being labeled a synthetic chemical preservative. Such preservatives include bacteriocins, dimethyl dicarbonate (Velcorin), competitive microbial inhibition, controlled and modified atmospheres, and irradiation. Bacteriocins are not new; however, like nisin, they are now being used to extend shelf life and enhance the safety of a variety of food products. The use of bacteriocins is likely to be expanded in the future. Dimethyl dicarbonate, a relatively new preservative used in beverages such as wine, tea, and juices, is particularly effective in preventing spoilage caused by yeasts. Competitive microbial inhibition relies on the fact that many harmless bacteria, notably lactic acid bacteria, can inhibit the growth of both spoilage bacteria and pathogens. Inhibitory strains of lactic acid bacteria can be selected for use in dairy cultures or be added to refrigerated foods to extend shelf life and enhance safety. Modified and controlled atmosphere packaging are already widely used by the food industry. They have the potential for even wider use, particularly with fresh fruits and vegetables sold at retail. These methods rely on inhibiting microbial growth by excluding oxygen or by inhibitory concentrations of carbon dioxide. Carefully selected gas mixtures can also delay the ripening of certain fruits and vegetables and extend the shelf life of fresh meats. Finally, irradiation, also not a new technology, is poised for widespread use to enhance the safety and shelf life of many foods. With proper controls, irradiation could be a valuable means of reducing Salmonella contamination of poultry and *Escherichia coli* O157:H7 contamination of ground beef.

A Scientist's View of Consumer Trends

One of the most obvious consumer trends is a dramatic increase in the consumption of fresh foods, particularly fruits and vegetables. This increase is the result of the well-publicized value of a high-fiber diet and betacarotenes as an aid in preventing colon cancer. The number of meals eaten away from home has increased dramatically. The trend toward dining outside the home is likely rooted in lifestyle changes such as households with two working parents. The number of home-delivered meals, the ultimate convenience food, has also increased, even though the most popular foods consumed today (pizza and hamburgers) are generally the same as those of 20 years ago. This indicates that the types of foods consumed do not change rapidly, but the way these foods are consumed has changed. Finally, the population is getting older on average. Aging may not be a consumer trend, but it has a profound effect on food safety considerations. An older population means a more susceptible population.

New food processing and preservation technologies and wider applications of older technologies have, for the most part, had little impact on most processed foods. Adoption of new technologies will likely continue at a slow pace. Consumers consistently buy foods on the basis of value and taste, not processing technology. Technologies that add value will be the first to gain consumer acceptance. The demand for convenience foods will probably increase. Demands on our time are increasing, and we have less time to spend on food preparation, and more meals will be eaten away from home, in part because of convenience but also because of a trend for new tastes and variety in the diet.

Finally, the trend toward foods that claim to enhance performance, rooted in an aging population's need for better health during longer life-spans, will continue. With increased demand, the pressure on the food industry for better processing and preservation methods will also increase and may result in safer food.

Impact of Changing Consumer Lifestyles on the Emergence/Reemergence of Foodborne Pathogens

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Foodborne illness of microbial origin is the most serious food safety problem in the United States. The Centers for Disease Control and Prevention reports that 79% of outbreaks between 1987 and 1992 were bacterial; improper holding temperature and poor personal hygiene of food handlers contributed most to disease incidence. Some microbes have demonstrated resistance to standard methods of preparation and storage of foods. Nonetheless, food safety and public health officials attribute a rise in incidence of foodborne illness to changes in demographics and consumer lifestyles that affect the way food is prepared and stored. Food editors report that fewer than 50% of consumers are concerned about food safety. An American Meat Institute (1996) study details lifestyle changes affecting food behavior, including an increasing number of women in the workforce, limited commitment to food preparation, and a greater number of single heads of households. Consumers appear to be more interested in convenience and saving time than in proper food handling and preparation.

Reporting of foodborne and waterborne diseases in the United States began more than 50 years ago (1). At that time, state and territorial health offices were concerned about the levels of morbidity and mortality caused by typhoid fever and infantile diarrhea; cases were to be investigated and reported. The underlying purpose of reporting was to obtain information regarding the role of food, milk, and water in outbreaks of intestinal illness to provide a basis for public health action.

In 1923, the Public Health Service began publishing summaries of outbreaks of gastrointestinal illness attributed to milk; in 1938, it added summaries of outbreaks due to any foods. In 1966, the present system of surveillance of foodborne and waterborne diseases began to incorporate into an annual summary all reports of enteric disease outbreaks attributed to microbial or chemical contamination of food or water. Comprehensive surveillance should result in greater awareness of the most important food-protection methods.

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Between 1983 and 1987, the etiologic agent in foodborne disease outbreaks was not determined in 62% of the outbreaks (2); between 1988 and 1992, the foodborne disease was of unknown etiology in 59% of the outbreaks (1). Bacterial pathogens caused the largest percentage of outbreaks (79%) when etiology was known—Salmonella caused 69% of bacterial outbreaks. For each year from 1983 through 1992, the most commonly reported food preparation practice that contributed to foodborne disease concerned improper holding or storage temperatures. The second most common practice was poor personal hygiene of the food handler. Food from unsafe sources was the least commonly reported factor in each of the 10 years of reporting. It is now time to examine food handling and determine how to reverse the trend.

Foodborne disease surveillance has traditionally served three purposes. The first is disease prevention and control. Prevention and control measures include early identification and removal of contaminated products from the commercial market and correction of faulty food-preparation practices in both food-service establishments and the home. Surveillance also provides knowledge of disease causation. The responsible pathogen is not identified in more

than half of the foodborne disease outbreaks for various reasons, including late initiation of laboratory investigation, inability of available technology to identify the pathogen, and lack of identification of the pathogen with a particular food. Finally, surveillance assists in administrative guidance. Information enables assessment of trends in prevalence of outbreaks caused by specific etiologic agents and in vehicles of transmission. This information assists in identifying common errors in food handling. In July 1995, the Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), and Food Safety and Inspection Service (FSIS) began a comprehensive effort to track major bacterial pathogens that cause foodborne illnesses (3). CDC provides the overall management and coordination with state health departments in the five survey sites of the FSIS/CDC/FDA Sentinel Site Study. The program actively seeks out specific cases of foodborne illness to identify whether a food was of concern and to better establish frequency and source of foodborne disease outbreaks and cases. CDC will use the data to identify emerging foodborne pathogens and monitor incidence of foodborne illness; FSIS will use the data to evaluate the effectiveness of new food-safety programs and regulations to reduce foodborne pathogens in meat and poultry; FDA will use the data to evaluate its efforts to reduce foodborne pathogens in seafood, dairy products, fruits, and vegetables.

According to a recent report to Congressional committees (4), experts believe that the risk for foodborne illness is increasing. The food supply is changing in ways that can promote foodborne illness, and there are no comprehensive data to explain at what point pathogens are introduced into food. Further, because of demographic changes, more people are at a greater risk of contracting a foodborne illness.

According to Ollinger-Snyder and Matthews (5), changes in agricultural practices, a growing population susceptible to infectious diseases, lifestyle changes, the emergence of new foodborne pathogens, and the high turnover rate reported for workers in the food-service industry indicate that new approaches are needed to allay consumers' fears and to prevent the spread of foodborne disease in the United States. They recommend implementation of Hazard Analysis and Critical Control Points (HACCP) systems and certification of food-service managers.

The food processing industries are developing and implementing HACCP systems; the meat and poultry industries are mandated to do so beginning in January 1998 (6). Hazard analysis has been defined as the identification of sensitive ingredients, critical processing points, and human factors that affect product safety. Critical control points have been described as processing determinants whose loss of control would result in an unacceptable food-safety risk.

Most contend that the HACCP system approach must be implemented at each stage of the farm-to-family continuum. Where are the critical control points and the HACCP system development in the home, food-service or retail establishments, or the car when food is carried from one location to another? The consumer is a complex and critical control point in the process.

Take the case of the barbecued chicken served to 260 guests at an outdoor barbecue in 1983. Guests were served chicken that was parboiled in the morning by one set of cooks and then placed in a large container and refrigerated. The evening cooks assumed the chicken had been adequately cooked, so they basted it in barbecue sauce and warmed it over the fire. Some 71% of the guests got sick from the chicken that was insufficiently cooked and improperly held (5). What of the infected bakery worker who stirred a vat full of buttercream frosting with a bare hand and arm? Some 5,000 cases of viral gastroenteritis were caused by the infected worker who claimed he had washed his hands. Other more recent outbreaks (7) appear in Table 1.

Recent data (1) indicate that 80% of reported foodborne illness outbreaks occur outside the home. Even though illnesses would be expected to be reported more often when they occur as a result of eating in restaurants, the numbers are large. National standards for restaurant safety are contained in the Food Code (8). FDA has the legal authority to impose the standards on state and local jurisdictions. The Food Code, which is updated every 2 years, includes temperatures for cooking, cooling, refrigeration, reheating, and holding food in food-service establishments. County or city employees are generally charged with responsibility for inspecting restaurants; each state or locality has its own laws governing restaurant safety.

Table 1. Foodborne illness reports from restaurants, 1996

Date	Description	Cause
6/96	Salmonella-contaminated food, 38 cases	Employees did not wash hands before handling food
9/95	*Escherichia coli* O157:H7 "beef," 11 cases	Raw food cross-contaminated other
8/95	*Salmonella* Newport "chicken," >850 cases	Raw meat on cutting board with vegetables
1/95	Hepatitis A, contaminated food, 95 cases	Human fecal matter from handling—handwashing
8/94	Salmonella, hollandaise sauce, 56 cases	Holding temperature too low for 9 hours
1/93	*Clostridium botulinum*, canned cheese sauce, 7 cases, 1 death	Unrefrigerated storage of opened container

Source: Center for Science in the Public Interest, 1996

Food service outside the home is big business, with sales of more than $300 billion (9) and nearly 10 million employees. The restaurant industry's share of the food dollar is 43%, and the typical consumer more than 8 years of age had more than four meals per week away from home in 1996. Given those statistics, it is clear that food-service establishments play a critical role in food safety. The Center for Science in the Public Interest (7) conducted a survey of 45 agencies across the country to determine if state and local agencies were enforcing 12 key food-safety standards in the FDA Food Code. The standards chosen for the study affect consumer health and safety and include such areas as food cooking and refrigeration temperatures, frequency of inspections, and consumer warnings for raw foods. Not one of the 45 agencies surveyed was following all of the Food Code recommendations.

In the survey, only 13% of agencies enforced the Food Code and recommended cooking temperatures for pork, eggs, fish, and poultry; only 64% of agencies required hamburgers to be cooked to 155°F. Recommendations for cooling cooked food were followed by only 20% of the agencies, and only 11% required refrigeration of food at FDA-recommended temperatures.

Every restaurant can take steps to ensure the safety of the food it prepares and serves to its customers. Continuous employee training and institution of HACCP-type systems should assist restaurants and other food-service institutions in improving their food-safety records. Programs available through the national restaurant trade organization could assist even the smallest establishments in achieving food-safety goals.

For more than 25 years, the Food Marketing Institute (10) has surveyed consumers about their changing needs and priorities in food attitudes and behavior. The 1996 trends report has an expanded focus on the primary grocery store or supermarket, including questions to help retailers learn more about take-out foods (Table 2). In 1996, nearly 40% of the 2,000 shoppers surveyed purchased fresh deli items from their primary supermarket at least once per week, and more than 10% reported purchasing ready-to-eat take-out foods as frequently. Three-fourths of these shoppers purchased food from the deli at least once per month, and half bought take-out food from the supermarket as often.

According to the survey, fast-food restaurants dominate (48%) all food outlets as the primary source of take-out food; only 12%

Table 2. Sources of take-out food (%)

Source	86	87	88	89	90	91	92	93	94	95	96
Fast-food restaurant	43	44	41	41	46	51	55	46	46	41	48
Restaurant	38	33	38	33	27	23	24	27	25	22	25
Supermarket	10	9	11	12	14	14	12	15	15	17	12
Deli/pizza parlor/bagel shop/coffee shop/donut shop	*	*	*	*	*	*	*	*	*	8	4
Gourmet or specialty shop	*	*	*	*	*	*	*	*	*	*	3
Convenience store	*	*	*	*	2	2	2	2	2	2	1
Some other place	2	7	3	6	2	1	*	1	1	1	2
It varies	1	1	1	3	4	5	3	5	3	2	0
Don't eat out	*	*	*	7	6	4	4	4	4	3	2
Not sure	3	3	4	1	*	1	1	1	2	3	2

*Data not collected for this year.
Source: Food Marketing Institute, 1996

purchase take-out foods from the supermarket. A recent article in Food Processing Magazine (11) states, "Somewhere on their way to the supermarket, consumers have been getting lost." Home-meal replacement, ready-made meals approximating what Mom used to make, have begun to rapidly compete for the food dollars of time-pressed consumers. According to Hollingsworth (12), consumers are eating more meals at home, but they are not cooking more. Consumers want to get food in a take-out location and go home to eat it (Figure).

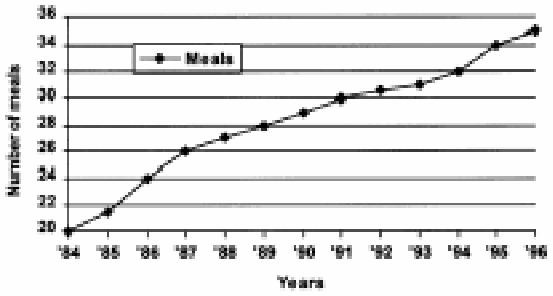

Figure. Annual meals (including snacks) purchased at commercial restaurants per person and consumed at home.

These take-out or eat-at-home foods have built-in food-safety hazards. Consumers are time-pressed, and they are buying these foods. Are they treating them as perishable? The U.S. Department of Agriculture (13) has expressed concerns about these foods; they say that take-out foods need to be handled with care. Hot foods need to be picked up or received hot and eaten within 2 hours. If eaten later, hot foods should be divided into shallow containers, covered loosely, and refrigerated immediately.

Are consumers ready for all of this food handling? Most consumers are confident that the food they purchase is safe to eat (10). Spoilage of foods is considered the greatest threat to food safety by the largest group (49%) of respondents. They count on freshness and expiration dates (22%) and increasingly see bacteria and contamination as threats (17%). It is interesting to note, however, that between 1992 and 1996 these shoppers were less likely (15% vs. 7%) to see spoilage as a threat; similarly, processing and preparation of foods was less an issue in 1996 than in 1992 (8% vs. 10%).

Consumers are concerned about handling of foods by other shoppers and by supermarket employees. Consumers rely increasingly on food stores (16%), manufacturers (21%), government (21%), and themselves (25%) for food-safety protection. Consumers apparently are willing to share responsibility for food safety with others, but they want to know that steps are taken during the processing and distribution of foods to reduce the likelihood of pathogen or other bacterial contamination.

According to Technomics (14), these supermarket issues noted in the Food Market Institute trends data (10) will be shared with food-service operators as the share of consumer food expenditures changes from 51% vs. 49%, 48% vs. 52%, and 45% vs. 55% (projected) for retail expenditures versus food-service expenditures in 1991, 1996, and 2001, respectively.

The number of households earning more than $75,000 annually continues to grow, and these households exhibit the highest levels of spending on food service. Consumer demands are changing the way that food-service operators and suppliers of food services must react. The area of convenience, highly prized by consumers today, has profound implications for food. Consumers want fast service with easy-to-eat foods and no stress, which means a far greater emphasis on portable foods.

Technology has conditioned us to demand and receive near-immediate satisfaction. There will be even greater emphasis on faster service, meaning more emphasis on convenient food formats to expedite preparation. Packaging and storage will greatly affect product quality and safety. According to Technomics (14) packaging will need to be temperature-tolerant and breathable. Preparation and processing technologies will need to have greater ability to rapidly cool and chill. And then there is the food-safety concern associated with dispensing equipment. Food will be required to have an extended shelf life. The safety factors associated with these new formats will also change.

Consumers want easy access to portable foods. Accessibility to variety in food options translates to a proliferation in nontraditional locations. These smaller sites may include back-of-house preparation facilities. This easy access to smaller operations also suggests a need for more of such operations and more variety in

menu options. While to the consumer this may translate to upscale menus with indulgence foods such as new and different bakery items, microbrewery beverages, and gourmet coffees, to the food-service operator it may mean greater cross-contamination with cream fillings, unpasteurized fermented drinks, and spoiled milk. New menu options create new challenges for service and for safety.

According to Steve Harrison, brewmaster of the Sierra Nevada Brewing Company (Chico, CA), "The concept that a beer will automatically go bad in 'X' number of days is a very untrue one." Consumers do not know that. What is "skunky beer"? Starting in late 1996, Anheuser-Busch began a freshness strategy in their advertising. Other large brewers are catching on, so freshness is associated with quality and safety. Imagine freshness dating, "born-on dating," as a quality parameter in brewing.

Consumers' increased emphasis on food-safety issues directly affects food service. The perceived healthfulness and quality of foods affects food sales; the increasing considerations of cleanliness as healthfulness and quality as safety become even greater shared responsibilities as food-service operators take over the roles historically associated with home kitchens. "On-the-spot exhibition" cooking is of increasing interest to today's consumers.

In June 1996, the Food Marketing Institute (15) published a review of foodborne illness. They note that the organisms that cause foodborne illnesses are found throughout nature and that mishandling and poor refrigeration are responsible for most contamination. The most common causes are cross-contamination of cooked foods with raw foods, contaminated utensils or serving plates, poor hygiene of food handlers, and time or temperature abuse.

Agreement is widespread that the most serious food-safety problem is foodborne illness of microbial origin (Table 3). Foodborne pathogens include a wide array of microorganisms, which have various physiologic effects on people, ranging from mild to severe, and are associated with a wide array of foods. Cross-contamination and association of foods within mixed dishes complicate environmental control. Further, some of the microbes have evolved and become more resistant to food preparation and storage techniques. Several industry and government publications (1,2,8,15,16) summarize biologic hazards associated with foodborne illness.

Mishandling can occur at any point in the food chain—in processing, at supermarkets or restaurants, or in homes. Many food manufacturers and retailers have HACCP plans in place, and over the next few years that number will increase. Consumers, however, must assume responsibility for the safety of food in the home. Proper preparation and sanitation methods are key to preventing foodborne illness in the home as in other areas of food handling. The messages for each of the segments of the food chain are the same—keep it clean (e.g., wash your hands) and control the temperatures (keep hot things hot and cold things cold) (Table 4).

For the food-service industry, a number of programs have been developed to educate food

Table 3. Sources of reported outbreaks with confirmed causes (%)

	Restaurant		Other Known Place		
	1983-87	1988-92	1983-87	1988-92	1996
Salmonella	50	60	46	58	30
Escherichia coli	<1	<1	2	1	5
Hepatitis A virus	6	7	3	4	
Staphylococcus aureus	2	3	10	5	
Campylobacter	1	2	6	3	45
Shigella	8	2	6	2	17

Sources: Centers for Disease Control and Prevention and U.S. Department of Agriculture for 1996, the 1996 Sentinel Site Study

Table 4. Pathogen control in foods to reduce foodborne illness

Pathogen	Control mechanism
Campylobacter	Heat foods ≥ 140°F
	Proper handling
Salmonella	Rapid chilling <40°F
	Hot storage >140°F
	Cooking >165°F
Escherichia coli O157:H7	Heat foods >155°F
	Avoid cross-contamination
Staphylococcus aureus	Rapid cooling <40°F
	Personal hygiene
Clostridium botulinum	Boil food 10-15 minutes
Clostridium perfringens	Refrigerate <40°F
	Proper handling
Listeria	Pasteurization of milk
	Adequate cooking

Source: Food Marketing Institute, 1996

handlers about food-related and personal behaviors that affect the safety of foods. For example, the Food Marketing Institute (17) has a Food Protection Certification Program for supermarket personnel to learn about the FDA Food Code requirements regarding food handling and hygiene. Similarly, the National Restaurant Association has developed a food-safety program called Serve Safe, intended to educate food-service workers about safe food handling.

Who or what teaches the average consumer about food safety? Common sense? Family? Health and fitness magazines? In May 1996, the Food Marketing Institute (17) conducted a series of consumer focus groups to establish the importance of food safety to consumers and to identify barriers to consumers' safe food purchase, handling, and preparation. They report that how consumers manage food safety reflects years of conditioning, observation, and reinforcement from mothers and grandmothers. In some cases, the more often consumers shop, the less concerned they seem to be about food safety when it comes to shopping, storage, and handling. Consumers link safety to fresh food, and they assume that when they shop more often, they purchase food in smaller quantities and food safety is less an issue. Respondents in the study also tended to think that cooked food was generally "safer" than raw food. For example, they believed that recontamination of unrefrigerated food was less a problem with cooked than with raw food.

Some safe food practices are observed for convenience, esthetics, or taste rather than for food safety. Thawing meat is messy; covering food prevents it from drying out; separating foods in the refrigerator is tidier. These kinds of behavior improve safety, but consumers may not understand the food-safety implications.

Overall, the consumers in the Food Marketing Institute study (17) find food-safety messages generally are "common sense," "basic," "practical," and "believable." Messages about such subjects as the order in which to choose foods in the supermarket, sell-by dates, storage and freezing of products, ways of keeping hot foods hot and cold foods cold are not considered too elementary. They also believe that storage times for food safety do not apply equally across food groups; they do not understand hazards from vegetables or fruits. Barriers to safe food-handling behavior in this study included historical (and cultural) practices, feeling of invulnerability, taste preferences, timing and planning, and space and convenience.

A 1992 survey conducted at Cornell University and designed to assess consumer food-safety awareness documented a substantial lack of knowledge about safe home food preparation practices. Seventy-five percent of those surveyed knew that Salmonella is associated with meat, poultry, and eggs, but only 65% would refrigerate a roasted chicken breast immediately; 29% would leave it on the kitchen counter until it reached room temperature. Further, 18% said they would not be concerned or were not sure about the safety of cooked meat left unrefrigerated for more than 4 hours; 14% said the same for cooked poultry.

In April 1996, the American Meat Institute (16) commissioned a study of 1,000 adults in the United States. Compared with 98% of respondents in the study who know that harmful bacteria can be present on meat and poultry products, only 74% made the link to dairy products and eggs; two in five respondents (43%) recognized that fruits and vegetables may contain harmful bacteria. These conclusions could be drawn for consumers who responded to the American Meat Institute (1996) questions. While the U.S. population is growing (up 10% since 1980), households are becoming smaller. In the 1980s, the number of households grew 17%, while the average household size decreased from 2.8 to 2.6 persons. This shift in family size and the increase in single heads of households has resulted in increased stress in the family with less time for shopping and food preparation. In addition, more women are in the workforce. Today, 70% of women ages 25 to 44 years are in the workforce; 75% work full time. Therefore, no adult is likely to be in the home for 70% of American households, and many children are preparing food for themselves. Finally, consumers spend less time on food preparation. More than 85% of employed women shop and cook, but most spend less than 30 minutes preparing every meal and 20% spend less than 15 minutes. Consumers are using convenience foods and quick methods of food preparation, including partially cooked foods that may require special handling.

The study results provided further documentation that the risk for foodborne illness is increasing, largely because of societal changes that affect the way consumers purchase and

prepare food. Contributing to this are changes in the family structure, more women in the workforce, and less available time for food preparation. Consumers in this study were not able to correctly separate home preparation issues from food service, nor did they know correct cooking temperatures to use in their own homes.

The ways in which consumers spread microorganisms to one another and to themselves include more than just coughing and sneezing. Not washing hands before, during, and after handling foods clearly contributes to the spread of foodborne infections and intoxication. Hands can spread disease-causing microbes to foods from other foods and from infected persons.

In a comprehensive review of 91 scientific articles published after 1986, Bryan et al.(18) attempted to link hand washing and infections. They report that hand washing has become an integral component of the tradition and ritual of prevention practice for the spread of infection, but several factors confound the ability to establish the effectiveness of hand washing for reducing infectious disease. Hand-washing practices were shown to significantly reduce infections transmitted by the fecal-oral route and in situations of poor personal hygiene. Hand washing is clearly a critical step in reducing personal contamination of food and cross-contamination between foods. Hand washing is but one practice that could dramatically affect risk, if not incidence, of foodborne disease.

According to data provided by the American Society for Microbiology (19), people do not wash their hands as often as they think they do (Table 5). In telephone surveys, 94% of respondents claim they always wash up after using the rest room; however, researchers contend that almost one-third of people do not wash their hands after using the bathroom. Of the more than 7,000 people nationwide who participated in the study, 81% said they wash their hands before handling or eating food. However, most say they do not wash up after petting an animal (48%), coughing or sneezing (33%), or handling money (22%).

Table 5. American Society for Microbiology/Bayer hand-washing survey, 1996

Behavior/Location	What they say[a] (%)	What they do[b] (%)
Wash hands:		
After using public restroom	94	68
Women		74
Men		61
New York (Penn Station)		60
Chicago (Navy Pier)		78
New Orleans (casino)		71
San Francisco (Golden Gate Park)		69
Atlanta (Braves game)		64
Women		89
Men		46

[a]1,004 adults; [b]6,330 adults
Source: American Society for Microbiology

In early 1997 (8), the U.S. Departments of Agriculture and Health and Human Services and the U.S. Environmental Protection Agency developed a program intended to coordinate a food-safety initiative among federal agencies, immediately after an announcement by U.S. President Clinton (January 1997) to promote an initiative designed to improve the safety of the nation's food supply. The president charged the federal agencies to work with consumers, producers, industry, states, tribes, universities, and the public to identify ways to improve food safety through government and private sector action, including public-private partnerships. The interagency response is a multifaceted program designed to include surveillance, coordination of activities within the various programs and agencies, risk assessment, research, inspections, and education. The underlying premise upon which this program was developed is that foodborne infections remain a major public health problem. Further, sources of food contamination are said to be almost as numerous and varied as the contaminants; bacteria and other infectious organisms are pervasive in the environment.

The current systems for protecting food in the United States include a broad range of government agencies and industries, many of which have been discussed in this paper. Responsibilities are shared among the U.S. Department of Agriculture (Food Safety and Inspection Service and Animal and Plant Health Inspection Service), the U.S. Department of Health and Human Services (FDA and CDC), and the U.S. Environmental Protection Agency. These responsibilities include oversight on the farm, in the processing facilities, during transportation and distribution (including food from foreign countries), and in food marketing channels including restaurants, supermarkets, and institutional food services (such as schools and hospitals).

Surveillance of foodborne illness outbreaks and their causes is a responsibility of FDA and

CDC. Education is shared among the agencies and is not the primary concern or responsibility of any one of the agencies. Pivotal to this new initiative is the element of education. Specifically, the program is intended to reinvigorate education of all those involved in food preparation, focusing on the use of safe practices. According to USDA et al. (8), educating people about steps they must take to prevent and control foodborne illness is a vital link in the food preparation chain. In spite of the education efforts of the government, both state and federal, consumer groups, and industry, which have occurred historically, foodborne illness occurs from a lack of knowledge of the risks involved at all stages of food preparation. Choices consumers make about how they handle food at home and about eating food that increases the risk for illness can have an important effect on foodborne disease incidence.

USDA et al. (8) will develop a program to improve consumer education; retail, food service, and institutional education; veterinary and producer education; and industry education in the transportation area. They propose developing an alliance among industry, consumer groups, and governmental agencies to mount a comprehensive food-safety awareness campaign for consumers. Highly focused messages and tactics for the general public and consumers at high risk will be developed. This thrust is in perfect harmony with the strategies and tactics proposed by the American Meat Institute (16) as an outcome to a series of studies and roundtable discussions held with medical doctors, dietitians, educators, and others. The ability of industry and consumer groups to work with the government in a program with common themes and elements is critical to the positive outcome of the effort. As one of the focus group members in the Food Marketing Institute (17) said, the more often the message is repeated, the more likely is the listener to hear it.

The broad-based approach to education, which includes data from surveillance and inspections, should provide the foundation for changes in consumer behavior. It is critical that consumers not only take responsibility for their actions regarding food safety, but that they also take seriously the learning that must occur for consumers of all ages to prevent contamination, cross-contamination, and mishandling of foods at home and in restaurants. Convenience, taste, and variety are welcome qualities in foods that we enjoy; safety in foods is critical to the public health and safety of consumers and to the government and businesses that support those consumers.

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Historical Overview of Key Issues in Food Safety

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Foodborne transmission of pathogenic and toxigenic microorganisms has been a recognized hazard for decades. Even half a century ago we knew about the dangers of botulism from underprocessed canned foods; staphylococcal poisoning from unrefrigerated cream-filled pastries, sliced ham, meat, and poultry salads; and salmonellosis from infected animal products. Despite new protective measures, changes in preservation techniques and failure to follow recognized procedures have created new dangers. Moreover, we now recognize new organisms that can cause foodborne illness—*Listeria monocytogenes, Escherichia coli* O157:H7*, Campylobacter jejuni, Vibrio parahaemolyticus, Yersinia enterocolitica*, and others. Controlling these organisms will require widespread education and possibly new regulatory initiatives.

When I was growing up on my parents' farm in East Texas, we never thought about food poisoning or unsafe food. The only foods we bought were sugar, salt, flour, and oatmeal; everything else we produced and preserved on the farm. My mother spent all summer canning fruits and vegetables for winter. We had no refrigeration; we cured our own meat and drank raw milk. But I never heard of botulism, staph poisoning, or salmonellosis or perfringens poisoning until I studied bacteriology in college. Only then did I wonder how we survived with no refrigeration in a hot climate. Finally, the answer came to me. We just did not give the bacteria time enough to develop so they could hurt us. Leftovers from breakfast—hot biscuits, eggs, ham, bacon or sausage, oatmeal, coffee or milk—went right out to the chickens. Lunch leftovers—biscuits, cornbread, vegetables, or fried chicken—were saved for a cold supper 4 or 5 hours later. Any food left went to the pigs. The bacteria had only a maximum of 3 or 4 hours to grow, and that usually is not enough. I survived and went on to study food microbiology, which included what was known then about food poisoning. The guru of food poisoning in those days was professor Gail M. Dack at the University of Chicago. Dr. Dack was a protégé of Professor E.O. Jordan, who in 1917 published a 107-page book entitled Food Poisoning. Dr. Dack took over the book and published his first version of Food Poisoning in 1943. In 1949 and 1956, subsequent editions appeared in which certain truisms became apparent.

Botulism was considered a problem of canners, both home and commercial. Thus, adequate heat processing would seem to solve the problem. Perhaps it did for the canner, but now we know that heating will not eliminate all botulism. Many foods, including salmon eggs, smoked fish, garlic in oil, vacuum packaged lotus roots, and baked potatoes, can support growth and botulinum toxin formation if the storage temperature is suitable. Similarly, we thought staphylococcal poisoning was limited to cream-filled pastries and cured ham. In recent years, outbreaks of staphylococcal poisoning have been traced to cheese, whipped butter, ham salad, fermented sausages, and canned corned beef. We now know how to prevent staphylococcal poisoning, but not all food handlers understand and fully comply with the appropriate control measures.

Salmonellosis was once thought a problem with meat from infected animals. Now we know that a variety of food products can serve as vehicles of this disease. As early as World War II, we found that dried eggs from the United States could transmit this disease to our British allies. Thousands of cases of human salmonellosis in the United States and other industrialized countries

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have been transmitted by ice cream, chocolate, potato salad, cheddar cheese, raw milk, black pepper, pâté, aspic, ham, pasteurized milk, and drinking water.

Clostridium perfringens, known since the 1940s, causes a problem only when there is gross temperature abuse of cooked food. *Clostridium botulinum, Staphylococcus aureus, C. perfringens*, and the salmonellae were well known in Dr. Dack's day, although the food vehicles might have changed. Not so well known were many of the organisms that preoccupy us today. For example, we used to think of *Escherichia coli* as merely an indicator organism that suggested insanitary handling. Now we know forms of *E. coli* can kill. Thirty years ago, *Listeria monocytogenes, Campylobacter jejuni, Aeromonas hydrophila, Plesiomonas shigelloides, Vibrio parahaemolyticus,* and *Yersinia enterocolitica* were not known; now these are well-established foodborne pathogens that we must control.

Although not part of a historical overview, other key issues deserve attention during this meeting. For example, we once thought that fresh, uncracked eggs were essentially sterile and safe to eat. We did not recognize the ability of *Salmonella* Enteritidis to invade the laying hen and thereby the yolk of an egg. An outbreak of *S.* Enteritidis at a Chicago hotel taught us not to rely on the safety of eggs merely because the shell was intact. *S.* Enteritidis in shell eggs is still a serious health problem and a growing concern to egg and poultry producers.

Of equal, if not greater, concern is *Salmonella* Typhimurium strain DT 104. Widely distributed in cattle herds of England, Scotland, and Wales, this organism is resistant to several antibiotics, including ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. Between 1990 and 1995, the number of *S.* Typhimurium DT 104 isolated from humans in Britain increased from 259 to 3,837 per year—a 15-fold increase. Moreover, the percentage of drug-resistant isolates increased from 39% in 1990 to 97% in 1995. *S.* Typhimurium DT 104 has been isolated in the United States from sheep, pigs, horses, goats, emus, cats, dogs, elk, mice, coyotes, ground squirrels, raccoons, chipmunks, and birds. American egg and poultry producers are concerned about its entry into U.S. poultry flocks. *S.* Typhimurium DT 104 infection in humans has been associated with the consumption of chicken, sausage, and meat paste as well as with the handling of sick animals. More than one-third of the patients have required hospitalization, and 3% have died; these figures are very unusual for ordinary Salmonella infections and indicate serious problems ahead.

Quantitative Risk Assessment: An Emerging Tool for Emerging Foodborne Pathogens

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New challenges to the safety of the food supply require new strategies for evaluating and managing food safety risks. Changes in pathogens, food preparation, distribution, and consumption, and population immunity have the potential to adversely affect human health. Risk assessment offers a framework for predicting the impact of changes and trends on the provision of safe food. Risk assessment models facilitate the evaluation of active or passive changes in how foods are produced, processed, distributed, and consumed.

The changing epidemiology of foodborne diseases is a result of complex interactions and changes in pathogens, foods, food distribution, food consumption, and population immunity (1-3). Predicting the impact of a trend in one part of the food continuum presupposes understanding of the whole system. Aspects of the food processing and distribution system can amplify or attenuate the trend as it grows into a potential health hazard. While a full understanding of pathogen contamination, infection, and survival is difficult, a systematic approach to assessing the impact of the pathogen on health may improve the quality of public health decisions (4,5).

Quantitative risk assessment is a possible approach for designing programs to address emerging foodborne diseases. The use of risk assessment in environmental toxicology illustrates the potential advantages of applying quantitative risk assessment in a new field.

Risk Assessment Defined

The essence of microbial risk assessment is describing a system in which a microbial hazard reaches its host and causes harm. Risk assessment consists of four steps: hazard identification, exposure assessment, dose-response assessment, and risk characterization (6). The knowledge in each step is combined to represent a cause-and-effect chain from the prevalence and concentration of the pathogen to the probability and magnitude of health effects. In risk assessment, risk consists of both the probability and impact of disease. In this way, risk reduction can be achieved in either dimension—by reducing the probability of disease or by reducing its severity.

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Hazard Identification

In hazard identification, an association between disease and the presence of a pathogen in a food is documented. The information may describe conditions under which the pathogen survives, grows, causes infection, and dies. Epidemiologic and surveillance data, challenge testing, and scientific studies of pathogenicity also contribute information. Data collected during hazard identification are later used in exposure assessment, where the impact of processing, distribution, preparation, and consumption of the food are incorporated.

Exposure Assessment

Exposure assessment describes the pathways through which a pathogen population is introduced, distributed, and challenged in the production, distribution, and consumption of food. This step differs from hazard identification in that it describes a particular food-processing pathway. Depending on the scope of the risk assessment, exposure assessment can begin with

pathogen prevalence in raw materials (e.g., a "farm-to-fork" risk assessment), or it can begin with the description of the pathogen population at subsequent steps (e.g., as input to a food-processing step). In any case, the intent of risk assessment is to track the pathogen population and estimate the likelihood of its being ingested by the consumer. By completing the pathway to the consumer, we incorporate the important issues of dose-response assessment.

Dose-Response Assessment

Dose-response assessment is used to translate the final exposure to a pathogen population into a health response in the population of consumers. This step is very difficult because of the shortage of data on pathogen-specific responses and because those responses depend on the immune status of the host (consumer). However, even limited knowledge of the shape and boundaries of a dose-response function can be informative in comparing the efficacy of alternate controls. The differences in response among various susceptible populations are important features in this step (7).

Risk Characterization

Risk characterization involves integrating the information gathered in the previous steps to estimate the risk to a population, or in some cases, to a particular type of consumer. In this step, by modifying the assumptions in the parameters of previous steps, we can study the effects of these alternate assumptions on ultimate health risk. Assumptions can be changed to study the impact of lack of knowledge and the potential gains through further research or to suggest the impact of a suspected trend. For this type of analysis, risk assessments are typically done in a computer environment to ease the computational burden and provide rapid responses to "what-if" questions using alternate assumptions and situations. Current spreadsheet applications and available "add-ins" allow generation of complicated probabilistic models that had previously only been available through expensive custom software.

Risk Assessment in Environmental Toxicology

In environmental toxicology, quantitative risk assessment has emerged as the predominant paradigm for describing the public health consequences of human exposure to environmental contaminants (8). Within this paradigm, existing situations are measured and compared according to a measure of population health risk. Similarly, proposed interventions are compared according to the reduction in population health risk that each intervention confers.

The adoption of risk assessment was primarily a result of legal and administrative challenges to regulatory authority during the 1970s (6). Regulatory agencies were required to provide a clear connection between an imposed regulation and an expected health benefit. If the expected health benefit could be quantified, the regulatory agencies were required to demonstrate that it was substantive. Quantitative risk assessment has since become widespread for different reasons. It is now used proactively to support decisions such as selection of waste treatment technologies, contaminated site cleanup operations, and state and municipal priority setting for public health initiatives.

The shift of environmental health issues into a framework of risk reduction opened the field to a broader set of analytic tools and prompted a broader spectrum of professionals to examine the complex problems in the field. Scientific societies have emerged with the sole mission of focusing on the general techniques of risk assessment and their role in public health decisions. In addition, environmental health risks can be compared with concurrent public health risks from other sources through the use of common measures. While this type of comparison is not always performed, scrutiny of the cost-effectiveness of various regulatory programs is increasingly required on the basis of risk reduction. Microbial food safety, as a relative latecomer to the field of risk assessment, can take advantage of its successes and failures and the wealth of constructive criticisms of frameworks, decisions, and methods for addressing pervasive uncertainties (4,8).

Opportunity for Technology Transfer to Microbial Health Risks

Quantitative risk assessment is an emerging tool in the field of microbial food and water safety (9-12). Recognizing the deficiencies of current approaches to evaluating the risk for human illness from pathogens in food, the Council for Agricultural Science and Technology recommended that risk assessment provide the basis for establishing food safety priorities and policies

(5). Because of recent initiatives advocating the widespread implementation of Hazard Analysis and Critical Control Points (HACCP) systems, quantitative risk assessment has been proposed as a means of providing health-outcome–based specification of microbial criteria for HACCP plans (12-14). Concurrently, international trade agreements have advocated that demonstration of increased domestic health risk (in a risk assessment) is the only acceptable basis for barriers to international trade in food (15-18). However, one of the most important benefits in the adoption of quantative risk assessment is improved understanding of the many factors that determine the safety of the food supply.

Some resistance to the adoption of risk assessment is likely. Good manufacturing practices and standard operating procedures carry a long history of reasonably safe production when properly applied. The return on investment in producing a quantitative risk assessment may not be high for an individual food company with a very conservative production process. However, good manufacturing practices and outbreak data are not particularly useful in predicting the impact of new products, newly recognized pathogens, and changes in food processing or in comparing international food systems. Whether changes in the food supply are planned (as in refocused inspection systems and minimally processed foods) or are occurring passively (as in changed pathogens, demographics, and consumer behavior), tools are required to assemble the information that describes the impact. Quantitative risk assessment may provide the only systematic means to interpret the impact of changes or trends before they become a source of epidemiologic data.

In a quantitative risk assessment of broad scope, there is a place for all the data from diverse information gathering activities relevant to microbial food safety. Recent analyses of pasteurized liquid egg (19) and ground beef contamination (20) incorporated evidence from farm-based studies of pathogen prevalence, technology assessments comparing decontamination methods, process-specific parameters of lot size and raw material mixing, growth and death models from predictive microbiology, monitoring studies of transportation and retail temperature control, and studies of consumption amounts and cooking preference.

By designing the quantitative risk assessment process as an intelligent information bank, we can develop a model to accommodate the breadth of available information. The model provides a focus for discussions among workers from diverse disciplines: farmers, veterinarians, food-processing experts, microbiologists, and consumer behavior experts. The model also allows for consideration and comparison of control strategies for which experimentation would be very difficult in a "live" environment. The impact, for example, of an aging population or a shift in cooking practices can be simulated by a variety of assumptions that reflect the extent of the change. By placing all of the information together, we can delineate gaps in knowledge and provide estimates of the benefits of proposed research.

The most obvious users for quantitative risk assessment as applied to microbial food safety are agencies responsible for food inspection, disease surveillance, and food standards. These agencies have the most to gain from models that incorporate existing and new data, capture knowledge of the relevant features of the food processing and distribution continuum, and capture knowledge of the variability in consumer behavior and immune system responses. If models are constantly updated and improved, decisions made to research, monitor, and control foodborne pathogens can be made with information that lends itself to multidisciplinary discussion and best describes what is currently known and unknown. Without such a model, there is little common ground for the type of collaboration often advocated for addressing the inherent complexity of foodborne disease.

Risk Assessment Case Examples

Two case examples illustrate the prospects of using risk assessment to support decisions regarding emerging foodborne diseases.

Escherichia coli O157:H7 in Ground Beef

A model of *E. coli* O157:H7 in ground beef has been developed to support comparative assessment of control strategies (20). The model describes the pathogen population from the production of ground beef (including carcass processing) to consumer cooking and consumption. The variability and uncertainty in the model are accommodated through the use of probabilistic representations for many of the parameters.

To generate a representative distribution of risk, the model is simulated many times with different values selected from the probability distributions. This is a technique known as Monte Carlo simulation (20-22).

While the direct output of the model is a distribution of health risk from eating ground beef hamburger patties, a more important use of the model is to describe the changes in health risk associated with changes in various parameters. By changing parameters describing, for example, pathogen prevalence and concentration in raw material, temperature abuse in transportation and retail, consumer cooking preference, infectious dose, and size of susceptible populations, we can study the impact of trends in disease risk factors. Because this model includes the farm-to-fork continuum, it is possible to assess the efficacy of interventions that would otherwise not be compared in the same analysis. In addition, the importance of improved data at different points in the process can be estimated.

Toxoplasmosis

A probabilistic model describing the incidence of toxoplasmosis was generated (23). While this model did not begin at the raw material level, valuable insights were gained in studying the impact of trends in exposure to *Toxoplasma gondii*. In congenital toxoplasmosis, the impact of maternal exposure to *T. gondii* depends on whether the mother has previously been infected (24). If this is the first exposure, the impact further depends on the trimester of pregnancy. If detected at an early stage and treated with certain drug therapies, the infection may have a smaller impact.

With such a model, the impact of varying risk factors can be studied. Since the most serious consequences of toxoplasmosis occur during pregnancy, a key variable is seroprevalence as a function of age. The protection offered by prior infection complicates disease therapy; a reduction in exposure to *T. gondii* could increase incidence of congenital toxoplasmosis by reducing the prevalence of immune women of childbearing age. This may be further complicated by changes in the age profile of pregnancy since younger women are less likely to have been exposed. In addition to the complexities of the population immunity profile, various trends in risk factors can be simulated, such as trends in cat ownership, consumption of implicated products, and the age distribution of pregnancy. The emergence of toxoplasmosis as one of the leading causes of death in the human immunodeficiency virus–positive population can be studied concurrently. The effectiveness of mitigation strategies (e.g., education and screening programs designed for pregnant women) can be compared to food-processing strategies intended to reduce overall exposure.

The model of *T. gondii* infection provides insight into the importance of detailed hazard identification to understand the complex mechanisms of disease, exposure modeling to understand the time-dependent nature of exposure, and intervention modeling to understand the potential negative consequences of a reduction in overall exposure. Moreover, the results underline the importance of performing all of the above tasks in the same overall exercise if the implications of trends and interventions are to be fully understood. It is unlikely that a sound decision could be made without a full microbial risk assessment involving modeling of the complex nature of population immunity and exposure.

Conclusions

One of the key benefits of quantitative risk assessment is the development of models describing the complex nature of pathogen populations in the food supply. Improved understanding of the efficacy of pathogen reduction is the most important side effect of this approach. Studies assessing the health impact of a foodborne pathogen often include extensive documentation of pathogen levels at unconnected points in the food and consumer pathway. In contrast, a microbial risk assessment based on a model provides a repository of knowledge describing health risk outcomes and control strategies. The model improves with each new related study and each critical review as more and more relevant data are uncovered. Furthermore, when a decision is required, a description of the system is already available in which assumptions and proposed interventions can be tested.

Initially, models can be expected to be crude. However, as a base for discussion, a model can be very effective at soliciting input from experts in the food industry and the public health community. Input from epidemiologists, microbiologists, and industry safety managers can be merged into the model until it represents the best available understanding of the interacting

features of the food supply and their effect on the distribution of health risk. Once the model has been developed, the impact of various control strategies and trends can be simulated. Our current inability to compare control strategies at different points of the food supply chain is evidence of the need for a system-level understanding that will improve decision-making capacity.

Decisions to address foodborne pathogens cannot wait for scientific certainty. Large degrees of uncertainty require that decisions be made with great caution; however, there is no excuse for not making the best decision on the basis of available information. Model-based quantitative risk assessment can provide the decision-maker additional insights not typically evident in "piece-meal" considerations of data. The ability to represent the essentially probabilistic nature of emerging foodborne disease is another risk assessment attribute not typically achieved by traditional approaches.

Many gains in decision support can be achieved through model-based risk assessment. Given that many current concerns are focused on emerging pathogens, it may be timely to adopt risk assessment as a tool that is well equipped for studying changes and interventions in the race against pathogens.

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Communicating Foodborne Disease Risk

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The food industry, like many others, has a risk communication problem. That problem is manifested in the public's desire to know the truth about outbreaks of foodborne diseases; ongoing concern about the safety of foods, additives, and food-processing procedures; and continued apathy regarding aspects of routine food hygiene. If these concerns are addressed in a coherent and trustworthy way, the public will have better and cheaper food. However, sloppy risk communication can itself cause public health damage. Because citizens are ill-equipped to discriminate among information sources, the food industry as a whole bears responsibility for the successes and failures of its individual members. We review risk communication research and practice for their application to the food industry.

The guardians of the world's food supply face a communication challenge of extraordinary complexity. They need to be ready at short notice to deal with various crises, often involving baffling combinations of foods, pathogens, handling and distribution practices, dietary norms, and interactions with medical conditions and medications. Their response to this challenge may have important health, economic, and even political implications. Conflicting pressures may come from groups that bear the cost when the public health response is too swift or too slow. Quick, confident explanations are expected after outbreaks that may never be fully understood. When consumers (and producers) need information, they cannot wait for more research. Consumers can read between the lines, especially when they perceive their lives or livelihoods at risk. If they misread messages, the communicators may still be held responsible. Moreover, consumers know that silence is also a form of communication.

At the same time, the guardians of the food supply must wage a continuing struggle to improve the handling of food. In the United States, campaigns are under way for cooking beef more thoroughly, separating raw meat from salad ingredients, and improving the sanitation of food handlers (e.g., Operation Clean Hands). To some extent, these campaigns are the incarnations of old messages that have not been communicated effectively. At the same time, the campaigns are responses to changes in the food supply that have increased the risks associated with conventional practices. For example, as the incidence or severity of foodborne disease pathogens increases, the effectiveness of customary food-handling practices decreases.

This article briefly reviews risk perception and communication research as a possible resource for better understanding (and perhaps meeting) the public's needs (1-3). Communication research provides a set of general tools and theories, as well as a body of results, showing a complex picture of strengths and weaknesses in lay understanding of risk. We explore here the implications for anticipating public response to emerging foodborne pathogens and offer a proposal for how an effective communication campaign might be organized.

Although risk communication research does not directly address emerging foodborne pathogens, it is compatible with the model of risk assessment that the food industry seems to be adopting (4). Drawn from the National Research Council's (5) volume, Improving Risk Communication, the model involves overlapping processes of assessing the magnitude of risks (through analytical procedures), managing their level (through practical measures), and communicating with the public about them.

Like many other risks, emerging foodborne pathogens are of primary concern to some specialists but one more thing to worry about for ordinary citizens. The thought processes that

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people rely on for making decisions are the focus of much research (Table; 6).

How much does the public know and understand? The answer to this question depends on the risks consumers face and the opportunities they have to learn about them. The next section discusses strategies for improving those opportunities.

Table. Thought processes involved in decision-making

People simplify. Many decisions require people to deal with more details than they can readily handle at any one time. To cope with the overload, people simplify. People want to know if foods are "safe," rather than treating safety as a continuous variable; they demand proof from scientists who can provide only tentative findings; and they divide the participants in risk disputes into good guys and bad guys. Such simplifications help people cope, yet also lead to predictable biases (7).

Once people's minds are made up, it's hard to change them. People are adept at maintaining faith in their beliefs unless confronted with overwhelming evidence to the contrary. One psychologic process that helps people to maintain their current beliefs is underestimating the need to seek contrary evidence. Another process is exploiting the uncertainty surrounding negative information to interpret it as consistent with existing beliefs (8).

People remember what they see. People are good at keeping track of events that come to their attention (9,10). As a result, if the appropriate facts reach people in a credible way before their minds are made up, their first impression is likely to be the correct one. Unfortunately, it is hard for people to gain firsthand knowledge of many risks, leaving them to decipher the incomplete reports they get.

People cannot readily detect omissions in the evidence they receive. It is unusual both to realize that one's observations may be biased and to undo the effects of such biases. Thus people's risk perceptions can be manipulated in the short run by selective presentations. People will not know and may not sense how much has been left out (11). What happens in the long run depends on whether the missing information is revealed by other experiences or sources.

People may disagree more about what "risk" is than about how large it is. One obstacle to determining what people know about specific risks is disagreement about the definition of "risk" (12-15). For some risk experts, the natural unit of risk is an increase in probability of death; for others, it is reduced life expectancy; for still others, it is the probability of death per unit of exposure. If lay people and risk managers use the term "risk" differently, they may agree on the facts of a hazard, but disagree about its riskiness.

Abridged from Fischhoff & Svenson (15).

Communicating Risk

An overarching theme of risk communication is that people understand risks that draw their attention and are presented comprehensibly. Whether the public's attention is aroused spontaneously or as a result of a message, the opportunity must be seized. The right information must be selected and communicated appropriately (1,16,17).

The hallmarks of effective communication should be used. Match the audience's level of technical sophistication. Do not talk down. Clarify terms (e.g., virus) that are used in everyday speech but not very precisely (e.g., risk). Organize information. Provide the audience with a quick logical overview. Make the desired level of detail easy to read. Use numbers to communicate quantities. Avoid ambiguous verbal quantifiers, such as "rare" or "likely". Ensure source credibility. Realize that messengers are a part of the message and essential to its interpretation. Use knowledgeable sources that will not misrepresent the message. Avoid risk comparisons with rhetorical implications. Comparing one uncontrollable accident risk with another, more familiar one (e.g., half as likely as being injured by lightning) can be useful; however, people dislike comparisons that imply they should accept one risk because they accept another, e.g., comparing the risks of nuclear power with those of eating peanut butter (from aflatoxin).

However useful communication research may be, there is no substitute for empirical testing of messages. With heterogeneous audiences, any fixed message will work better for some people than for others. In such cases, universal understanding may require providing the opportunity for the public to ask questions through public information sessions, agricultural extension services, science teachers, or toll-free numbers.

What To Say

The effort to communicate is wasted if the information is not worth communicating, either because people already know it or because it makes no difference to them. Indeed, communication can backfire if consumers think that their

time is being wasted with useless messages while they are being denied pertinent information. An analytical effort to determine what is worth knowing and a coordinated empirical effort to determine what people know already are required. These efforts take different forms in situations where consumers face well-formulated decisions and need only a few quantitative estimates before making choices, and in situations where consumers are trying to understand the processes creating and controlling a risk, in order to follow public discussion, devise decision options, or understand quantitative estimates.

Identifying Relevant Estimates

The tools of decision analysis provide ways to determine how sensitive well-structured choices are to uncertainty in different decision parameters (18,19). The more sensitive parameters should receive more attention, unless consumers know them already (and need no reminder). If conditions do not permit sensitivity analyses for individual decision makers, one can model the information needs of a population similar to the intended audience. Merz et al. (20) demonstrated this approach for communicating to carotid endarterectomy candidates. Scraping out the main artery to the brain reduces the probability of stroke for patients with arteriosclerosis. However, the procedure can cause many problems, including strokes. Decision analysis computed the attractiveness of surgery for a hypothetical population of patients, with a distribution of physical states (e.g., stroke risks) and values (e.g., time horizons). The analysis found that three of the potential complications (stroke, facial paralysis, and persistent headaches) posed sufficient risk that learning about them should dissuade about 30% of candidates from surgery. Learning about the other side effects should affect few additional patients. Therefore, physicians trying to secure informed consent should (while not hiding other information) make sure that patients understand the risks of these three complications.

Identifying Relevant Processes

Risk analysis provides one way to identify the critical processes in creating and controlling risks. Figure 1 shows a simple model for the risks of foodborne pathogens. It uses the formalism of the influence diagram (21,22). Such a model can be used both to assess risks and to characterize the comprehensiveness of lay understanding. In this model, people incur food-related risks as a result of decisions, which possibly lead to actions or exposures. These decisions concern such actions as eating a bite of suspicious food, choosing a particular diet, or opting for school (or home) lunch. Those decisions depend, in part, on the perceived risks of those actions as well as other nonrisk factors (i.e., other costs and benefits). Exposure may follow, if a pathogen is actually present; it can lead, in turn, to transmission of the pathogen and to changed health states, depending on the resistance to disease that the person's health provides.

Figure 2 elaborates on this model. It shows that food pathogenicity depends on both the prevalence of pathogens in the environment and the quality of food handling. A person's own health influences risk perceptions through the intermediate variable of perceptions of health, which in turn is influenced by the person's history of food consumption (or avoidance). Actual pathogenicity influences risk perceptions through awareness, a variable that communicators might affect. Nonrisk factors include visceral factors (e.g., hunger), external social factors (e.g., social pressure to eat any food offered by a host), norms (e.g., not eating dog), and the expected benefits of consumption (e.g., taste, texture, and other gustatorial pleasures). Computing risks with this model would require specifying each variable and estimating the contingencies by using statistical sources or expert judgment. For risk communication purposes, even a qualitative model can define the universe of discourse and allow approximate estimates of the most important relationships (23).

Identifying Current Knowledge

Determining what people already know about quantitative estimates is relatively straightforward, although there are various pitfalls (24,25). Eliciting knowledge of processes is more difficult. Respondents should be given the focus of the problem and maximum freedom to express their ideas and reveal which of the processes in Figure 2 are on their minds. Studies using open-ended techniques often find that people speak the language of risk without understanding its terms. For example, in a study about radon, we found that respondents often knew that it was a colorless, odorless, radioactive gas that caused lung cancer. However, when

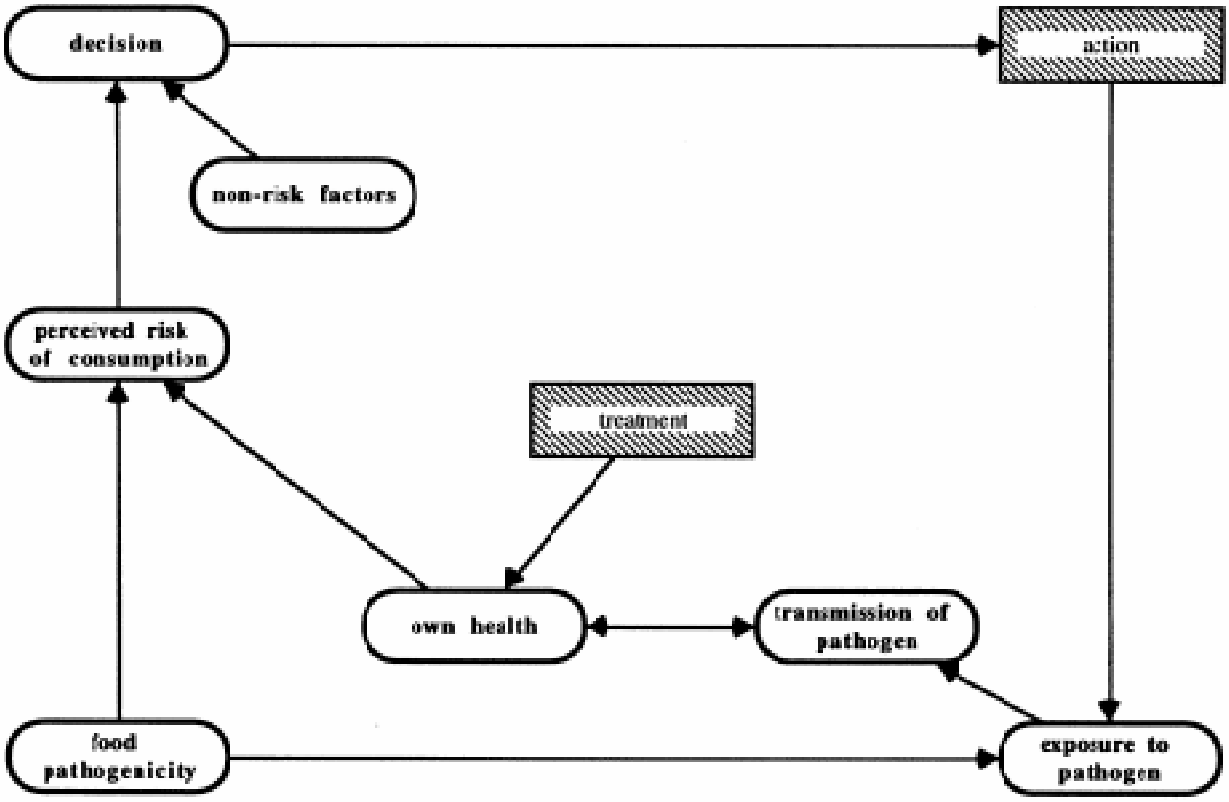

Figure 1. Diagram of the general model.

pressed, respondents often revealed inappropriate notions of radioactivity, believing that anything radioactive would permanently contaminate their homes. Some told us that they would not test for radon because there was nothing that they could do if they found a problem (26). In studies with adolescents, we found other forms of false fluency; for example, teens used terms such as "safe sex" and "clean needles" without understanding them (23). Without open-ended probing, we miss misconceptions that a technical expert never would have imagined, or we use language that does not communicate effectively with our audience (27).

Food Industry Communication Strategies

Technical experts (in any industry) generally want to get the facts in order before saying anything. Although that is an appropriate norm within the scientific community, refusing to address a concerned public can evoke mistrust and anger, as can failing to arouse an apathetic public. To steer an appropriate course, communicators need an explicit policy that balances the risks of saying too much with the risks of saying too little. The policy must consider both what to say and when to say it. From a decision theory perspective, citizens need information critical to identifying actions that will help them achieve personal goals. As a result, any recommendations should reflect both scientific knowledge and citizens' values. That is, consumers need to know what is the best gamble, given the trade-offs between, for example, the risks of throwing out good food and the risks of eating food that might make them sick.

At times, there may be a temptation not to tell it like it is. For example, one might argue that risks should be exaggerated when a frank report would leave people unduly apathetic, as judged by their own standards. That is, people should say "Thanks for getting my attention" once the grounds for the overstatement were made clear. Such gratitude requires a public that not only recognizes the limits of its own understanding, but also accepts paternalistic and manipulative authorities. That acceptance seems more likely for misrepresentations intended to get a

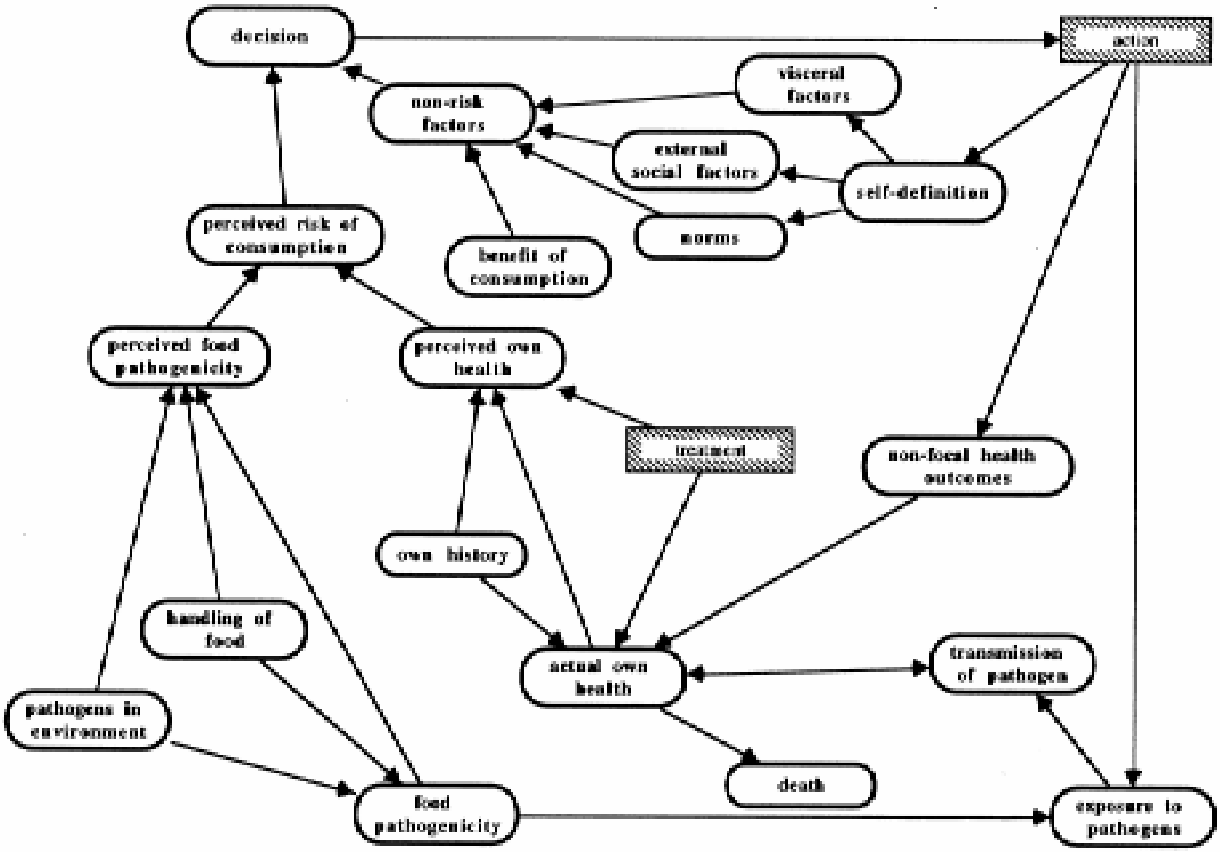

Figure 2. Diagram of the foodborne model.

complacent public moving than for ones intended to allay a hysterical public's fears. In either case, once the secret is out, all future communications may be subjected to second guessing ("How seriously should we take them this time?").

One situation in which paternalistic authority is needed arises when a single message must be sent to a heterogeneous audience—for example, when officials must decide whether to declare a particular food "safe." Safety is a continuous variable, and any cut-off represents a value judgment. For any given food, different groups may face different risks, derive different benefits, and want to make different trade-offs. For example, a few people are strongly allergic to sulfur dioxide as a dried food preservative. Marketing such foods signals their safety to all. Labels that declare preservatives in foods allow consumers to customize their risk levels, but only if they know their own risks (i.e., whether they are strongly allergic, which they may learn only through a bad reaction whose source they identify).

The Food and Drug Administration faces a similar challenge in its effort to standardize risk labels for over-the-counter drugs. For example, other things being equal, producing bilingual labels will require either reducing print size or omitting information about some side effects. These modifications would, in turn, increase the risks for consumers with limited vision (e.g., some of the elderly) or those particularly sensitive to the omitted effects. Whatever labeling, warning, or communication strategy is chosen will leave some residual risk, with an uneven distribution depending on the heterogeneous sensitivities of the audience. Thus, the strategy reflects the authorities' notion of the "acceptable level of misunderstanding" (28).

What that acceptable level should be is a political and ethical question, which could be resolved by properly constituted public or private groups, and a scientific question, partially resolvable by research of the sort described here. Rigorous empirical testing is needed to deter-

mine whether communications fulfill the hopes placed in them (27). Emerging foodborne pathogens provide a particular challenge to safety communications—and a particular need for evaluation. Their novelty and ability to produce outbreaks in diverse places in the world and the food chain encourage treating them as unique. If a communication strategy is improvised only when a crisis hits, or as it evolves, the chances for a misstep increase. Those chances are especially large if the outbreak is the first major risk problem for the health authorities involved (16).

As a result, communications about these unique situations should be routine. A standard format for reporting risk information should be adopted. Funtowicz and Ravetz (29) propose a notation that includes a best-guess risk estimate (expressed in standard units), a measure of variability, and a "pedigree" (indicating the quality of the research). Although new, such notation might become familiar, much as degrees Fahrenheit, miles per gallon, probability of precipitation, and recommended daily allowance have become familiar.

Another part of communication planning is to adopt standard scripts for reporting complex procedural information regarding what citizens should do and what food specialists are doing. The adoption process should include empirically testing the comprehensibility of concrete messages with an audience like the intended audience. Influence diagrams offer one template for organizing procedural information. Risk analyses provide one way to identify the crises most likely to occur and may allow not only testing the most likely messages, but also identifying the persons most likely to do the communicating and preparing them accordingly. The chemical industry's Community Awareness and Emergency Response program might provide some useful lessons in how to organize for unlikely events, although the challenges of dealing with the relatively identifiable community surrounding a chemical plant are different from those presented by dealing with the diffuse national (or even international) audience concerned about a food. The chemical industry's experience may also provide guidance on how to achieve voluntary industry compliance with a set of communication principles. Public goodwill is eroded every time an industry spokesperson violates the public trust by misrepresenting, or just explaining inadequately, the state of affairs. Reducing misrepresentation requires institutional discipline; reducing inadequate communication requires a scientific approach to communication.

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Animal Diseases of Public Health Importance

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The Food and Agriculture Organization's (FAO) interest in emerging diseases caused by foodborne pathogens derives from its role as the leading United Nations agency with a mandate for food quality and safety matters. The Food Quality and Standards Service of FAO's Food and Nutrition Division is active in all areas related to food safety and implements the FAO/World Health Organization Food Standards Program. Its activities include providing assistance to FAO's member nations in addressing problems,strengthening infrastructure, promoting standardization as a means of facilitating trade, and safeguarding the interests of consumers. This paper considers the importance of emerging foodborne diseases from the perspectives of the consumer, international trade in food, producers and processors, and developing countries and addresses prevention and control measures.

In recent years, public concern regarding food safety has increased as a consequence of the outbreak of bovine spongiform encephalopathy (BSE) in cattle, the prevalence of *Salmonella* serotype Enteritidis illnesses (from poultry, meat, eggs), and the more localized outbreaks of illnesses associated with *Listeria monocytogenes* (from dairy products, pâté, salads) and *Escherichia coli* O157:H7 (from ground or minced beef, unpasteurized apple juice, vegetables). Emerging pathogens and the appearance of problems such as BSE have resulted in enactment of specific controls in many countries, while the general heightening of interest internationally has prompted health professionals and the food industry in many countries to scrutinize the control of emerging infectious agents.

Animal Feeding and Food Safety

The Food and Agriculture Organization (FAO) of the United Nations has had a long-standing interest in the area of food safety and food quality. Because of problems such as BSE and emerging pathogens, FAO convened an Expert Consultation on Animal Feeding and Food Safety in Rome in March 1997 to address these issues and provide the scientific basis for improving practices in the feeding of animals for the production of food.

The ultimate objective of food industry and safety regulators is to ensure that food reaching the consumer is safe and wholesome. This objective does not imply that food can ever be completely free of risk but rather that the level of risk to the consumer can be acceptable. Foods generally expected to be safe may become unsafe as a result of hazards introduced during production, processing, storage, transport, or final preparation by the consumer. For food derived from animal sources, the hazards may originate from a number of sources, including the consumption of contaminated feed.

Hazards in food that may relate to animal feed include salmonellosis, mycotoxicosis, and ingestion of unacceptable levels of veterinary drugs and agricultural and industrial chemicals. In addition, if the postulated link between BSE and new variant–Creutzfeldt-Jakob disease is established, this disease would also be an example of contamination originating from animal feed.

The FAO consultation limited its considerations to food safety matters that pertained strictly to animal feeds; it did not consider plant toxins, radionuclides, or parasites spread by human sewage. The risk to human health from other infectious agents that may

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contaminate either feed or forage appears to be negligible or nonexistent and was, therefore, not considered by the consultation. Only the standard domestic animals from which food is derived in large quantities, such as meat and meat products, milk and milk products, and eggs and egg products, as well as fish products derived from aquaculture that involves the feeding of fish, were considered. All aspects of animal feed, other than natural unrestricted grazing, were considered. The consultation concluded that emerging pathogens are generally not identified through traditional animal surveillance and epidemiology.

Hazards Associated with Animal Feed

Mycotoxins are secondary metabolites produced by fungi of various genera when fungi grow on agricultural products before or after harvest or during transportation or storage. Some fungi such as *Aspergillus* spp. and *Penicillium* spp. can invade grain after harvest and produce mycotoxins, while others, such as *Fusarium* spp., typically infest grains and produce mycotoxins before harvest. In some circumstances, Aspergilli can grow and produce mycotoxins before the crop is harvested.

Both intrinsic and extrinsic factors influence fungal growth and mycotoxin production on a substrate. Intrinsic factors include water activity, pH, and redox potential; extrinsic factors are relative humidity, temperature, and availability of oxygen.

Many mycotoxins with different chemical structures and widely differing biologic activities have been identified. Mycotoxins may be carcinogenic (e.g., aflatoxins B1, ochratoxin A, fumonisin B1), estrogenic (zearalenone and I and J zearalenols), nephrotoxic (ochratoxins, citrinin, oosporeine), dermonecrotic (trichothecenes), or immunosuppressive (aflatoxin B1, ochratoxin A, and T-2 toxin). Much of the published information on toxicity comes from studies in experimental animals, and these may not reflect the effects of mycotoxins on humans and other animals. In addition, their significance in human foods of animal origin is incompletely understood. Mycotoxins are regularly found in animal feed ingredients such as maize, sorghum grain, rice meal, cottonseed meal, groundnuts, legumes, wheat, and barley. Most are relatively stable compounds, are not destroyed by feed processing, and may even be concentrated in screenings.

Various animal species metabolize mycotoxins in different ways. In pigs, ochratoxin A can undergo enterohepatic circulation and is eliminated very slowly, whereas in poultry species it is rapidly excreted. The polar mycotoxins such as fumonisins tend to be excreted rapidly. Mycotoxins, or their metabolites, can be detected in meat, visceral organs, milk, and eggs. However, their concentration in these food products is usually considerably lower than in the feed consumed by the animals; at these levels, mycotoxins are unlikely to cause acute intoxication in humans consuming these products. Residues in animal products of carcinogenic mycotoxins, such as aflatoxin B1, M1, and ochratoxin A, pose a threat to human health, and their levels should be monitored and controlled.

In most instances, the principal source of mycotoxins for humans is contaminated grains and cereals, rather than animal products. This means that the hazard is much greater in developing countries in which maize and other grains form the staple diet and the intake of animal products, including meat, is low.

Only limited information is available regarding mycotoxin residues in animal products intended for human consumption. The metabolism of mycotoxins by animals and the residues of mycotoxins and their metabolites in animal tissues should be studied further.

Infectious Agents

Agent Causing Transmissible Spongiform Encephalopathies in Ruminants

Transmissible spongiform encephalopathies are nonfebrile neurologic diseases with a long incubation period and are fatal. These diseases are associated with incompletely defined agents termed prions, which are resistant to normal heat treatments of feed and food. Sheep scrapie has been recognized for over 250 years. BSE was first recognized in the United Kingdom during 1986. For BSE, the infectious agent enters the feed primarily through rendered infected tissues (notably the central nervous system and the reticuloendothelial system) under insufficient heat to reduce the concentration of the infectious agent to an ineffective dose. In the case of sheep scrapie, infection is naturally maintained by transmission between sheep. Humans have likely been exposed to the scrapie agent by eating

brain and other tissues, although there is no evidence that Creutzfeldt-Jakob disease in humans has been associated with scrapie.

Humans can potentially be exposed to BSE through consumption of infected tissues. The occurrence of a new variant of the human transmissible spongiform encephalopathy, Creutzfeldt-Jakob disease, has raised the possibility of an association with the BSE agent. With the limited number of cases now, there is no proven link between this new variant and the possible transmission of the agent from infected bovine tissue to humans. The FAO consultation recommended risk reduction measures to address the elimination of BSE from cattle.

Salmonella enterica

The more than 2,000 *Salmonella* serotypes can be divided into three groups: species-specific, such as gallinarum (in poultry); invasive, which may cause systemic infections in their host, such as Enteritidis (in laying hens); and noninvasive, which tend to remain within the intestinal tract. Members of the first group are infrequently feedborne pathogens. Among the second group, the principal manifestation of human infection is gastroenteritis, with septicemia occurring in some patients. The third group may be associated with subclinical infections in farm livestock; it sometimes causes disease in livestock and is associated with food poisoning in humans.

Salmonellae are widely distributed, and animal feed is only one of many sources of infection for farm animals. Animal feed ingredients of both animal and plant origin are frequently contaminated with salmonellae, although the most common serotypes associated with human disease, Enteritidis and Typhimurium, are rarely isolated from animal feed. Feed can be contaminated from raw ingredients.

Toxoplasma gondii

The protozoon *T. gondii* is found in cats and, according to serologic surveys, also in birds and other domesticated species including sheep, pigs, goats, and horses. The primary source of infection for animals is feed contaminated with feces of cats and possibly with rodent tissues.

Cats are an important source of infection for humans; however, some human infections may be due to the handling or consumption of raw meat. Pregnant women may miscarry or give birth prematurely, and infants often get central nervous system disorders and ocular disease.

Trichinella spiralis

T. spiralis is a nematode that parasitizes the intestinal tract of mammals, particularly pigs. The larvae encyst in the tissues, particularly the muscles, which act as a source of infection for humans who consume raw or partially cooked meat. The clinical manifestations include fever, muscle pain, encephalitis, meningitis, myocarditis, and (rarely) death.

The cysts in infected carcasses can be killed by freezing (-18°C for 20 days) or traditional rendering temperatures. Adequate cooking of raw meat and table scraps before feeding to farm animals would eliminate this hazard.

The FAO consultation also addressed potential hazards associated with veterinary drugs and agricultural and other chemicals and recommended risk reduction measures to prevent, eliminate, or reduce the hazards to acceptable levels. The consultation participants prepared a draft Code of Practice for Good Animal Feeding to be considered by the Codex Alimentarius Commission (CAC).

Codex Alimentarius Commission

Since 1962, CAC has been responsible for implementing the Joint FAO/World Health Organization (WHO) Food Standards Program. "Codex Alimentarius," whose name is taken from Latin and translates literally as "food code" or "food law," was founded in response to the worldwide recognition of the importance of international trade and the need to facilitate trade while ensuring the quality and safety of food for the world consumer.

It follows, therefore, that the commission's primary objectives are the protection of the health of consumers, the assurance of fair practices in the food trade, and the coordination of all food standards. Food standards, guidelines, and recommendations are the work of CAC. With the adoption of the World Trade Organization's Agreement on the Application of Sanitary and Phytosanitary Measures and the Agreement on Technical Barriers to Trade, a new emphasis and dimension have been placed on Codex standards.

Codex Committee on Food Hygiene

The Codex Committee on Food Hygiene (CCFH) has overall responsibility for all

provisions of food hygiene prepared by Codex commodity committees and contained in commodity standards, codes of practice, and guidelines. CCFH also develops general principles, codes of practice, guidelines for food hygiene, and microbiologic criteria for food to be applied horizontally across Codex committees. Food hygiene is defined as "all conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain."

According to the deliberations at the 29th session of CCFH, the microbiologic safety of foods is principally ensured by control at the source, product design, process control, and good hygienic practices during production, processing, handling, distribution, storage, sale, preparation, and use, preferably in conjunction with the application of the Hazard Analysis and Critical Control Points (HACCP) system. This preventive system offers more control than end-product testing because of the limited effectiveness of microbiologic examination to assess the safety of food.

When they have been established by Codex or national risk managers, objectives for food safety can be taken up by industry; by applying HACCP (or an equivalent food safety management system), industry can ensure that these objectives are met. This is the use of HACCP as a corrective risk management option: a risk is identified, and a management option is selected and implemented. HACCP is also used as a preventive risk management tool. In this case, hazard analysis identifies potential hazards in raw materials, production line, and line-environments to the consumer. Hazard analysis is defined as "The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan." Input concerning the potential hazards and their control could come from risk analysis, but often such information is not available and industries need to apply their best judgment.

The Revised Principles for the Establishment and Application of Microbiological Criteria For Foods states, "Microbiological criteria should be established according to these principles, and be based on scientific analysis and advice, and where sufficient data are available, on a risk analysis appropriate to the foodstuff and its uses." These criteria may be relevant to the examination of foods, including raw materials and ingredients of unknown or uncertain origin, and may be used when no other means of verifying the efficacy of HACCP-based systems and good hygienic practices are available. Microbiologic criteria may also be used to determine that processes are consistent with the General Principles of Food Hygiene. Microbiologic criteria are not normally suitable for monitoring critical limits as defined in the HACCP system.

Establishing microbiologic criteria and food safety objectives in general is difficult because of the considerable knowledge gap relating to biologic hazards and their relationship to human illness. This has led to many evaluations by CCFH, which are based on subjective or qualitative assessments and serve as the basis for recommendations. Although aware of these limitations, CCFH is now developing a framework of principles and guidelines for the application of microbiologic risk assessment. CCFH's action was in response to the recommendation of the 1995 Joint FAO/WHO Consultation on the Application of Risk Analysis to Food Standards relating to the application of risk assessment within the Joint FAO/WHO Food Standards Program. International Commission for Microbiological Specifications for Foods and CCFH delegations are also in the process of developing background papers on a number of foodborne pathogens to better conduct quantitative risk assessments and set subsequent food safety objectives. Notwithstanding the development of risk analysis approaches by these groups, the work of CCFH and all Codex committees would benefit from advice from an expert body on foodborne biologic hazards for purposes of risk management. The committee could be modeled on the FAO/WHO Joint Expert Committee on Food Additives and Joint Meeting on Pesticide Residues, allowing for the unique consideration of epidemiologic and clinical data related to pathogens causing human illness, and of the dynamics of microbial populations in food throughout the food chain.

Control of *Listeria monocytogenes* in foods is an example of the need to consider a structured risk management approach. Listeria are frequently consumed in small amounts by the general population without apparent ill effects. Only higher levels of Listeria are thought to cause serious disease problems. It is believed that Listeria will always be present in the environment. Therefore, the critical issue may

not be how to prevent Listeria in foods, but how to control its survival and growth to minimize the potential risk. In many foods, complete absence of Listeria is unrealistic and unattainable; trying to achieve this goal can limit trade without having any appreciable benefit to public health. A relevant risk management option, therefore, is to focus on foods that have historically been associated with human disease and support the growth of Listeria to high levels, rather than focusing on foods that do not support growth. Thus, establishing tolerably low levels of Listeria in specific foods may be one food safety objective achieved by risk managers after a rigorous and transparent risk analysis. Such an approach is now being considered by CCFH after an initial risk assessment by the International Commission for Microbiological Specifications for Foods and CCFH delegations.

Although Listeria presents unique challenges in terms of its widespread occurrence and the particular susceptibility of vulnerable groups, pathogens such as *E. coli* O157:H7, *Salmonella*, and *Campylobacter* are also being addressed. These microbial pathogens produce acute foodborne illnesses and can cause severe chronic sequelae, creating an important public health problem and food safety concerns.

Codex Codes of Hygienic Practice are based on good manufacturing practices, HACCP principles, and risk analysis. CCFH is responsible for coordinating and overseeing the work of specific Commodity Committees in this area. In the specific area of food hygiene, Codex has revised its main document, Recommended International Code of Practice: General Principles of Food Hygiene, to incorporate risk assessment principles and include specific references to the HACCP system.

FAO Programs on Food Quality and Safety

The Food Quality and Standards Service is a service within the Food and Nutrition Division of the FAO, located in Rome. The Secretariat of CAC is also located there. The Regular Program of the Food Quality and Standards Service provides the technical and scientific basis for FAO for all food quality matters, including food safety. This includes providing the Secretariat for the Joint Expert Committee on Food Additives and participation in both the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues and in the Joint Expert Committee on Food Irradiation.

The Food Quality and Standards Service develops and publishes guidelines and manuals (including the FAO Food and Nutrition Paper Series and Manuals of Food Quality Control), arranges expert consultations and conferences (e.g., the Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety, September 30 to October 4, 1996; the Joint FAO/WHO Expert Consultation on the Application of Risk Management to Food Safety Matters, January 27-31, 1997; the Joint FAO/WHO Consultation on Food Consumption and Exposure Assessment to Chemicals, February 10-14, 1997; and the FAO Consultation on Animal Feeding and Food Safety, March 10-14, 1997), and has a major and continuing program of providing technical assistance regarding food standards and food control to member countries, particularly developing countries and countries in transition from a centrally planned to a market economy.

The Joint Expert Committee on Food Additives, the Joint Meeting on Pesticide Residues, and the Joint Expert Committee on Food Irradiation are expert committees that provide independent scientific advice that forms the basis for the development of food safety recommendations used in international trade. These committees are forums in which independent, invited experts assess the state of scientific knowledge of food additives, pesticide and veterinary drug residues in food, mycotoxins, other chemical contaminants in food, and food irradiation treatments and make recommendations to member governments and to Codex.

FAO's Food Quality and Standards Service also develops and publishes Manuals of Food Quality Control. These manuals provide recommendations for the development and operation of food quality and safety systems. While aimed primarily at providing advice to developing countries, the manuals document modern approaches, including the development of quality control programs throughout the food chain that apply to all countries. Such an approach is instrumental in facilitating international trade in food. Key titles in the series include Food Inspection, Food for Export, Management of Food Control Programs, Imported Food Inspection, and Quality Assurance in the Food Control Laboratory.

The program of technical assistance projects undertaken by the Food Quality and Standards

Service handles assistance in food quality control, including safety; such projects have established or strengthened the food control systems in a number of developing countries. Typically, they assist in establishing the infrastructure for an enhanced food control program, assessing laboratory service requirements, providing guidance to develop legislation and procedural manuals, setting up reputable inspection and certification systems, and providing training and staff development. In these assistance projects, the standards established by the CAC are basic guides to international requirements.

Conclusion

Food will always represent some biologic risk; it is the task of the food industry to maintain the level of risk at the minimum that is practical and technologically feasible. It should be the role of regulatory bodies to use risk assessment to determine realistic and achievable risk levels for foodborne hazards and to base their risk management and food safety policies on the practical application of the results of these analyses.

Foodborne illnesses are preventable. Adherence to good manufacturing practices and good hygienic practices and application of the HACCP system can result in food safety and ensure food quality. Food safety is the shared responsibly of governments, academia, the food industry, and the consumer.

Codex standards, guidelines, and recommendations have the objective of protecting the consumer and facilitating international food trade. Adherence to Codex provides the basis for food safety and quality and meets the requirements of international trade.

Foodborne Disease Control: A Transnational Challenge

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"Disease knows no boundaries and borders are porous to disease."(1)

In the globalized political economy of the late 20th century, increasing social, political, and economic interdependence is occurring as a result of the rapid movement of people, images, values, and financial transactions across national borders. Another consequence of the increase in transnational trade, travel, and migration is the greater risk of cross-border transmission of infectious diseases. As the world becomes more interconnected, diseases spread more rapidly and effectively. With more than one million people crossing international borders every day, and with the globalization of food production, manufacturing, and marketing, the risk of infectious disease transmission is greater. Economic globalization has also increased the need for governmental budget austerity, and consequent national preparedness has been eroded. The emergence of new infectious diseases, as well as the reemergence of old ones, thus represents a crucial transnational policy issue. These problems cannot be resolved by national governments alone; they require international cooperation. This article analyzes the role of foodborne disease surveillance programs, nationally and internationally, in the control of foodborne diseases.

In the past two to three decades, public health authorities in industrialized countries have been faced with an increasing number of food safety problems. In 1983, a Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Safety concluded that illness due to contaminated food was perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity (2). More recent data from industrialized countries indicate that annually up to 10% or more of the population may have a foodborne disease. The situation is equally serious in developing countries, where infant diarrhea causes many illnesses and deaths. In addition to known foodborne diseases, public health communities are being challenged by the emergence of new or newly recognized types of foodborne illnesses, often with serious and chronic health consequences. Certain populations (e.g., pregnant women, the elderly, infants and children, immunocompromised persons, and the undernourished) are particularly vulnerable. In economic terms, foodborne illnesses are very costly for industry, health services, and society as a whole.

Many factors have contributed to the increase in foodborne disease. Industrialization, leading to increased wealth and urbanization, has revolutionized the food supply system, resulting in mass production and an explosive increase in the number of food service establishments and food outlets. Mass production, environmental factors, and inadequate knowledge on the part of food handlers have contributed to increased contamination of primary foodstuffs.

The increase in international trade has increased the risk for cross-border transmission of infectious diseases. The globalization of food (and feed) trade, facilitated by the liberalization of world trade, while offering many benefits and opportunities, also presents new risks (3). Food, a major trade commodity, is also an important vehicle for transmission of infectious diseases. Because food production, manufacturing, and

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marketing are now global, infectious agents can be disseminated from the original point of processing and packaging to locations thousands of miles away. This multinational approach to food production and distribution and the progressive opening up of world markets have allowed the international food trade to flourish. The value of food trade, U.S. $266 billion in 1994, was more than 300% greater than it was 20 years ago and continues to grow rapidly (4).

The globalization of foodborne diseases also results from increased travel. International travel is more accessible today. The World Tourism Organization estimates world tourist arrivals at 567 million in 1995, and this figure is expected to rise to 660 million by the year 2000. Over the past 200 years, the average distance traveled and the speed of travel have increased 1,000 times while incubation periods for diseases have not changed. As a result, a person can be exposed to a foodborne illness in one country and expose others to the infection in a location thousands of miles from the original source of the infection (5). Depending on their destination, travelers are estimated to run a 20% to 50% risk of contracting a foodborne illness.

As international trade and travel increase, foodborne disease outbreaks of the same origin are more likely to occur in different parts of the globe. Food safety in the late 20th century represents a transnational challenge requiring enhanced levels of international cooperation in setting standards and regulations and in strengthening surveillance systems. Effective food safety programs, built on a clear understanding of the epidemiology of foodborne disease, must be developed and implemented. The globalization of the world's economy has been accompanied by intense economic competition and increased pressure on governments to downsize. Public sector austerity has reduced disease surveillance in many countries (6). For example, in Great Britain, the failure to maintain public health infrastructures has, in the words of the British Medical Association, resulted in "Britain returning to the 19th century in terms of public health, with problems such as dirty water, contaminated food, and old infectious diseases reemerging" (7). Failing a reversal of this trend, public health authorities and health services may be overwhelmed in the near future by outbreaks or epidemics of foodborne diseases. The 1991 epidemic of cholera in Peru and the 1996 outbreak of *Escherichia coli* O157 in Japan demonstrate how one single foodborne disease epidemic or outbreak may disrupt the functioning of a health-care system.

Epidemiologic surveillance of foodborne illness is fundamental to the planning of food safety programs and the development of a strategy for prevention and control. There are different methods of surveillance: death registrations and hospital discharges; disease notification; laboratory-confirmed cases; sentinel surveillance; surveillance of investigated outbreaks; population-based surveillance; and case-control studies of sporadic cases (8). This article examines the role of foodborne disease surveillance programs, nationally and internationally, in the control and prevention of foodborne disease.

Foodborne Disease Awareness of Public Health Authorities

Data on the incidence of foodborne illnesses collected through notifications, laboratory confirmations, and sentinel or population-based studies can provide a measure of the magnitude of the foodborne disease problems, their economic consequences, and over the years, an indication of the trend. Although several weaknesses are associated with the collection of such data—particularly those collected through notification and laboratory confirmations (since they represent only the tip of the iceberg)—they can nevertheless be useful in raising the awareness of public health authorities about the importance of food safety.

Surveillance data collected in some industrialized countries confirm that foodborne diseases constitute one of the most widespread health problems and that they have increased over the last two or three decades (Figures 1-4). Part of the increase may be attributable to recent improvements in information reporting and collection systems, improved diagnoses, or greater publicity and concern about food safety in general. However, a real increase of foodborne disease incidence is not disputed. First, the increase has been steady and cannot be explained by a one-time improvement in the surveillance system. Second, increases have been observed in different countries, including those with no improvement in reporting and surveillance programs. The general increase, as demonstrated by the results of surveillance data, has led many public health authorities to take stringent regulatory and

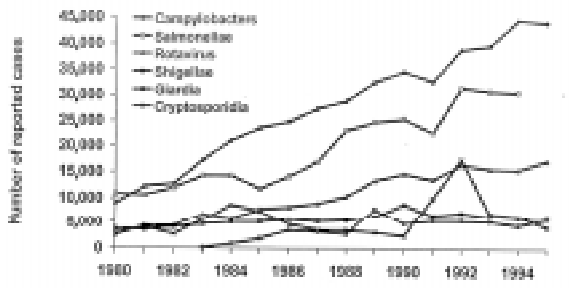

Figure 1. Laboratory reports of gastrointestinal infections in England and Wales.

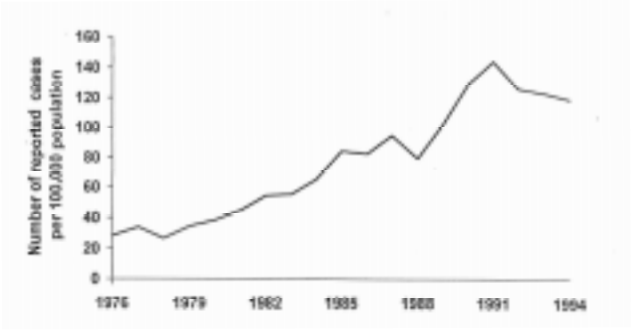

Figure 2. Incidence of foodborne diseases in Venezuela.

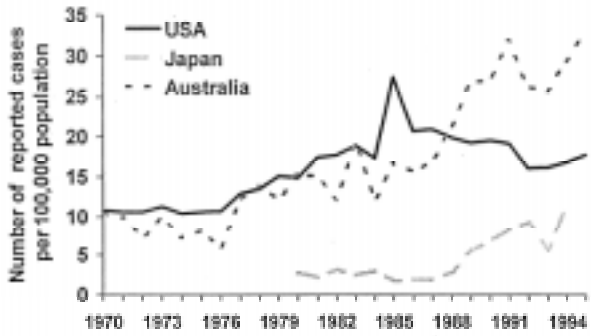

Figure 3. Incidence of salmonellosis in the United States, Japan, and Australia.

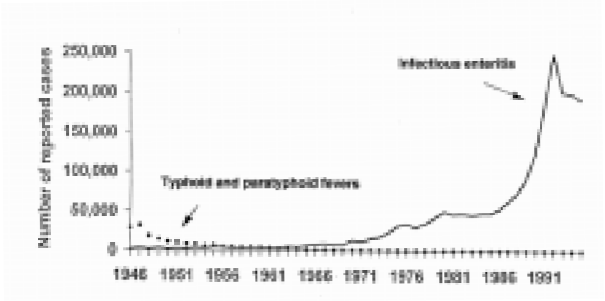

Figure 4. Incidence of infectious enteritis and typhoid and paratyphoid fevers in Germany.

educational measures to improve food safety, with some successful results (9). For instance, in the United States, active surveillance of foodborne listeriosis has led to concerted efforts by industry and government to prevent the disease. As a consequence, the number of cases and deaths has decreased by 44% and 48%, respectively (10).

Public health authorities must be aware of the magnitude and trend of foodborne illness so that necessary resources can be mobilized to improve food safety programs. Lack of reliable epidemiologic data in many parts of the world has impeded the recognition of the public health importance of food safety and consequently the emphasis on food safety programs.

Early Detection Of Foodborne Disease Outbreaks

Surveillance of foodborne diseases plays an important role in the early detection of foodborne disease outbreaks and their control. Early identification of the source of the outbreak is becoming increasingly important as countries move towards industrialization. Increased mass production means outbreaks can change from being small and confined to a family to large, affecting hundreds or even thousands of people (Table).

Rapid investigation of foodborne disease outbreaks is crucial to prevent them from taking on massive proportions. In the 1993 French outbreak of listeriosis due to potted minced pork (affecting 39 persons and causing eight miscarriages and one death), public health authorities traced its source within 1 week and thus prevented the outbreak from spreading by removing the implicated food product from the market and informing the group at risk about its unsafe nature (11). In an outbreak of botulism in

Table. Examples of large foodborne disease outbreaks

Country	Year	Disease	No. cases
United Kingdom	1985	Salmonellosis	1,000
United States	1985	Salmonellosis	>168,000
United States	1993	Salmonellosis	224,000
China	1988	Hepatitis A	>310,000
Germany	1993	Salmonellosis	1,000
Australia	1991	Norwalk-like agent	>3,050
United States	1992-93	*E. coli* O157 infection	>500
Japan	1996	*E. coli* O157 infection	>6,000

the United Kingdom traced to hazelnut yogurt, the source was identified within 3 days, and the product was withdrawn from the market (12).

Because of global food distribution and worldwide travel, an international exchange of information on foodborne disease incidences and outbreaks and the foods involved is extremely important to identify international clusters originating from a common source. For instance, Salm-Net, a network for the international surveillance of human salmonellosis, has demonstrated the value of such an interactive international collaboration. Individual countries with apparently isolated outbreaks can feed their information into the network and ascertain whether the outbreak is confined to their country or is of wider international importance. The identification and investigation of several international outbreaks have been simplified by the Salm-Net network.

Food, the Transmission of Diseases, and the Identification of Associated Risk Factors

Information collected through investigation of foodborne disease outbreaks or case-control studies of sporadic cases provides a better understanding of the role of food in the transmission of communicable diseases and in the identification of risk factors leading to disease. Epidemiologic data from foodborne disease surveillance can provide public health authorities with important information about the types of food implicated in outbreaks; populations at risk; practices that lead to contamination, growth, and survival of foodborne pathogens; and places where foods are often mishandled. Such data are essential for designing effective intervention programs. Such programs in industrialized countries, for example, have demonstrated the relatively greater prevalence and incidence of foodborne diseases of microbial origin over those of chemical origin and the role of food handlers in the transmission of diseases; they have identified campylobacteriosis and salmonellosis (particularly infections caused by *Salmonella* Enteritidis) as the leading foodborne diseases. The emergence of other diseases, such as infections due to *E. coli* O157 and *Listeria monocytogenes*—often with serious sequelae—has been pinpointed as a major public health problem. These surveillance programs have also alerted public health authorities to the foods most often implicated and the major risk factors in food preparation.

Because of the lack of epidemiologic data, the role of food in the transmission of diseases has been poorly acknowledged, particularly in developing countries. Diarrheal diseases in infants and children and diseases such as shigellosis and cholera have been perceived as being water-borne for many years. For instance, after the cholera epidemic in Peru (where epidemiologic investigations implicated, among other foods, seafood, and an embargo was placed on trade in foodstuffs), the role that food plays in the transmission of the disease began to be fully recognized.

Increased trade in food, international travel and migration, and economic and technologic development have changed dietary habits. New foods, food preparations, and dietary habits are introduced into different regions, and as a consequence, foodborne diseases are emerging or reemerging. Dietary habits are also changing as a result of nutritional recommendations and campaigns or may be influenced by food policy, production systems, or environmental changes that lead to increased access to certain foods. These changes in dietary habits influence the epidemiology of foodborne illnesses and contribute to the emergence of foodborne diseases. In the United States, public information campaigns promote an increased consumption of fruits and vegetables. To meet the increased demand, these products have to be imported on a seasonal basis. At certain times of the year, more than 75% of the fresh fruits and vegetables available in grocery stores and restaurants are imported (13). Epidemiologic data have shown that, partly as a consequence of the increased consumption of fruits and vegetables, the proportion of foodborne disease outbreaks has doubled (14).

Data collected through foodborne disease surveillance programs permit the monitoring of changes in the epidemiology of foodborne diseases and the identification of new pathogens and new dietary or food preparation habits that may present a health risk. The data can also determine if existing programs need to be readjusted to ensure that the food safety program is adequate and relevant.

A method used in recent years to complement epidemiologic data in identifying risky practices and behavior is the Hazard Analysis and Critical Control Points system (HACCP). Application of HACCP to food preparation permits the

identification of practices that may be potentially hazardous and need to be modified or those that are critical for ensuring the safety of foods and require specific monitoring. However, the first principle of HACCP—to conduct a hazard analysis—calls for epidemiologic data on foodborne diseases, as the process involves an appraisal of the possibility of hazards and the severity of their effects; the qualitative and quantitative evaluation of the presence of hazards; the survival and multiplication of microorganisms of concern; the production or persistence of toxins, chemicals, or physical agents in foods; and, conditions leading to the above.

As demonstrated in the decision tree for hazard analysis (Figure 5) (15), access to information would be difficult without epidemiologic surveillance of foodborne diseases. Similarly, epidemiologic data are also needed to develop sampling plans of food, as demonstrated in the decision tree for *Listeria monocytogenes* sampling plans of foods (Figure 6) (16).

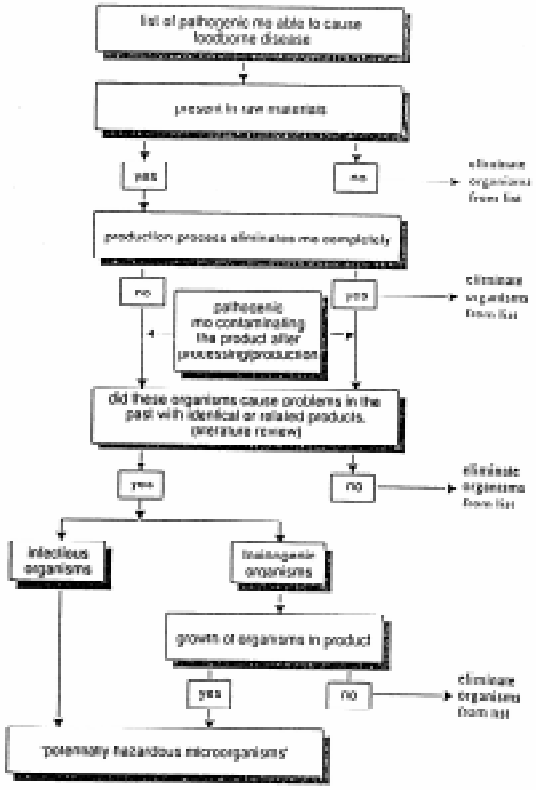

Figure 5. Hazard identification: identification of potentially hazardous microorganisms (15).

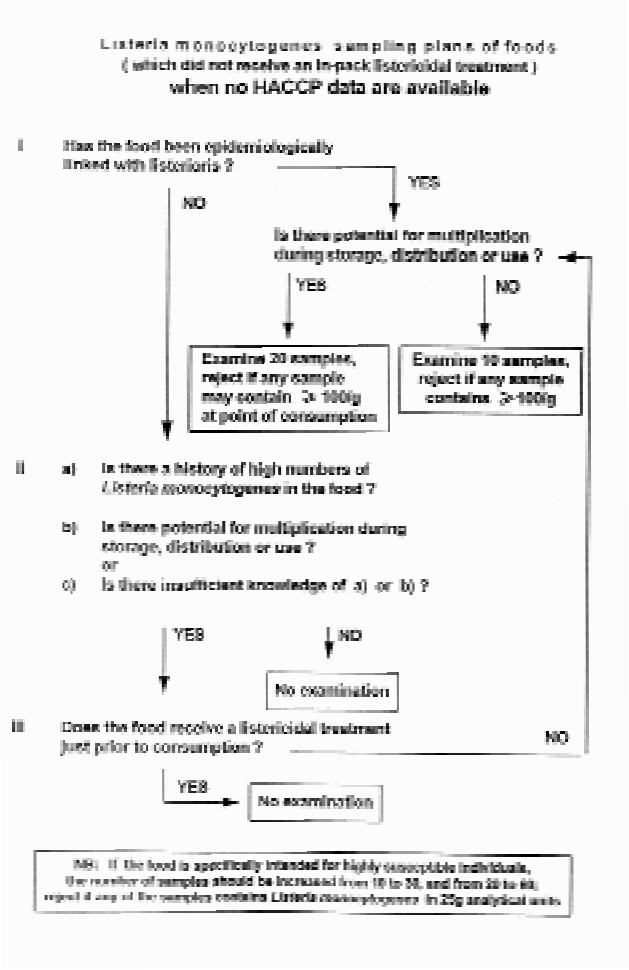

Figure 6. *Listeria monocytogenes* sampling plans of foods that did not receive an in-pack listericidal treatment (16).

Planning and Evaluating the Effectiveness of Food Safety Programs

The collection of epidemiologic data is important in planning interventions and setting priorities. Countries with scarce resources, facing an abundant number of foodborne diseases and food safety problems, need to prioritize food safety issues. Epidemiologic data provide a basis for identifying foodborne diseases, groups at risk, or even priority points in the food chain.

Evaluating the effectiveness and impact of an intervention is an important element of any plan. Data collected through disease notification or sentinel studies permit an evaluation of the effectiveness of interventions and their impact on health, and if necessary, the adjustment of a program to improve its efficacy and impact. Data

on the rising incidence of foodborne illnesses in many countries demonstrate that present prevention strategies, mainly based on regulatory measures, are inadequate and emphasize the need for additional measures (e.g., additional regulatory initiatives and health education about food safety).

Risk Assessment and International Food Standards

The movement of ever-increasing quantities of food across borders has resulted in a transnationalization of disease risk (17). Therefore, the globalization of food trade and the open access to foreign markets need to be accompanied by effective means of health protection for populations. In the food sector, international regulatory instruments need to be integrated with strengthened surveillance and monitoring.

As a result of the Uruguay Round of Multilateral Trade Negotiations and the increased liberalization of trade facilitated by this agreement, concern about the safety of imported food has grown. However, provisions in the Agreement on the Application of Sanitary and Phytosanitary Measures, which entered into force with the establishment of the World Trade Organization on January 1, 1995, are designed to address these concerns: according to the work of the Codex Alimentarius Commission, its standards, guidelines, and recommendations are recognized as the reference for national food safety requirements. Countries that are members of the World Trade Organization may no longer be able to reject foods that meet Codex standards, guidelines, and recommendations without providing justification.

Moreover, the increased volume of the global food trade underscores the need for sound epidemiologic information and international risk assessment. In this regard, Article 5 of the Sanitary and Phytosanitary Measures agreement explicitly requires World Trade Organization members to conduct scientific and consistent risk assessments. Furthermore, the World Health Organization has recommended that the application of the HACCP system at every stage of the food chain represents an effective approach for governments to meet the terms outlined in the agreement (18).

Another issue receiving more attention from regulatory agencies and underlined during the Food and Agriculture Organization/World Health Organization Conference on Food Standards, Chemicals in Food, and Food Trade (1991), is the scientific basis of the Codex standards. The Conference recommended that the Codex, in its norm-setting work on health and safety, place greater emphasis on risk assessment (19). Epidemiologic data on foodborne diseases have an important role in risk assessment. One example is assessing the risk of contracting listeriosis associated with different levels of *Listeria monocytogenes* in smoked fish and meat products (16). However, the need for risk assessment as the basis for setting standards has shown a great gap in knowledge about foodborne pathogens and their relation to human illness (20-22). To address the national/transnational risks caused by foodborne diseases, this gap must be narrowed.

Risk Assessment Approach

Risk assessment is defined as a scientifically based process that has the following steps: 1) Hazard identification—The identification of biologic, chemical, and physical agents present in a particular food or group of foods that can cause illness. 2) Hazard characterization—The qualitative or quantitative evaluation of the nature of the illness associated with biologic, chemical, and physical agents that may be present in food. For chemical agents, a dose-response assessment should be performed. For biologic or physical agents, a dose-response assessment should be performed if the data are obtainable. 3) Exposure assessment—The qualitative or quantitative evaluation of the likely intake of biologic, chemical, and physical agents in food as well as exposures from other sources. 4) Risk characterization—The qualitative or quantitative estimation, including uncertainties, of the probability of and severity of known or potential illness in a given population on the basis of hazard identification, hazard characterization, and exposure assessment.

In many cases, data are not available to support a quantitative risk assessment of biologic hazards. We discuss next the types of challenges that make quantitative risk assessment difficult for pathogenic organisms associated with food and the role of epidemiologic surveillance.

Hazard Identification

Because only some foodborne disease outbreaks are adequately investigated and have the

etiologic agents identified, many foodborne pathogens remain unidentified. Most of the available epidemiologic data are furnished by industrialized countries, while the situation in developing countries is largely unknown. The epidemiologic database must be extended to include information from developing countries. However, investigation and surveillance systems in developing countries need to be strengthened before the database can expand.

Hazard Characterization

For many foodborne pathogens, dose-response data are limited or nonexistent. Information on which dose-response estimates can be based is difficult to obtain and may be inaccurate for various reasons: host susceptibility to pathogens is highly variable, attack rates from a specific pathogen may vary widely, virulence of a pathogenic species is highly variable, pathogenicity is subject to genetic variation resulting from frequent mutation, antagonism from other bacteria in foods or the digestive system may influence pathogenicity, and foods may modulate the ability of bacteria to infect or otherwise affect the host.

Exposure Assessment

An exposure assessment will give an estimate of either the number of pathogenic organisms or the level of toxins consumed in food. Although the levels of chemical agents in food may change only slightly due to processing, the population of bacterial agents is dynamic and may increase or decrease dramatically. Changes in populations of bacteria are affected by complex interactions of these factors: ecology of the bacterial pathogen; processing, packaging, and storing of food; preparation steps, such as cooking, which may inactivate bacterial agents; and cultural factors relating to consumers.

In addition, for some of the emerging foodborne pathogens, the sources of exposure are still not fully understood. Information on foodborne disease outbreaks provides an opportunity to learn about the types of foods that may harbor the pathogen.

Risk Characterization

Characterizing the risk associated with biologic pathogens depends on information gained in the previous steps. Risk characterization will result in a qualitative or quantitative estimate of the potential for adverse effects from a particular pathogen on a specific population. Whether a quantitative risk assessment approach is possible and appropriate for characterization of risks associated with foodborne pathogens is not known. Thus, the qualitative approach to characterizing risk may be the only alternative.

International Travel

International travel and migration are contributing factors in the spread of foodborne diseases in some countries. For instance, 80% to 90% of the incidence of salmonellosis in Scandinavian countries is attributed to international travel. Surveillance of travel-related foodborne diseases provides a mechanism for appreciating the relative prevalence of foodborne diseases in various countries. It also provides a basis for informing physicians and health services about unfamiliar diseases contracted by travelers returning from distant places. In this way, advice on precautionary measures can also be given to travelers. The only foodborne disease now covered by the International Health Regulations is cholera, which is reported to the World Health Organization. Since the purpose of these regulations is to help provide maximum security against the international spread of diseases with a minimum of interference with world traffic (i.e., trade and travel) (23), it is timely to consider whether the regulations should cover additional foodborne diseases.

Conclusion

The globalization of the risks associated with foodborne illness, specifically increased international travel and trade in food, has resulted in greater interdependence in terms of food safety. Therefore, internationally agreed-upon food safety standards and other types of agreements are becoming increasingly important in addressing the complex transnational challenge of foodborne disease control. Epidemiologic data provide a common ground for reaching international consensus on food safety issues.

As Morris Potter has said, "If one recognizes that ensuring food safety is inherently uncertain, foodborne illnesses become opportunities to learn rather than failures to predict. Foodborne disease will occur, and we must be prepared to react quickly to reduce the risk of new foodborne hazards" (24).

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Consumer Concerns: Motivating to Action

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Microbiologic safety is consumers' most frequently volunteered food safety concern. An increase in the level of concern in recent years suggests that consumers are more receptive to educational information. However, changing lifestyles have lessened the awareness of foodborne illness, especially among younger consumers. Failure to fully recognize the symptoms or sources of foodborne disease prevents consumers from taking corrective action. Consumer education messages should include the ubiquity of microorganisms, a comprehensive description of foodborne illnesses, and prevention strategies. Product labels should contain food-handling information and warnings for special populations, and foods processed by newer safety-enhancing technologies should be more widely available. Knowledge of the consequences of unsafe practices can enhance motivation and adherence to safety guidelines. When consumers mishandle food during preparation, the health community, food industry, regulators, and the media are ultimately responsible. Whether inappropriate temperature control, poor hygiene, or another factor, the error occurs because consumers have not been informed about how to handle food and protect themselves. The food safety message has not been delivered effectively.

Consumer Knowledge and Concern

Consumers are receptive to information about microbiologic hazards. Nationwide surveys by the Food Marketing Institute indicate that more people volunteer concerns about microbiologic hazards than about any other potential food safety issue. From 1992 to 1996, volunteered concern about microbiologic safety increased from 36% to 49% (1). Specifically, concern about contamination by bacteria or other microorganisms was 77%, more than concern about pesticide residues (66%), product tampering (66%), antibiotic residues (42%), or any other food safety risk.

Food-Handling Practices

Although many consumers recognize the potential seriousness of foodborne bacteria, they lack information on safe handling and storage of food products (2). Williamson et al. (3) found that consumers under 35 years of age knew less about food safety terms and concepts than those over 35. Specific safe food handling was not practiced by 15% to 30% of survey respondents. For example, consumers did not cool cooked food rapidly, with 29% indicating they would let roasted chicken cool completely before refrigerating. Only 32% indicated they would use small, shallow containers to refrigerate leftovers. Consumers did not know that failure to refrigerate may jeopardize safety, with 18% not concerned or uncertain about the safety of cooked meat and 14% not concerned about poultry left unrefrigerated for more than 4 hours. The need for sanitation was not recognized, with only 54% indicating they would wash a cutting board with soap and water between cutting raw meat and chopping vegetables.

Food safety experts have identified the most common food-handling mistakes made by consumers at home. These mistakes include serving contaminated raw food, cooking or heating food inadequately, obtaining food from unsafe sources, cooling food inadequately, allowing 12 hours or more between preparation and eating, and having a colonized person handle implicated food or practice poor hygiene (4). The same factors were identified in mishandling associated with specific pathogens (5).

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Changing Lifestyles

Many factors have contributed to consumers' lack of familiarity with safe food handling and increased foodborne illnesses. Increased participation in the paid labor force has lessened the exposure of young people to food-handling practices in the home; few schools offer or require food preparation classes; and partially prepared foods may have different, less familiar handling requirements (2,6).

People eat out more frequently today, thereby increasing their exposure to the food service industry, noted for high turnover rates and minimal job training in personal hygiene (7). Furthermore, the population is shifting, with an increased percentage of persons at higher risk for foodborne illness because of age or health status (8,9). Additionally, some food safety recommendations related to temperature and acidity do not eliminate risks from some pathogens (2).

Nature and Source of Foodborne Illness

Consumer perceptions and behavior related to foodborne illness changed little between 1988 and 1993 (10). Consumers misperceived the nature of foodborne illness and the most likely pathogen source. Consumer belief about the type of food responsible for foodborne illness—meat, poultry, seafood, eggs—was consistent with expert opinions; however, consumers believed that foodborne illness was generally mild, without fever, and occurred within a day of eating contaminated food. Infections caused by Salmonella and Campylobacter, the most common foodborne illnesses in the United States (11), are not consistent with the symptoms consumers described, because these organisms have longer latency and cause fever.

Most consumers believed that their foodborne illness was caused by food prepared somewhere other than the home. Williamson (3) found that about one-third of consumers thought food safety problems most likely occurred at food manufacturing facilities, and one-third blamed unsafe restaurant practices. Only 16% thought mishandling was most likely to occur in the home. Fein et al. (10) found that 65% of consumers attributed foodborne illness to food prepared at a restaurant, 17% to mishandling at the supermarket, and 17% to mishandling at home. In contrast, food safety experts believe sporadic cases and small outbreaks in the home are far more common than recognized outbreaks (2).

Failure to recognize the home as a likely source of foodborne illness is not unexpected because illness traced to a food establishment affects many people and may receive widespread publicity (12). Illness that occurs at home is rarely reported unless severe (2).

If consumers misperceive the nature and origin of foodborne illness, they underestimate the frequency of serious consequences and are less motivated to change. Schafer et al. (13) found that motivation for proper food handling requires viewing the mishandling of food as a direct threat to one's health. The failure to associate mishandling of food in the home with foodborne illness interferes with foodborne disease education efforts (10).

Ubiquity of Organisms

Consumers do not seem to be aware of the ubiquity of microorganisms in the environment. During foodborne disease outbreaks, press accounts focus on fecal contamination of food. Government standards classify natural microorganisms as contaminants, which suggests that microorganisms are only present as a result of mishandling. In contrast, Hazard Analysis and Critical Control Points (HACCP) programs recognize and attempt to control potential dangers related to pathogenic microorganisms.

When consumers in a national sampling were asked on whom they rely for product safety, the percentage responding "myself as an individual" decreased from 48% in 1989 to 25% in 1996 (1). As self-reliance decreased, consumer reliance on food manufacturers and supermarkets increased. This may be a response to the message that if raw food contains microorganisms, it is contaminated. It suggests some consumers are shifting the responsibility for safe food to manufacturers and retailers.

Consumers may not realize they can introduce pathogens during food handling. In 1990, the Food Marketing Institute asked consumers what steps they took at home to ensure the safety of food (14). Respondents volunteered refrigeration (58%), proper storage (35%), checking expiration dates (26%), washing and cleaning the food (25%), cooking properly (22%), and wrapping food properly (20%). No one volunteered washing hands or preventing cross-contamination.

Labeling

Products must contain safety labels instructing consumers how to handle food. In 1989, the National Advisory Committee on Microbiological Criteria for Foods recommended a mandatory uniform logo for perishable refrigerated foods, uniform labeling for frozen food, "Use by" dates, and time/temperature indicators wherever possible (15).

Although products are currently labeled when they require refrigeration, the label is ineffective because the warning is difficult to find or read. As the proportion of older people increases, print must be larger. Labels should also display symbols to further enhance the effectiveness of the message.

Safe-handling labels on meat products appear to have made a difference. The Food Marketing Institute (1) found that 60% of survey respondents had seen the labels. Of those aware of the labels, 65% said the labels increased their awareness of safety, and 43% said they changed their behavior as a result of the information. The most common volunteered change was washing the counter and utensils after contact with meat (approximately 40%), followed by washing hands more frequently (approximately 20%) and cooking to the proper temperature (approximately 20%).

Labels do not consistently contain needed information. When foodborne illness was related to consumption of unpasteurized apple juice, consumers were not able to determine from the label which products were pasteurized. Many major manufacturers do not indicate whether their fresh juice product is pasteurized.

Consumers are not advised about potential risks for special populations. Raw milk sold in California must contain a warning statement, but other states may not have this requirement. Because of inconsistencies in labeling, unpasteurized juice products may be given to infants. Also, products that contain honey do not include a warning about potential risk for infant botulism.

Processing Technology

Consumers do not realize that pathogens can survive minimal processing, as evidenced by a recent *Escherichia coli* outbreak associated with fresh apple juice, which demonstrated that processors also may not recognize potential risks. A fresh apple juice manufacturer in northern California claimed its product was safe because the juice was squeezed in small batches and frozen immediately.

Freezing is not effective against *E. coli* O157:H7, but other methods are protective. Several methods have been developed to reduce pathogens and increase the safety of foods. Once these methods are verified as effective and safe, the food industry should be free to use them, and consumers should have the opportunity to select safer foods. In some cases, the regulatory approval process appears to hinder rather than facilitate the safer handling of food.

Food irradiation, exposing food to high levels of electromagnetic energy for specific purposes, has been approved for selected uses. A petition before the Food and Drug Administration to permit irradiation of meat and other muscle foods appears to have satisfied safety concerns, but approval has not yet been granted. The requirement to seek approval for each application of irradiation prevents rapid response in cases of foodborne outbreaks. Although this regulatory procedure may have been reasonable when irradiation was first introduced, it warrants a fresh look in view of the wealth of data now available on the safety and wholesomeness of food irradiation (16).

Attitude surveys and marketing experience consistently demonstrate that consumers will purchase irradiated food (17). National surveys indicate consumer concern about irradiation was lower than other food-related concerns. When specifically asked what they considered a serious health hazard, 29% identified irradiation, 77% identified bacteria, and 66% identified pesticides (1). The percentage of consumers concerned about irradiation has decreased significantly over time. In the late 1980s, 42% to 43% classified irradiation as a serious concern, decreasing to 29% in 1996. A relative ranking of food processing methods surveyed by the Gallup Organization found that irradiation, food preservatives, and chlorination generated similar concern (18).

In a nationwide Food Marketing Institute survey, 69% of consumers indicated they were very or somewhat likely to purchase products irradiated to kill bacteria or other microorganisms (1). Surveys completed in several areas of the country indicate 60% to 70% of consumers would prefer irradiated food (17). In one study, information about irradiation increased interest in purchasing to 90%, and education plus food samples increased purchase intent to 99%.

Consumers have purchased irradiated food in select locations across the United States since 1992 (17). Fruits from the Mainland and Hawaii have sold well in the Midwest and California (L. Wong, pers. comm.). Irradiated chicken gained over 60% of market share when priced 10% lower than nonirradiated chicken and 47% when priced the same (J.A. Fox, pers. comm.).

Irradiated food is not widely available. Special interest groups threaten companies that exchange information about irradiation processing. Consumers, however, appear to prefer irradiated foods when the benefits of these foods are endorsed by health professionals. Food manufacturers and retailers should offer consumers the choice of safety-enhanced, energy-pasteurized irradiated food.

Communicating with the Public

To respond to consumers' need for information, a multifaceted program is needed. The HACCP strategy, which teaches consumers to critically think through the food safety process to determine how foodborne illness could occur, has been effective (19,20). The HACCP approach to home food preservation is logical and highlights key control points (21).

Consumers should be informed that microorganisms are ubiquitous in the environment, found on raw products of animal or plant origin. Pathogens may survive minimal processing and preservation treatments. People may introduce pathogens during any stage of food processing or handling, including just prior to consumption. Foodborne illness can range from mild to severe and life-threatening with chronic complications. People have control and can reduce risks.

Communicating food safety information to the public effectively is another challenge. Consumers obtain most of their information on food, nutrition, and science from the media; television is cited most frequently, and newspapers and magazines follow (22,23). Brochures enforce messages and serve as useful references, although they are not as widely seen as media stories.

Developing messages with the press should be a primary activity of a food safety education program. Consumers judge a message by the credibility of the person conveying it, its appeal to their common sense, and the frequency of the message (24). Media presentations can motivate people to listen and change behavior. Consultants from the USDA hotline say, "We've seen an explosion in media coverage of food safety, and callers want more detailed explanations of things they read and hear" (25).

Information on safe food handling must be motivating and memorable. Stories that capture the public's attention are personal. They relate life experiences of people with whom the public can identify. Stories of the consequences of mistakes are memorable. They can be touching, humorous, or grotesque. It is easy to visualize and remember the infected bakery worker who made 5,000 people ill when he mixed a vat of buttercream frosting with his bare hands and arms despite bouts with diarrhea (26).

Stories can be heartrending, as in the experiences of a family who lost a child to *E. coli* O157:H7 infection. It is difficult to document the effectiveness of vivid accounts of doing things right or wrong. However, when Washington state carried extensive coverage linking the outbreak to undercooked hamburgers, 13% of the men said they ate undercooked hamburger, compared with 38% in Colorado (25).

Conclusions

Consumer concerns about foodborne illness can motivate change in regulatory and industry use of technology, product labeling, and consumer education.

New Technologies

The food industry has both a right and a responsibility to use safe and effective technology to enhance the safety of the food supply. Regulatory authorities should expediently evaluate and facilitate new technologies, such as food irradiation, laser light treatment, and high-pressure processing, which enhance food safety. Health professionals, the food industry, and regulators should challenge special interest groups that distort information and strive to limit consumer choice.

Improved Labeling

Processed and packaged food should bear labels that clearly indicate how food should be handled. Labels should include warnings about special risks to select populations. Benefits from special processing that can reduce microbes should also be encouraged.

Consumer Education

Consumers need to appreciate the seriousness of foodborne diseases. They must learn to

recognize unsafe food-handling practices, the latency period for some microbes, and the symptoms of foodborne disease. They need to understand how to protect themselves through kitchen and personal hygiene, including thoroughness and frequency of hand washing, temperature control, and safe food choices such as foods processed by heat or energy pasteurization. Young people should be reached through age-specific school curricula, such as personal hygiene and special "living skills" units that address food safety and diet. Food industry and health educators should work with the media to develop interesting and timely messages to increase consumer knowledge about safe food handling. Messages must be consistent, science-based, frequent, and personalized.

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Identifying and Controlling Emerging Foodborne Pathogens: Research Needs

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Systems for managing the risks associated with foodborne pathogens are based on detailed knowledge of the microorganisms and the foods with which they are associated—known hazards. An emerging pathogen, however, is an unknown hazard; therefore, to control it, key data must be acquired to convert the pathogen from an unknown to a known hazard. The types of information required are similar despite the identity of the new agent. The key to rapid control is rapid mobilization of research capabilities targeted at addressing critical knowledge gaps. In addition, longer-term research is needed to improve our ability to respond quickly to new microbial threats and help us become more proactive at anticipating and preventing emergence. The type of contingency planning used by the military in anticipating new threats serves as a useful framework for planning for new emergence.

The microbiologic safety of food has been advanced substantially by the introduction and implementation of Hazard Analysis and Critical Control Points (HACCP). HACCP provides a systematic conceptual framework for identifying hazards and focusing efforts on the proper functioning of key food production, processing, and marketing steps. When applied appropriately, HACCP is a cost-effective means of controlling known hazards in foods. Its successful implementation depends on knowledge of such issues as the pathogenic microorganisms' virulence, cultural characteristics, ways in which they contaminate the food, effects of food processing and preparation on their survival, and food consumption patterns. Because it requires substantial knowledge, HACCP cannot be expected to control unknown hazards, such as emerging foodborne pathogens. Therefore, controlling a new foodborne microbial threat requires moving the hazard as quickly as possible from being unknown to being known. The key to this transition is the timely acquisition of needed research data. This article identifies classes of research information needed and discusses a conceptual approach for addressing unknown microbial threats.

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Anticipating the Next Emerging Pathogen

Two types of emergence are encountered with pathogenic foodborne microorganisms. A true emergence, where a microorganism that had not been identified as a public health threat begins to cause disease, is relatively rare. More common is reemergence, where a known microorganism causes disease in a new way, for example, by causing new types of infections, being associated with new foods, or appearing in new geographic locations. For both types, the operational requirement is to control an unanticipated public health threat. The timeliness of response is critical since the public health and economic costs of an emerging pathogen are directly related to the time between its emergence and its control.

The events that lead to emergence are often complex, with the cause often being obscure and only indirectly related to the new agent. Past emergence of foodborne pathogens has been associated with changes in microbial genotypes, demographics, food production and processing methods, marketing and preparation practices, medical diagnostics, globalization of the food industry, changes in consumer education, and general socioeconomic trends (1-3). Planning for a microbial threat is a challenge because one does not know what the agent will be, what food it will be associated with, or where or when emergence will occur. While there are several potential ways of anticipating and responding to microbial

threats, the contingency planning used by the military to anticipate threats seems well suited for emerging pathogens. Military contingency planning can be viewed as having four major components: intelligence, personnel and facilities, rapid response, and strategic planning.

Intelligence is the gathering of medical, scientific, and other information that allows emergence to be identified. In the United States, this role is filled to a great extent by the Centers for Disease Control and Prevention (CDC). In addition to providing information on known foodborne pathogens, CDC works with local public health agencies and the medical and scientific communities to investigate new disease syndromes and identify unrecognized foodborne pathogens. This type of intelligence gathering played a pivotal role in the recent recognition of *Cyclospora* as a cause of foodborne gastroenteritis. CDC's new sentinel site program, FoodNet, is expected to greatly enhance the identification of new foodborne diseases. However, these surveillance activities are largely limited to the United States, whereas an effective intelligence system for foodborne disease must be worldwide. For example, *Cyclospora* was identified as a likely foodborne or waterborne pathogen in Asia and South America before an outbreak was reported in the United States. Intelligence related to foodborne disease can be acquired from several sources: the World Health Organization's surveillance program, the U.S. military's international network of laboratory and medical investigators, medical and scientific reports, and the Internet. The Internet is increasingly an important source of intelligence related to emerging pathogens; through news groups and bulletin boards such as ProMed, scientists and public health practitioners share their experiences on almost a real-time basis. Such advances in intelligence gathering are critical to reducing the time between emergence and control. However, limiting intelligence to medical considerations is not enough: intelligence gathering must include awareness of changes and advances in food production methods, agricultural practices and conditions, veterinary medicine, environmental and water microbiology, food technologies, consumer trends, and general socioeconomic conditions.

The second component of contingency planning is ensuring sufficient personnel and facilities to characterize a new biologic agent and develop control strategies. The inability to predict the agent or the associated food, coupled with the degree of specialization required of investigators, requires a broad range of capabilities and resources. However, no one organization is likely to maintain the capabilities needed to deal with all contingencies. If we were to follow the military pattern, we would have reserve groups that could be mobilized as needed. However, even this approach requires planning and support to ensure the needed expertise and facilities. For example, the number of researchers and laboratories studying *Clostridium botulinum* has dropped to a point where it would be difficult to rapidly mobilize a research team, despite this pathogen's history of reemerging in a surprisingly wide range of foods.

Rapid mobilization of resources is the third component. This component is particularly important for free-living infectious agents because one goal is to limit their dissemination to prevent them from establishing secondary reservoirs. It is much easier to fight a small, contained war than a global one. The mobilization of resources to respond to an emergence must be appropriate to the severity of the threat. Overreacting hurts the credibility of the entire system, while underreacting increases both the public health and economic impact. Rapid response efforts have focused at identifying new agents and removing suspect food from the marketplace, two key initial steps. However, research to prevent another occurrence of the emerging pathogen has been much less organized and timely.

The fourth component of contingency planning, strategic planning, is actually the first chronologically. This is the phase where members of war colleges pose "what we would do if" scenarios and plan appropriate responses. This type of contingency planning has generally received attention in relation to emerging pathogens only in connection with the use of biologic warfare agents. This process relies on futurist thinking to consider how changes in society, economics, technology, agriculture, medicine, and international trade may affect the microbiologic safety of the food supply. Such a broad view is needed because more general events or trends in society cause most disease emergence. This type of strategic planning is undertaken with the realization that the probability of any specific "what if" scenario is

low, but the probability that one scenario will materialize is extremely high.

Research Needs

Research, an integral part of responding to a new foodborne microbial threat, is the key for moving a new or reemerging biologic agent from being an unknown pathogen to being one for which control measures are available. Two areas of research can be classified on the basis of time constraints. Acute research needs are deficiencies in knowledge that must be addressed to establish control of an emerging pathogen. This research is highly targeted and specific for the microorganism and food of concern; it must be accomplished as quickly as possible. Acute needs generally require applied research, although basic research may have to be conducted if the deficiencies in knowledge are great. The second class encompasses longer-term basic and applied research needs not mandatory to immediate control.

Acute Needs

While the data needed for any single emerging biologic agent are highly specific, acute research needs fall into general categories that are virtually the same for all new pathogens. Common research questions include the following: Are methods available for detecting and categorizing the agent? What food is the vehicle for the pathogen? How do the implicated foods become contaminated? What is the pathogen's reservoir in nature? Is the pathogen's presence in contaminated food the result of an error or breakdown in normal controls? Does the pathogen grow in foods? Does the pathogen survive normal food processing, distribution, and preparation? How infectious/toxigenic is the pathogen? Are there subpopulations of consumers at increased risk for this pathogen? Is the pathogen's ability to cause disease restricted to specific strains with identifiable virulence characteristics? Answering these questions requires specific data that do not differ substantially from pathogen to pathogen (Table).

The criteria for classifying needs as acute are reasonably straightforward: Is the research needed to prevent a recurrence of the disease or to modify current HACCP plans? However, these questions have different priorities, which depend on when the information is needed. To deal with emerging pathogens, we should learn from modern business practices, especially the concept of "just-in-time" research. Little consideration has been given to how to assess and set research priorities for emerging foodborne pathogens. One attempt was provided as an appendix of the U.S. Pathogen Reduction Task Force. A relatively simple decision tree used a series of questions to

Table. Research data needed for most emerging foodborne pathogens

Research area	Knowledge gaps
Detection methods	Sampling and enrichment techniques
	Cultivating
	Biochemical/taxonomic char.
	Antibodies for capture and differentiation
	Subtyping
	Virulence-associated char.
	Detecting injured or viable-but-nonculturable cells
Microbial ecology	Contaminated foods
	Reservoirs and routes of transmission
	Life cycles
	Geogr. range and seasonality
	Route of contamination and location of pathogen in food
Pathogenicity	Dis. char. and diagnosis
	Sequelae
	Host range
	Infectious dose
	Subpopulations at risk
	Animal models
Growth characteristics	Free-living vs. obligate parasite
	Growth requirements
	Temperature
	pH
	Water activity
	Oxygen
Survival characteristics	Heat resistance
	D-values
	Z-values
	Susceptibility to anti-microbial food additives
	Acid resistance
	Sensitivity to disinfectants or dessication
	Sensitivity to radiation
	UV
	Ionizing
Control	Effectiveness of food preservation
	Inspection systems to segregate contaminated materials

identify what research was the limiting step in responding to the foodborne pathogen (4).

The timeliness of addressing research needs must be an integral part of the planning process, but has been generally overlooked. Past research mobilization efforts for new foodborne microbial threats can be best described as haphazard, likely because they reflect the way research is funded. The traditional means of ensuring strong research programs, competitive funding of projects after proposals have undergone extensive peer review, is time consuming and often not appropriate for the acute phase of responding to an emerging foodborne pathogen. Further, the peer-review process tends not to select the often mundane research needed during the acute phase of an emergence. Two alternative approaches may be more effective. The first is to have a series of designated laboratories that have as part of their mission and funding the task of being able to modify their research programs to address acute research needs. Such laboratories would need to have a critical mass of facilities and expertise in various aspects of food safety microbiology. The second approach is to have a group of reserve scientists with unique expertise or access to facilities not available at the designated laboratories or needed to supplement those capabilities. Funds could be earmarked to noncompetitively fund such reserve scientists on an as-needed basis, with the understanding that research needs designated as acute would take precedence over other research needs.

Longer-Term Needs

The three areas of longer-term research associated with emerging pathogens are amenable to more traditional funding. The first area, specific to the new pathogen, consists of research for improvements or alternatives to the detection and control methods initially devised. With initial disease control established, basic and applied research can seek to understand the microorganism and develop more optimal approaches for its prevention, control, or elimination. The second area concerns activities to help reduce the time between the emergence of a pathogen and its initial control (e.g., improved surveillance through the development of new diagnostic methods and further identification and characterization of virulence determinants and modes of pathogenicity to accelerate detection of new agents). Just as important as acquiring research data is rapid data dissemination. The continuing development of computer-based information networks is a component of this second research area.

The third area focuses on identifying research factors that will allow new microbial threats to be anticipated. Of necessity, the current response to emerging pathogens is almost entirely reactive. The public health community detects a new syndrome, and only then is research mobilized, often during a crisis. While reactive response will always be part of dealing with emerging microbial threats, a more proactive approach is needed if prevention is to be even partially realized. In military terms, war is the last resort and represents the failure of diplomats to predict and prevent a crisis. Microbial threats, like wars, do not spontaneously emerge but are the result of a series of events or conditions. There is a need to reexamine how food is produced, processed, marketed, and prepared to identify conditions that contribute to emergence. For example, organic acids are used extensively throughout the food industry to control spoilage and pathogenic microorganisms. Archer (5) hypothesized that over time, exposure to pH conditions that stress but do not kill may lead to the emergence of hardier and possibly more virulent foodborne pathogens. It is already well established that the induction of acid tolerance can enhance both the survival and virulence of foodborne pathogens (6). Further, one of the basic tenets of microbial genetics is that conditions that kill most, but not all, of a bacterial population foster the development of resistance. This is supported by recent studies that suggest that bacterial stress responses may select for hypermutability (7,8). While these findings do not mean that organic acids should not be used as a tool for controlling foodborne pathogens, they suggest that proactive research should be conducted to find ways of using these agents that minimize the potential for resistance. Proactive research, including research that might appear unrelated to the emergence of foodborne pathogens, can draw on the already substantial body of basic research related to the conditions and requirements for gene transfer among biologic agents. For example, Baur et al. (9) reported on the conditions that led to the competence of *Escherichia coli* for genetic transformation in freshwater environments.

Maximal competence occurred when the bacterium was exposed to 2 mM Ca^{2+} as temperatures increased from 10°C to 20°C. With such information, researchers could examine food processing operations to determine the presence and importance of such conditions. For example, fruits and vegetables are often treated with calcium under fluctuating temperatures to enhance the texture during later processing.

A key to being more proactive in addressing the threat of microbial foodborne pathogens—consideration of root causes—will likely require food microbiologists to become involved in nontraditional research areas. If new biologic agents arise as the result of changes in technology, society, or global economics, predicting and preventing emergence will ultimately require better understanding of how such factors influence pathogen introduction and dissemination.

Conclusions

One of the critical lessons of the past 10 years is that we cannot become complacent about infectious diseases (1). Only a few diseases (e.g., smallpox) have actually been eliminated. The rest, including virtually all foodborne diseases, we hold in check, winning battles but not the war. Eventually, our weapons (e.g., antibiotics) become obsolete; pathogens (e.g., *E. coli)* become more dangerous; or we become complacent. Contingency planning must be developed and undertaken with a long-term commitment. Without that commitment and without understanding that planning is successful when problems are avoided or minimized, programs of this type lapse quickly. In the long term, the costs of planning, both in terms of economics and human suffering, are a fraction of those incurred as the result of the emergence of a major microbial threat.

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Maximizing the Usefulness of Food Microbiology Research

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Funding for food microbiology research often follows disease outbreaks: botulism from vacuum-packed white-fish chubs, listeriosis from soft cheeses, or illness due to *Salmonella* Enteritidis or *Escherichia coli.* As a consequence of research, detection, identification, and subtyping methods improve, and more is learned about pathogenicity and virulence. Research also explores the organisms' capacity to multiply or survive in food and to be killed by established or novel processes. However, rarely is there a critical overview of progress or trustworthy statements of generally agreed-on facts. That information is not maintained in a form that can readily be used by regulatory departments and the food industry to ensure a safe food supply. A centralized system is urgently needed that is accessible electronically and carries information in a standardized format on the essential properties of the organisms, including pathogenicity, methods of detection, enumeration and identification, alternative prevention and control methods, and growth and survival characteristics.

Most countries aim to regulate food and the food industry to ensure a safe and wholesome supply. Although the technology of food processing is based on tradition (drying, freezing, fermentation), research has resulted in a wide range of processes to meet the ever-changing requirements of consumers. Canning of low-acid foods set an enviable example of microbiologic safety. Today, consumers demand less heavily processed foods that contain a minimum of chemical preservatives, have a long shelf life, and are "fresh" yet microbiologically safe. Competitive food retailing requires such products to be no more expensive than existing products. In the United Kingdom, there has been a swing toward convenience foods and foods with ethnic flavors, sometimes with a premium price. Commercial success is evident, but relatively few of those foods have been evaluated experimentally for microbiologic safety. Emphasis is on risk assessment, on Hazard Analysis and Critical Control Points (HACCP) and process control, all of which rely heavily on existing knowledge. That knowledge, and the raw data from which it is derived, could be better organized and easier to use.

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The International Commission on Microbiological Specifications for Foods (ICMSF)

The history of microbiologic aspects of food control is reflected in the publications of ICMSF. ICMSF was established in 1962 as foodborne disease greatly increased microbiologic testing of foods. Increased testing, in turn, created widespread practical and regulatory problems in the international food trade. At that time, analytical methods were not standardized, sampling plans were of doubtful statistical validity, and the interpretation of results applied different concepts of biologic significance and acceptance criteria that confused and frustrated industry and regulatory agencies alike.

ICMSF was founded to assemble, correlate, and evaluate evidence about the microbiologic safety and quality of foods; to consider whether microbiologic criteria would improve and ensure the microbiologic safety of particular foods; to propose, where appropriate, such criteria; and to recommend methods of sampling and examination.

The primary purpose of the commission remains to give guidance on appraising and controlling the microbiologic safety of foods, with due attention to microbiologic quality.

Meeting the commission's objectives would facilitate international trade; the work of national control agencies, the food industry, and international agencies concerned with the humanitarian aspects of food distribution; and the health of consumers.

An advisory body, the ICMSF provides basic information through extensive study and experience and makes recommendations, without prejudice, based on that information. Results of the studies are published as books or papers.

At its meetings, the ICMSF functions as a working group. Its membership consists of 18 food microbiologists from 11 countries whose interests include research, public health, official food control, education, product and process development, and quality control. Members are from government laboratories in public health, agriculture, and food technology; universities; and the food industry. ICMSF also seeks assistance from consultants specializing in particular areas of microbiology. Four subcommissions (Balkan and Danubian, Latin American, Middle East and North African, and Southeast Asian) promote activities similar to those of ICMSF among food microbiologists on a regional scale and facilitate worldwide communication.

ICMSF raises its own funds and pays for its activities and meetings. The commission obtained support from government agencies, the World Health Organization, International Union of Microbiological Sciences and International Union of of Biological Sciences, and the food industry (more than 80 food companies and agencies in 13 countries).

During its first 25 years, ICMSF devoted the major portion of its effort to methodology research, improved standardization of methods, and 17 refereed publications (1). One important finding was that in analyzing salmonellae, samples could be composited, making it practical to collect and analyze the large number of samples recommended in some stringent sampling plans. With the evolution of alternative methods, rapid test kits, and the everexpanding list of biologic agents involved in foodborne illness, the commission reluctantly discontinued its program of comparison and evaluation of methods.

The long-term objective of enhancing the microbiologic safety of foods in international commerce was addressed initially in books recommending uniform analytical methods (2), sound sampling plans and criteria (3), and the microbial ecology of foods (4,5), which familiarized the analyst and food technologist with processes used in the food industry and foods submitted to the laboratory. Knowledge of the microbiology of major foods and the factors affecting their microbial content helps interpret analytical results.

At an early stage, the commission concluded that no food sampling plan could ensure the absence of a pathogen. Testing foods at ports of entry, or end-product testing elsewhere in the food chain, cannot guarantee food safety. This led the commission to investigate the potential value of HACCP for enhancing food safety. A joint ICMSF/World Health Organization meeting in 1980 led to a report on the use of HACCP for controlling microbiologic hazards in food, particularly in developing countries (6-8).

The commission published the principles of HACCP and procedures for developing HACCP plans (9); the publication discussed the relative importance of controlling the production and harvesting, preparing, and handling of foods. Recommendations are given for the application of HACCP at each step in the food chain—from production and harvest to consumption.

The commission recognized that a major weakness in the development of HACCP plans is hazard analysis; knowing about the many biologic agents responsible for foodborne illness has become difficult. The commission's latest book (1) proposes to facilitate the development and assessment of HACCP plans, improve the safety of foods, and facilitate risk assessment. A thorough concise review of reports on growth and death responses of foodborne pathogens, it is intended as a relatively quick reference manual to assist in making decisions. The commission is revising Microbial Ecology of Foods: (Vol. 2) Food Commodities with publication planned as Microorganisms in Foods 6 in late 1997.

ICMSF submitted its recommendations for sampling foods and acceptance criteria for *Listeria monocytogenes* to the Secretariat of Codex Alimentarius in 1993. To stimulate discussion, the recommendations were initially provided to the Codex; they were subsequently published (10) and revised after further discussion (11). At the request of the Secretariat of Codex, the commission developed recommendations for the revision of Principles for the

Establishment and Application of Microbiological Criteria for Foods, which appears in the Procedural Manual of Codex.

Members of ICMSF believe that the original objectives of the commission still apply. The formation of the European Union, the many other worldwide political changes, the growth of developing countries seeking export markets, and the increased worldwide interest in international trade, as evidenced by the passage of the General Agreement on Tariffs and Trade and the North American Free Trade Agreement, all point to the continuing need for unprejudiced guidance and advice. Import and export policies must be as uniform as possible and based on scientific facts. The commission will strive to meet this goal through a combination of educational materials, promotion of HACCP, and recommendations, where appropriate, of sampling plans and microbiologic criteria. In addition, commission members will continue to transfer information on food safety through symposia, meetings, committee activities, and in their daily work. The future success of ICMSF will continue to depend on extensive support from consultants and those who provide the financial support essential to the commission's activities.

Research and Its Current Uses

Research Procedures and Funding

In most countries, funding for research has become more competitive, and has even decreased in recent years. The following observations are based on experience in the United Kingdom and the European Union.

The researcher first identifies the sources of funds and writes a proposal. If funds are granted, the research proceeds. The output may include confidential reports to the sponsor, refereed scientific publications, and, increasingly, a patent or a licensed process or technique. Because funding has so many different sources, writing proposals takes a substantial proportion of the researchers' time. In many cases, the best researchers also write the most persuasive proposals, but is this an efficient use of the researchers' time?

In the United Kingdom, research generally falls into two categories: research funded by the parent body and research supported by a contract. The Institute of Food Research falls under the Biotechnology & Biological Sciences Research Council (BBSRC), formerly the Agriculture and Food Research Council. A proportion of the institute's funds comes directly from the BBSRC, although these funds are declining and facing increased competition among BBSRC institutes.

In addition, funds may be obtained from the central government: the Ministry of Agriculture, Fisheries, and Food (MAFF) and the Department of Health, both of which have research programs focusing on food microbiology. In addition, Scotland and Northern Ireland have separate research budgets, which traditionally have been spent in those countries. Funds from these sources sometimes support projects for 3 years, but more recently for shorter periods.

The European Union supports research across very wide areas, of which only a small part is food technology and food microbiology, e.g., Food-Linked Agro-Industrial Research, now ended, and European Cooperation in Scientific and Technical Research. European Union programs run for 4 or 5 years, but funding is usually less than 100% (often 50% and dependent on financial support from the institute or on parallel funding from one of the above sources).

In the United Kingdom, research funds from the food industry are usually restricted either to very short trouble-shooting projects or to confidential investigations. MAFF encourages industrial support of research in LINK programs, with the government contributing up to 50% of the total funding, provided that the research contains elements of novelty and a consortium of companies, sometimes only three or four.

Not only is the researcher faced with attracting funds from a variety of sources, but the requirements of those funding sources differ greatly. Research on genetic or physiologic reasons for phenomena, such as the unusual acid tolerance of Vero cytotoxin-producing *Escherichia coli* strains, might meet BBSRC's stringent requirements of scientific quality. MAFF publishes a Requirements Document each year, identifying areas and topics that fit into its policy to maintain a safe and wholesome food supply. Some of the research is basic science, e.g., developing novel methods of detecting or identifying microbes; some is more mundane, e.g., improving the effectiveness of sanitizing surfaces in food processes. MAFF also supports a limited program of surveillance for particular foodborne pathogens. These funds are available for research institutes, universities, and other

laboratories, and proposals are judged by both MAFF staff and independent assessors from outside. Research is organized into programs on hygienic food processing, separation and detection of pathogens and their toxins, physicochemical principles underlying microbial growth, growth conditions for pathogens, ending in 1997-98, and new programs on assessing microbiologic hazards and risks and managing microbiologic hazards and risks of salmonellae and campylobacters in poultry. The Department of Health also supports food safety research—partly as surveillance and partly as programs targeted at a specific organism, e.g., *E. coli* O157:H7. The Department of Health also responds to topics identified by the Advisory Committee on Microbiological Safety of Foods.

University departments in England are subjected to a different form of scrutiny to determine the amount of research funding they receive from the central government, judged by the Higher Education Funding Council for England. The quality of publications is judged against the number of researchers, and the funding is directed toward research excellence. Newly established departments find it difficult to compete.

Research Output

Output from research has been regarded as the report to the contractor, sometimes confidential and sometimes public. More recently, funding agencies have encouraged exploitation of research through patents or licensing agreements. To the researcher, expertise must be recognized through refereed publications. Although in addition to detailed papers, overviews and sometimes summaries for the industry are provided, there is generally less consideration for the research user. In recent years, many reviews have become mere compilations of completed research. Moreover, most computerized databases terminate in about 1970, thus ignoring a wealth of earlier information. HACCP and risk assessment would be greatly facilitated if data on microbial responses (growth, survival, death) were collated in a more standardized form. Details should include the food commodity (e.g., meat, fish, vegetables, or fruit), the subgroup (e.g., for meat, beef, pork, or lamb), and the process applied (e.g., cooked, cured, smoked, or fermented). Details of the packaging should include the pack atmosphere (%CO_2, %O_2, %ballast gas). In the longer term, it might become important to know the exact identity of the organism including serotype, phage type, strain, and, in time, virulence factors and even sequences for 16S rRNA and for toxin genes. Other items that should be tabulated include inoculum concentration; prior history of the inoculum, especially if that history might alter the response; incubation temperature(s); measure of water (% brine, a_w); pH; other factors (e.g., nitrite, sorbate); microbiologic method(s); response (growth rate, D value, time to toxin); and estimate of the reliability of the measures of the responses.

Many readers assume that these details are included in most, if not all, publications. However, our experience in compiling published data for comparisons with models of growth, thermal death, or survival (nonthermal death) illustrates that much desirable information is lacking (12-15). Attempts to define the boundary between growth and no growth are not new (16-19). Despite different methods and strains of microbes, compilations of reported data often show a trend (Figure).

Research Users

Despite an overall neglect of the research user's needs, some useful listings are available

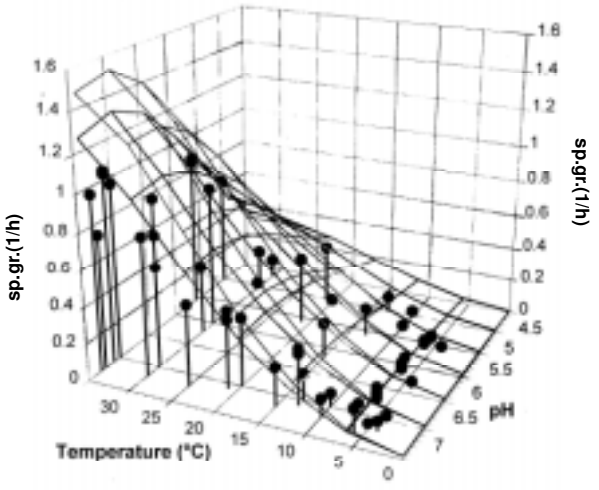

Figure. Specific growth rate of *Listeria monocytogenes* predicted by different models and independent observers (provided by J. Baranyi, Institute of Food Research, Reading Laboratory). Estimates of specific growth rate from published papers are shown vertically. The response surfaces were generated from predictive models and are not a surface fitted to the values.

(20) as are helpful overviews and discussions (21-26). There remains, however, an opportunity for the researcher to make a product more attractive to the research user, e.g., the food industry (both national and international), regulatory bodies, and informed consumers.

Additional Research Needs

Required research includes developing a database of reliable information on microbial responses to food processing conditions. The database needs to be easy to access and use. In addition, because understanding of growth responses and thermal death is greater than our understanding of survival, i.e., little or no loss of viability over many months, we need to have a better understanding of the factors determining that survival and of the underlying microbial physiology. In recent years, we have been faced with such new microbiologic challenges as *Salmonella* Enteritidis Phage Type 4, multidrug-resistant *S.* Typhimurium Definitive Type 104, verocytotoxigenic *E. coli*, Shiga-like toxin-producing *E. coli*, and increasingly vancomycin-resistant *Enterococcus faecium,* and we lack the capacity to anticipate emergence of new pathogens. Finally, new technologies make it possible to study genetic stability and variation in populations, perhaps identifying why particular strains are, or become, more virulent and more pathogenic.

Control of Food Safety

Food safety has traditionally been controlled by inspection and compliance with ordinances or codes of practice. Much faith has been placed in sampling plans and associated microbiologic criteria. However, this approach is retrospective, depending on the microbiologic examination of product that has often been dispatched or consumed. The number of samples that can be examined statistically is too small to detect low levels of "defectives." HACCP was a substantial step forward, with the concept that safety could be designed into a food process. However, HACCP demands substantial knowledge about the characteristics of the food, the process, and the microbes of concern. The knowledge required to implement HACCP effectively is not organized into systems that are easy to use.

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Epidemiology and Detection as Options for Control of Viral and Parasitic Foodborne Disease

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Human enteric viruses and protozoal parasites are important causes of emerging food and waterborne disease. Epidemiologic investigation and detection of the agents in clinical, food, and water specimens, which are traditionally used to establish the cause of disease outbreaks, are either cumbersome, expensive, and frequently unavailable or unattempted for the important food and waterborne enteric viruses and protozoa. However, the recent introduction of regulatory testing mandates, alternative testing strategies, and increased epidemiologic surveillance for food and waterborne disease should significantly improve the ability to detect and control these agents. We discuss new methods of investigating foodborne viral and parasitic disease and the future of these methods in recognizing, identifying, and controlling disease agents.

Emerging infectious diseases can be defined as infections that have newly appeared in a population or have existed but are rapidly increasing in incidence or geographic range (1). Agents for which a particular route of transmission is newly recognized and agents (previously unidentifiable) that are now known because of advances in detection methods should also be included in this definition. Advances in epidemiologic and detection methods during the last 10 to 20 years have placed food and waterborne human enteric viruses and protozoal parasites within this category.

Human enteric viruses and protozoa are parasitic agents that replicate in the intestines of infected hosts and are excreted in the feces. In general, the viruses are limited to human hosts, while the parasitic agents (in the form of cysts or oocytes) have a variety of human and nonhuman animal hosts. Both are transmitted primarily by the fecal-oral route, and as a result, 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, through contact with fecally contaminated water or other cross-contamination routes. Viruses and parasites differ from foodborne bacterial pathogens in important ways. Because they are environmentally inert, they do not replicate in food, water, or environmental samples. Additionally, unlike bacterial pathogens, human enteric viruses and protozoal parasites are environmentally stable (2), are resistant to many of the traditional methods used to control bacterial pathogens (2), and have notably low infectious doses (3,4). This allows virtually any food to serve as a vehicle for transmission and enables these agents to withstand a wide variety of commonly practiced food storage and processing conditions (2,5).

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Epidemiology

Human Enteric Viruses

Human enteric viruses are increasingly recognized as important causes of foodborne illness. A recent report issued by the Council for Agricultural Science and Technology ranked human enteric viruses as fifth and sixth among identified causes of foodborne disease in the United States (6). A review of U.S. national surveillance data for 1979 showed that 14 (44%) of 32 foodborne disease outbreaks in institutional settings were epidemiologically typical of viral

gastroenteritis (7). Viral gastroenteritis was reported as the most common foodborne illness in Minnesota from 1984 to 1991, predominantly associated with poor personal hygiene of infected food handlers (8). Furthermore, recent data indicate that 10% of the 4,617 outbreaks of foodborne disease of unconfirmed etiology reported from 1973 to 1987 met at least two of the clinical criteria for outbreaks of acute viral gastroenteritis (8,9). The apparent failure to confirm a viral etiology in such outbreaks has been due largely to the lack of available tests and the reluctance of public health officials to use epidemiologic criteria in the classification of foodborne viral disease (9-11). The unavailability of food specimens and the failure to report outbreaks of mild gastrointestinal disease have also contributed to reporting difficulties. All of these factors have resulted in a drastic underestimate of the true scope and importance of foodborne viral infection (8).

The most common types of foodborne viral disease are infectious hepatitis due to hepatitis A virus and acute viral gastroenteritis associated with the Norwalk agent and other related small, round-structured gastrointestinal viruses (12). The human enteroviruses may also be transmitted by foodborne routes (13) and are the most commonly isolated agents in surveys of naturally occurring viral contamination in foods (14). Foodborne outbreaks due to small round viruses, parvoviruses, and astroviruses are occasionally reported (15). Rotaviruses, some adenoviruses, and hepatitis E virus are important causes of waterborne disease outbreaks, particularly in developing countries (12). Foodborne outbreaks associated with human enteric viruses are almost always due to the consumption of fecally contaminated raw or undercooked shellfish and ready-to-eat products contaminated by infected food handlers (12). Postrecovery and secondary transmission are a concern (16,17). Recent outbreaks are summarized in Table 1 (18-24).

Parasitic Protozoa

Since 1981, enteric protozoa have become the leading cause of waterborne disease outbreaks for which an etiologic agent could be determined (5). A recent study reported that 21% of drinking water–associated outbreaks between 1991 and 1992 were attributable to parasitic agents (25). Furthermore, these agents are frequent contaminants of potable water supplies (26,27). The potential for transmission of these agents by foodborne routes is increasingly recognized (28). For instance, from 1988 to 1992, seven food-associated outbreaks of giardiasis, comprising 184 cases, were reported in the United States (29). However, since foodborne transmission is only recently documented and more than half of all reported foodborne disease outbreaks have undetermined etiology, the true importance of foodborne transmission of parasitic protozoa is unknown.

Table 1. Recent outbreaks of foodborne viral disease

Agent	Location	Date	No. Cases	Food	Confirmation[a]	Ref.
HAV	Shanghai, China	Jan. 1988	300,000 (4% total population)	Raw clams	Yes (IEM, Hybridization, Cell culture, Experimental infection)	18
HAV	U.S. (AL, GA, FL, TN, HI)	July-Aug. 1988	61	Raw oysters	Yes (Antibody capture-RT-PCR)	19
SRSV	U.S. (LA)	Nov. 1993	40	Raw oysters	No	21
SRSV	U.S. (LA, MD, MS, FL, NC)	Nov. 1993	180	Raw/steamed oysters	No	22
SRSV	U.S. (GA)	Dec. 1994	34 clusters	Steamed/ roasted oysters	No	22
SRSV	U.S. (FL, TX)	Jan. 1995	3	Oysters	No	20
Norwalk	U.S. (DE)	Sept. 1987	191	Commercial ice	No	23
Norwalk	U.S. (CO)	July, 1988	1440	Celery/ chicken salad	No	24

[a]Confimation of the virus, viral antigen, or viral nucleic acid in food specimens
HAV = hepatitis A virus; IEM = immune electron microscopy; RT-PCR = reverse transcriptase polymerase chain reaction; SRSV = small round-structured gastrointestinal virus.

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 protozoon that has recently been associated with the consumption of contaminated fruit (30). Large communitywide waterborne outbreaks of parasitic protozoa are usually associated with surface water supplies that are either unfiltered or subjected to inadequate flocculation and filtration (5). Two large waterborne outbreaks have occurred in the United States within the last 10 years (31,32); one of these was the largest recorded waterborne disease outbreak in U.S. history (32).

Limitations

Most of the information about viral and parasitic food and waterborne disease comes from outbreak investigations by state and local health departments and surveillance programs directed by the Centers for Disease Control and Prevention (CDC). However, since many of these diseases are not reportable and surveillance is based on voluntary reporting by state health departments, the magnitude of this disease problem is underestimated. This is exacerbated by a reluctance to use epidemiologic criteria in the classification of foodborne viral disease and the failure to report mild outbreaks of gastrointestinal disease. Furthermore, since investigation generally follows an outbreak, important information and samples may have been destroyed, consumed, or lost to inaccurate recall. Since many of these outbreaks are small and confined, epidemiologic investigation may be limited by the resources available to state and local health departments. Difficulties in investigating and reporting are further complicated by the fact that the enteric protozoa cause common opportunistic infections in the immunocompromised, and the role of foods in these diseases has not been studied. Likewise, the role of the foodborne transmission route in sporadic disease and the importance of carrier states and secondary illness after a primary foodborne disease outbreak are poorly characterized.

Detection

Clinical Samples

Illness caused by human enteric viruses can be suspected epidemiologically by considering incubation period and illness duration analysis, classic viral gastroenteritis symptoms, and the absence of bacterial or parasitic pathogens in stool samples (10,11). Laboratory confirmation of human enteric viral infection has been based on a rise in specific antibody to the virus, or alternatively, the demonstration of virus particles, antigen, or nucleic acid in stools. The detection methods most often applied to clinical samples have included immune electron microscopy, radioimmunoassay, and enzyme immunoassay (33). The usefulness of these assays has been reduced by low detection limits ($>10^4$-10^5 particles/ml) and the inability to cultivate Norwalk-like viruses in vitro, which has limited the supply of viral antigen available for developing reagents (8). In addition, the Norwalk agent is only one of several small round-structured gastrointestinal viruses that cause outbreaks with similar clinical and epidemiologic features (8).

Before 1981, parasitic disease in humans was diagnosed histologically by identifying the life cycle stages of parasitic agents in the intestinal mucosa (34). More recently, clinical diagnosis has involved methods to concentrate parasitic agents from stool specimens followed by a variety of fluorescent or immunofluorescent staining techniques and subsequent microscopic examination (34). Serodiagnostic methods have been developed (35,36), but additional evaluations are needed to confirm the diagnostic utility of these methods.

Environmental, Food, and Water Samples

Failure to confirm a viral and parasitic etiology in foodborne outbreaks has also been due to the lack of adequate methods to detect the causative agents in environmental samples. Like bacterial pathogens in food and water, viruses and parasites are frequently present in small numbers. However, unlike traditional food microbiologic techniques, which have relied on cultural enrichment and selective plating to increase cell numbers and differentiate pathogens in background microflora, techniques to detect human enteric viruses and parasitic protozoa require live mammalian cells for growth. For this reason, standard methods to detect enteric bacteria in foods cannot be used; instead, detection requires an initial concentration step, often from large volumes of food or water, followed by mammalian cell culture infectivity assay or immunofluorescent staining.

Concentration methods are usually cumbersome, and yields are less than optimal. Both cell culture infectivity and immunofluorescent staining are expensive and slow and require highly trained personnel. Furthermore, mammalian cell culture lines are largely unavailable for the epidemiologically important foodborne viruses. Alternative detection methods based on immunologic and molecular methods have been reported; recent methodologic developments have focused on overcoming barriers to detection, such as improving recovery efficiencies and detection limits and preventing inhibitions due to food-related compounds.

Human Enteric Viruses in Foods

Concentration

Two general schemes for the concentration of human enteric viruses from foods have been reported: extraction-concentration methods and adsorption-elution-concentration methods (Figure). The general purpose of concentration is to provide a high recovery of infectious virus in a low-volume aqueous solution free of cytotoxic materials. Both schemes employ conditions favoring the separation of viruses from shellfish tissues (most of the developmental work has used shellfish as model food commodities), primarily through the use of filtration, precipitation, polyelectrolyte flocculation, and solvent extraction. While either method can be used, adsorption-elution-precipitation methods have been favored in recent years (2,37). Virus yields after concentrations are 10% to 90% (38).

Detection

Traditional methods to directly detect viruses in foods after concentration have been based on the ability of enteric viruses to infect live mammalian cells in culture. Quantal and enumerative methods using a variety of mammalian cell culture lines, generally from primate kidneys, have been reported. Such approaches have been limited because levels of contaminating virus generally are low (1-200 infectious units per 100 grams of shellfish) (39), residual food components interfere with assays (38), and the epidemiologically important viruses do not replicate (small round-structured gastrointestinal viruses) or replicate poorly (hepatitis A virus) in mammalian cell culture (2). Alternative methods such as enzyme-linked immunosorbent assay (ELISA) and DNA/RNA probes have been reported but are limited by high detection limits (>10^3 infectious units), unavailability of reagents, and poor sample quality (2). These difficulties are illustrated by the confirmation of viral contamination in a food in only two reported instances (18,19).

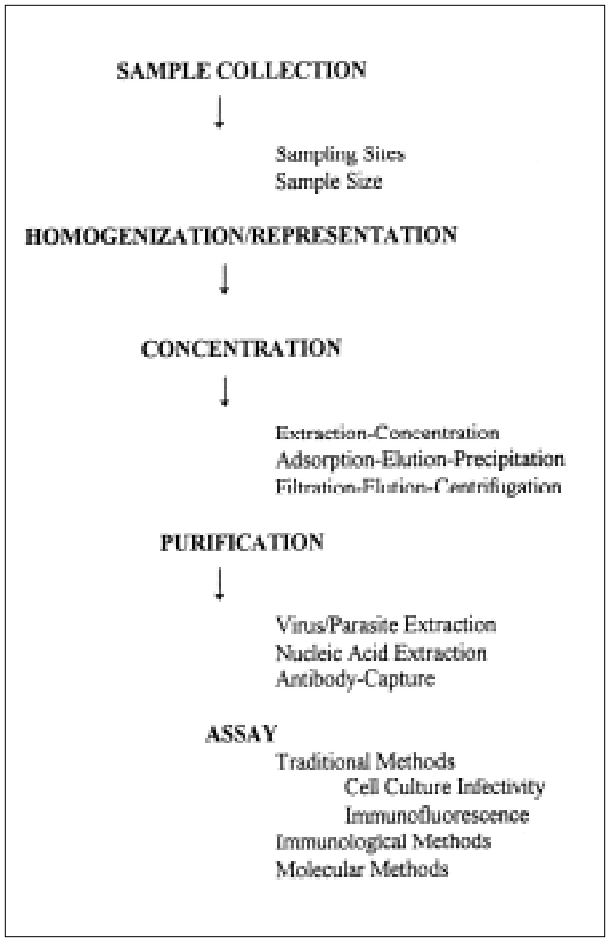

Figure. General steps in the isolation of human enteric viruses and parasitic protozoa from foods.

The in vitro enzymatic amplification method of polymerase chain reaction (PCR) offers an opportunity to enrich a single specific nucleic acid sequence up to a millionfold and hence provides a sensitive and specific method with a theoretical detection limit of one virus unit. This method is readily adaptable to the detection of RNA viruses by preceding the PCR with a brief reverse transcription (RT) step, hence the designation RT-PCR. The recent cloning of the Norwalk agent and related small round-structured gastrointestinal viruses has provided an opportunity to develop effective molecular

detection methods for these previously nondetectable agents (40,41).

The application of PCR methods to the detection of human enteric viruses in foods is an area of active research. However, the development of such methods is complicated by low levels of contamination, high sample volumes, and the presence of food components, which may interfere with enzymatic amplification reactions. To address these issues, three alternative approaches have been used to simultaneously reduce sample volumes and the level of interfering compounds. The most frequently applied approach involves isolating and purifying nucleic acids (RNA) from the food sample before RT-PCR (42-47). A second approach combines capture of the virus with specific antibody followed by nucleic acid amplification by using RT-PCR (19,48). In the third approach, the intact virus particle is concentrated and purified from the complex food matrix resulting in sample volume reduction and removal of inhibitors, followed by subsequent heat release of viral nucleic acid from the virion capsid and RT-PCR (49-51). All three methods have been applied to various shellfish species, and in some cases, to other food commodities and naturally contaminated field shellfish specimens. The methods are summarized in Table 2, along with optimized virus detection levels (19,42-51). A combined approach was reported by Chung et al. (51), who successfully detected human enteroviruses and hepatitis A virus in naturally contaminated oyster samples after viral amplification in mammalian cell cultures. Despite enormous strides in the ability to detect human enteric viruses with PCR, the technique is still limited by the absence of effective concentration methods, the presence of enzymatic inhibitors, and the inability to distinguish between infectious and noninfectious virions.

Table 2. Emerging detection methods for human enteric viruses in foods

Pathogen	Sample	Detection limit	Field app.	Ref.
Nucleic Acid Extraction/RT-PCR				
HAV	Clams	2000 particles/g (10 PFU/g)	No	44
Poliovirus	Oysters	38 PFU/20 g (2 PFU/g)	No	42
HAV	Clams/ Oysters	100 PFU/1.5 g (67 PFU/g)	No	43
Norwalk	Clams/ Oysters	5-10 PCRU/1.5 g (3-7 PCRU/g)	No	43
Poliovirus	Oysters/ Mussels	10 PFU/5 g (2 PFU/g)	No	45
SRSV	Oysters/ Mussels	Not Specified	Yes	46
Poliovirus, HAV	Clams	100 PFU/50 g (2 PFU/g)	No	50
Norwalk	Clams	1000 PCRU/50 g (20 PCRU/g)	No	50
Norwalk	Various	20-200 PCRU/10 g (2-20 PCRU/g)	No	47
Antibody-Capture/RT-PCR				
HAV	Clams/ Oysters	Not Specified	No	48
HAV	Oysters	Not Specified	Yes	19
Virion Concentration				
Poliovirus, HAV	Oysters	10 PFU/50 g (0.02 PFU/g)	Yes	49, 51
Norwalk	Oysters	4500 PCRU/50 g (90 PCRU/g)	No	49
Poliovirus, HAV	Clams	1000 PFU/50 g (20 PFU/g)	No	50
Norwalk	Clams	100 PCRU/50 g (2 PCRU/g)	No	50

HAV = hepatitis A virus; RT-PCR = reverse transcriptase polymerase chain reaction; PCRU = PCR-amplifiable units; SRSV = small round-structured gastrointestinal virus.

Enteric Parasitic Agents

Concentration

Like viruses, parasitic protozoa are usually present in low concentrations in contaminated water and hence must be concentrated from large volumes of water before detection (Figure). *Giardia* cysts and *Cryptosporidium* oocysts are concentrated from 100 to 1,000 liters of water by filtration through yarn-wound filters. Retained particulates are eluted from the filters and reconcentrated by centrifugation. The pelleted cysts and oocysts are then separated from particulate debris by flotation on Percoll-sucrose gradients, followed by subsequent detection. With this method, a concentrate of less than 5 ml can be provided for final detection (52). While recovery efficiencies as high as 100% have been reported (53), recovery is generally poor and greatly affected by water quality and particulate matter (53,54). Alternative methods using calcium carbonate precipitation can concentrate *Cryptosporidium* oocysts from water with concentration efficiencies as high as 63% (55).

Detection

Traditional methods to detect parasitic agents from water sample concentrates have been based on immunofluorescent staining of filtered sample concentrates (52). The sample concentrate is filtered through cellulose acetate filters and commercial kits that use fluorescein isothiocyante–labeled monoclonal antibodies applied for immunofluorescent staining. The stained filters are examined under an ultraviolet microscope, and cysts and oocysts are classified according to immunofluorescence, size, shape, and internal morphologic characteristics. The results are reported as presumptive and confirmed cysts and oocysts per 100 liters of water (52). Confirmation is based on the ability to visualize organelles under light microscopy. This method is extremely limited because it is time-consuming and expensive, requires highly skilled personnel, and does not indicate viability of the cysts or oocysts; in addition, cross-reactions of monoclonal antibodies with algal cells and debris interfere with the interpretation of results (56).

Because of the limitations of immunofluorescence assays, alternative strategies for the detection of protozoal parasites in environmental and water samples are being sought. Most of the approaches use the traditional methods of concentration, in conjunction with alternative detection methods. In many cases, the inclusion/exclusion of fluorogenic dyes is used to enhance morphologic examination; in particular, 4,6-diamidino-2-phenyl indole and propidium iodide have been used to facilitate detection and also assess viability (57). Two recent methodologic developments include cell sorting/particle counting approaches and molecular approaches. In many cases, a combination of approaches is used. Particle-counting approaches include the Fluorescence-Activated Cell Sorting system, a laser-based particle counter that is able to simultaneously sort particles, sense fluorescence, and determine size (58). This system is being used to detect *Giardia* and *Cryptosporidium* in water samples in England and Australia (55). Differentially stained cysts and oocysts may be visualized microscopically with cooled charge couple devices (58,59). Molecular approaches that apply DNA hybridization to the detection of *Giardia* species have been reported (60). A method that combines fluorescence and in situ hybridization with confocal microscopy has been applied to both detect and speciate *Giardia* cysts (61). PCR methods have been developed to detect *Giardia* and *Cryptosporidium* (62-64). While PCR methods have the potential to detect one single infectious unit and may be applied to discriminate pathogenic from nonpathogenic species, they remain limited because of enzymatic inhibition, the inability to discriminate between viable and nonviable organisms, and the current absence of quantitative assay.

Several methods in development are combinations of multiple detection approaches. A combined method, designated the electrorotation assay, couples filtration and subsequent elution with affinity immunocapture by using paramagnetic beads. By inducing an electric field on a microscope with a special stage attachment, the organism-bead complexes rotate in a characteristic pattern that enables detection of parasitic protozoa (52). Several investigators are also working on methods that couple cell culture infectivity with immunostaining, thereby providing detection with simultaneous indication of viability (M. Sobsey, pers. comm.). Clinical ELISA kits have been evaluated for use in environmental water samples with reported detection limits of fewer than 10 cysts or oocyts (65); however, cross-reaction with algae continues to be a problem. Emerging detection approaches are summarized in Table 3 (58-64). Methods to concentrate and detect parasitic protozoa specifically from foods are under development.

Considerations

Although prototype alternative and rapid methods to detect human enteric viruses and parasitic protozoa in foods and water have been reported, multiple barriers must be overcome before these methods are applicable to routine monitoring. To obtain sample representation and detection sensitivity adequate for the low levels of contamination found with these enteric pathogens in naturally contaminated environmental specimens, large sample volumes of food and water need to be processed. While some of the detection methods begin with large samples, many do not consider sample size, which limits the sensitivity of the assay procedure from the beginning. The approaches then applied to concentrate and purify the pathogens from the samples do not only need to be reasonably efficient but also need to produce a concentrate low in volume and free of inhibitory compounds.

Table 3. Emerging detection methods for parasitic protozoa in water

Pathogen	Detection limit	Viability	Differentiation	Ref.
Flow Cytometry with Fluorescent Imaging and CCD				
Cryptosporidium	NR	Yes, using differential fluorogenic vital dyes	NR	58, 59
DNA Hybridization				
Giardia (16s-like rDNA)	1 cyst	NR	No	60
Giardia (16s-like rDNA)	NR	NR	Yes-in situ hybridization with differential fluorescence *G. lamblia; G. muris*	61
Polymerase Chain Reaction				
Giardia (Giardin gene)	1 cyst	Partial, using mRNA as target; Depends on inactivation method	NR	62
Giardia (Giardin gene)	1 cyst	NR	Yes-primer sequence *G. duodenalis*	63
Cryptosporidium (Target not specified)	20 oocysts	No	Yes-by hybridization *C. parvum*	64

CCD = cooled charge couple device; NR = not reported

To complicate matters further, different food commodities have differing types and levels of inhibitors, many of which have remained recalcitrant to almost all removal processes.

The relationship between detection by molecular and immunologic approaches and the subsequent viability or infectivity of these enteric pathogens remains a concern. While investigators have addressed these issues using in vitro excystation, inclusion and exclusion of vital dyes, animal infectivity (parasitic protozoa) (57,66,67), and assays combining mammalian cell culture infectivity with alternate detection strategies (viruses) (51; M. Sobsey, pers. comm.), these methods are not useful for the nonculturable human enteric viruses; they are expensive, time-consuming, and not readily amenable to routine diagnostic work. Establishing quantitative detection methods also needs further research.

The development of detection methods is also limited by the current state of knowledge. For instance, while recent sequencing evidence indicates that the Norwalk-like small round-structured gastrointestinal virus group consists of multiple members having the same physical and genomic characteristics as other viruses in the family Caliciviridae (40,41), considerable sequence and antigenic diversity remain among the members of this group (68-70). While PCR primers for these genetically diverse agents have been reported (46,71), development of universal detection methods is clearly limited until more complete information about this group of human enteric viruses is available.

Research is needed to develop and refine the prototype protocols into collaboratively tested methods that could be routinely and expeditiously used to evaluate the microbiologic safety of food products. In general, future research needs for the routine application of alternative methods to detect enteric viral and parasitic protozoal contamination in foods requires development of the following: 1) simple, rapid, and cost-effective extraction and concentration procedures; 2) simple and reliable methods for the removal of inhibitors; 3) methods that are not restricted by food product; and 4) quantitative approaches for assessing the relative levels of contamination.

Conclusions

Improved epidemiologic surveillance, predominantly through the creation of population-based centers that will focus on the epidemiology and prevention of food and waterborne infectious disease (72), should improve knowledge regarding viral etiology in foodborne disease outbreaks

and the clinical and economic importance of viral and parasitic disease agents. Data obtained from these studies may elucidate the role of foods in sporadic disease as well as in secondary spread.

Current research programs under way at CDC, U.S. Department of Agriculture, and Food and Drug Administration, as well as extramural funding through the National Institutes of Health and U.S. Department of Agriculture programs, should promote the development, testing, and dissemination of rapid and accurate detection methods for viral and parasitic foodborne disease agents. Such research will continue to be important as testing regulations, such as the landmark Environmental Protection Agency Information Collections Requirement Rule, and the new U.S. Department of Agriculture Pathogen Reduction: Hazard Analysis and Critical Control Points (HACCP) Systems Rule, emerge in the future. From a clinical standpoint, the development of inexpensive and widely available reagents has been improved through recent developments in molecular biology (33). This will increase the number of facilities able to perform diagnostic testing, which will ultimately facilitate epidemiologic investigation. From a food safety standpoint, improved detection methods should eventually provide a regulatory option for monitoring food safety, particularly in the economically important shellfish species and in detecting viral contamination due to human handling, or parasitic protozoal contamination from animal wastes. The development of rapid detection methods will also aid in the evaluation of control strategies for viral and protozoal contamination of foods. For instance, depuration, thermal processing, and irradiation for the control of foodborne viral contamination in shellfish need to be further evaluated. In the case of contamination by infected food handlers and animal wastes, the availability of rapid methods will increase our understanding of this transmission route and help determine critical control points for HACCP approaches to control the transmission of these foodborne disease agents. This will allow the integration of a true farm-to-table food safety approach for the control of viral and parasitic food and waterborne disease.

In the United States, there is currently a clear regulatory mandate to evaluate food safety risks within a systematic conceptual framework in the form of quantitative microbial risk assessment. The application of quantitative risk assessment to food safety issues has been hampered by the lack of available data regarding prevalence, transmission, infectious dose, and behavior of microorganisms in foods; this has been particularly true for emerging pathogens. Improved epidemiologic and detection methods should dramatically affect the ability to evaluate food safety risks through risk assessment strategies. For instance, by using emerging epidemiologic data in conjunction with epidemiologic modeling, statistical overview analysis (meta-analysis), and geographic information systems, scientists should be able to improve hazard analysis and exposure assessment and provide a clearer picture of disease transmission patterns. More rapid, accurate, and readily available detection methods should allow determination of prevalence of contamination and disease, and when quantitative, should provide a means of assessing dose-response relationships. Together these will improve subsequent risk characterization, allowing regulators to quantitate the magnitude of the problem, evaluate risk reduction strategies, and prioritize competing risks.

The combination of increased surveillance, improved detection methods, and testing requirements should result in a marked improvement in the ability to detect, investigate, and control food and waterborne enteric viral and parasitic protozoal agents. Taken together, these approaches promise to provide increased information necessary to assess risks, control disease, and ultimately improve public health in the next century.

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Quantitative Microbiology: A Basis for Food Safety

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Because microorganisms are easily dispersed, display physiologic diversity, and tolerate extreme conditions, they are ubiquitous and may contaminate and grow in many food products. The behavior of microbial populations in foods (growth, survival, or death) is determined by the properties of the food (e.g., water activity and pH) and the storage conditions (e.g., temperature, relative humidity, and atmosphere). The effect of these properties can be predicted by mathematical models derived from quantitative studies on microbial populations. Temperature abuse is a major factor contributing to foodborne disease; monitoring temperature history during food processing, distribution, and storage is a simple, effective means to reduce the incidence of food poisoning. Interpretation of temperature profiles by computer programs based on predictive models allows informed decisions on the shelf life and safety of foods. In- or on-package temperature indicators require further development to accurately predict microbial behavior. We suggest a basis for a "universal" temperature indicator. This article emphasizes the need to combine kinetic and probability approaches to modeling and suggests a method to define the bacterial growth/no growth interface. Advances in controlling foodborne pathogens depend on understanding the pathogens' physiologic responses to growth constraints, including constraints conferring increased survival capacity.

Ensuring the microbial safety and shelf life of foods depends on minimizing the initial level of microbial contamination, preventing or limiting the rate of microbial growth, or destroying microbial populations. With many foods, these strategies have been practiced successfully for thousands of years. However, in the last decade, the incidence of foodborne disease has increased in the industrialized world (1), despite the introduction of the Hazard Analysis and Critical Control Points (HACCP) concept and the promulgation of regulations in food safety. The increased incidence of foodborne disease is caused by changes in agricultural and food processing practices, increasing international trade in foods, and social changes (which include changed eating habits and increased population mobility) (2).

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This article develops the propositions that available quantitative information, properly applied, is a basis for improved food safety; the information available, largely an empiric description of microbial behavior in foods, highlights a lack of understanding of the physiology of foodborne pathogens; and knowledge of the physiology may lead to more precise control of foodborne bacteria and novel protocols to ensure the microbiologic safety of foods.

Characteristics of Bacteria

Bacteria have inhabited the earth for approximately three and a half billion years and have colonized almost every conceivable habitat (3). In fact, the development of microbial populations is probably precluded only when liquid water is absent or conditions are so extreme that rapid denaturation of proteins outpaces their rate of replacement. The major characteristics that underpin the success of prokaryotes are small size and ease of dispersal, physiologic diversity, and tolerance of extreme

conditions (4). The temperature range over which microbial populations develop is -12°C (the temperature at which intracellular water freezes) to +112°C (the temperature at which liquid water is maintained only under elevated pressure). The pH range is pH 1 to pH 12, and the salinity range is zero to saturated (4).

Langeveld et al. (5), who studied microbial development in biofilms in a tubular heat exchanger used to pasteurize milk, report the exploitation of different ecologic niches by bacteria. Through the ascending temperature range of the tube (~20°C to ~90°C), the dominant microbiota changed from gram-negative bacteria such as *Acinetobacter,* to coliforms to *Streptococcus thermophilus* to thermophilic bacilli. At the highest temperature, the wall of the exchanger was colonized by *Thermus thermophilus*. Thus, it appears that contaminants deposited along the length of the tube were selected by the in situ temperature, with the fastest-growing organism dominating.

Factors Affecting Microbial Behavior in Foods

Most studies in food microbiology are concerned with the rapid growth of populations, but in many ecosystems, the survival characteristics of the population also need to be considered. The longevity of bacterial spores and their resistance to harsh conditions are well documented. However, the ability of vegetative cells to resist stressful conditions is increasingly recognized as an important ecologic trait (6). Attention also needs to be given to relatively slow-growing populations in various situations, e.g., when the shelf life of a product is extended by control of rapidly growing spoilage organisms. The behavior of foodborne microorganisms, be it the growth or death of microbial populations, is based on the time of exposure to environmental factors affecting population development; for example, equivalent kills of bacteria in milk are achieved by low temperature–long time pasteurization (60°C/30 min) and high temperature–short time pasteurization (72°C/15 sec). When populations are in the biokinetic range, the rate at which they develop is determined by factors such as temperature, water availability, and pH applied in food preservation procedures. The extent of microbial growth is a function of the time the population is exposed to combinations of intrinsic food properties (e.g., salt concentration and acidity) and extrinsic storage conditions (e.g., temperature, relative humidity, and gaseous atmosphere).

Different factors assume dominance in different foods and preservation strategies. In many foods, the full preservation potential of a single property is restricted because of considerations related to the esthetic, organoleptic, and nutritional properties of the product. However, several properties or conditions may be combined to provide a desired level of stability (7). In situations where the preservation strategy is designed to slow the rate of population growth, the effect will always be increased by storage temperature. Temperature control in processing, distribution, and storage (the cold chain) is crucial to ensure the adequate shelf life and safety of many common foods, including meat, fish, poultry, and milk. Newer technologies, including modified atmosphere packaging and sophisticated products such as sous-vide meals, do not obviate the need for strict temperature control. Indeed, the requirement for vigilance increases with increased shelf life and the possibility of growth of psychrotrophic pathogens over an extended period.

Predictive Microbiology

Predictive microbiology involves knowledge of microbial growth responses to environmental factors summarized as equations or mathematical models. The raw data and models may be stored in a database from which the information can be retrieved and used to interpret the effect of processing and distribution practices on microbial proliferation. Coupled with information on environmental history during processing and storage, predictive microbiology provides precision in making decisions on the microbiologic safety and quality of foods. The term "quantitative microbial ecology" has been suggested as an alternative to "predictive microbiology" (8).

The development, validation, and application of predictive microbiology has been extensively reviewed in the last decade (9,10). Modeling studies have concentrated on descriptions of the effect of constraints on microbial growth (rather than survival or death), often using a kinetic model approach (rather than probability modeling) and most often describing the effect of temperature as the sole or one of a number of controlling factors. For example, the temperature dependence model for growth of *Clostridium*

botulinum demonstrated a good fit to data, but the authors noted that "care must be taken at extremes of growth, as no growth may be registered in a situation where growth is indeed possible but has a low probability" (11).

The emphasis in modeling efforts on temperature (often in combination with other factors) may be justified, given its crucial role in the safe distribution and storage of foods. Surveys carried out over several decades in the United Kingdom, United States, Canada, and Australia point to the predominant role of temperature abuse in outbreaks of foodborne disease (12-14).

Problems with Predictive Microbiology and Research Needs

Several commonly perceived problems with predictive modeling (8) are reviewed below. While considerable progress has been made in defining philosophic approaches and experimental protocols for growth model development and many models have been developed and published, more validation studies are required, particularly involving independent and industry-based trials. More emphasis should be placed on modeling the death kinetics of foodborne pathogens with low infective doses.

Measurement of environmental factors (e.g., temperature) can be achieved with precision, but in some situations, (e.g., in chilling of meat carcasses), it is more difficult. Location of the sensor can be an important consideration (15,16). In meat chilling, where control of microbial development is a function of the combined effects of falling temperature and water activity, development of a technique to measure water activity in situ at the carcass surface would provide valuable information. Furthermore, development of techniques to measure constraints such as water activity, pH, or redox potential on a microscale might provide useful information for a complex food such as salami. This would allow definition of the role of the microenvironment in determining microbial behavior. The concept of a microenvironment is well developed in soil microbiology (17) but has been neglected in food microbiology.

The inherent variability of response times (generation time and lag phase duration) as an issue in predictive microbiology was first raised by Ratkowsky et al. (18), who related the variance of responses on a transformed rate scale such as $V(\sqrt{k})$ or $V(\ln k)$ to the variances of responses on a time (θ) scale. The variance was shown to be proportional to the square or cube of the response time. It was later confirmed (19-21) that nonnormal gamma and inverse Gaussian distributions described the distribution of response times in predictive microbiology, which indicate that variance is proportional to the square or cube of the response time, respectively.

The practical implication of these findings for the application of kinetic models is that inherent biologic variability increases markedly with increasing response times, and thus the confidence limits associated with predictions also increase markedly. However, if the probability distribution of the response time is known, one can determine the probability that an organism will grow more quickly than a predicted response time (21). Thus, kinetic models are appropriate to describe consistent microbial growth responses, but under extreme conditions a probability approach may be required.

Models are normally developed under static conditions (growth rates and lag times are measured at a series of set temperatures, water activity values, and pH levels), and the results are combined to describe the effects of each factor or a combination of factors on population development. Subsequently, models must be validated in foods under conditions that mimic situations encountered in normal practice, e.g., decreasing temperature and water activity during active chilling of meat carcasses or fluctuating temperatures during the distribution and storage of many food commodities.

Shaw (22) and later several other authors (23-26) reported on the effect of fluctuating temperatures. Depending on the magnitude of the temperature deviation, the organism may change its rate of growth to a rate characteristic of the new temperature, or it may stop growing if a lag phase is introduced. In both situations, *Salmonella* Typhimurium has responded nearly as predicted by the model (24,25). Baranyi et al. (26) presented similar results for the spoilage bacterium *Brocothrix thermosphacta.* When cycled between 25°C and 5°C, the model predicted behavior well in both the growth rate and lag phase duration. However, a temperature shift from 25°C to 3°C caused deviations from model predictions due to decline in viable cell numbers or extended lag phases. During the final extended phase of growth at 2.8°C, the rate

resumed at that predicted. Baranyi et al. (26) also examined the perceived problem of modeling lag phase duration. The difficulty in predicting lag phase duration in foods is not due to the lack of a suitable model: the difficulty comes from the lack of knowledge of the physiologic status of the organisms contaminating the food. The organisms may include cells that are actively growing, exhibiting a physiologic lag phase, damaged and under repair, exhibiting physiologic (endospores) or exogenous dormancy (VNC cells), damaged but unable to reproduce because of ineffective repair mechanisms, and dead. At least part of the confusion surrounding the measurement of lag phase duration arises from experiments in which inocula of different physiologic statuses were used (27,28).

Methods to define the physiologic status of foodborne contaminants under various conditions need to be developed. This will require observations on individual cells or small populations of cells either directly by microscopy or an indicator of single-cell metabolic activity (26). Luminescent *Salmonella* strains have been used as real-time reporters of growth and recovery from sublethal injury (29). Alternatively, a parameter to describe the suitability of cells to grow in a new environment may be incorporated in the model (26).

Current Status of Predictive Microbiology

Some problems with predictive microbiology have been discussed, and, for each, needed research has been suggested. Opinions vary on the efficacy of models to predict outcomes under real life conditions. At one end of the scale, accuracy such as that described for the growth of *Pseudomonas* in minced beef (Figure 1) can be

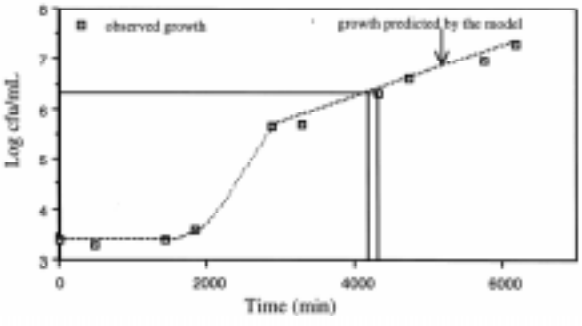

Figure 1. Validation of *Pseudomonas* predictor in minced beef. Printed with permission of G. Thomson, Defence Force Food Science Centre, Scottsdale, Tasmania, Australia.

obtained in trials conducted independently of the laboratory in which the model was developed. At the other end, models developed in laboratory broth systems have been reported to be inappropriate to describe growth on food (30). Dalgaard (31) reported similar discrepancies and suggested an iterative approach to model development using food, rather than laboratory media, as the growth substrate for model development. Such reports emphasize the need for rigorous validation of models under practical conditions. Deviations from predictions do not necessarily imply that the model is defective but more likely that knowledge of some food ecosystems is incomplete and factors other than those used in model development have an effect on microbial behavior.

The common theme of the problems in predictive microbiology discussed above is that of uncertainty—uncertainty in terms of the starting conditions (e.g., initial microbial numbers and types) and the microbial response in a given or changing environment. Uncertainty translates to variability if the distribution of response times is understood and the variance can be described. As we have indicated above, the variability associated with very long response times limits the utility of kinetic models and requires a probability approach. Thus, while in the last decade predictive modelers were justified in their selection of temperature as a primary factor to model in kinetic approaches, the next decade may see a return to probability modeling as pioneered by Genigeorgis (32) and Roberts (33). This shift will derive impetus from the emergence of dangerous pathogens with very low infective doses, and continued kinetic modeling will concentrate on survival and death rather than growth of populations.

The first kinetic death model to find widespread use in the food industry was for thermal destruction (34). One can consider a model describing a 12-log cycle reduction in *C. botulinum* spores in a short time with considerable certainty. However, as we move toward less severe processes with longer response times and the added complications of "shoulders" and "tails" to define the growth/no growth interface, biologic variability will again dictate a probability approach to describe the survival and slow decline of microbial populations.

Defining the Growth/No Growth Interface

Because growth of pathogenic bacteria in foods always increases the risk for foodborne disease, defining the conditions at which no growth is possible is of considerable practical significance for food manufacturers and regulators. Bacterial growth/no growth interface models quantify the combined effect of various hurdles on the probability of growth and define combinations at which the growth rate is zero. Increasing the level of one or more hurdles at the interface by only a small amount will significantly increase the probability of "fail safe" events and decrease the probability that a few cells in the population will resolve the lag phase and begin to grow (a "fail dangerous" event) (7). The growth/no growth interface also has great physiologic significance because at that point biosynthetic processes are insufficient to support population growth, and survival mechanisms are in place.

A procedure to derive the interface was proposed by Ratkowsky and Ross (35); it employs a logistic regression model to define the probability of growth as a function of one or more controling environmental factors. From this model, the boundary between growth and no growth, at some chosen level of probability, can be determined. The form of the expression containing the growth limiting factors is suggested by a kinetic model, while the response at a given combination of factors is either presence or absence (i.e., growth/no growth) or probabilistic (i.e., the fraction of positive responses in n trials). This approach represents an integration of probability and kinetic approaches to predictive modeling.

Microbial Responses to Stress and Microbial Physiology

Bacteria have physiologic mechanisms enabling them to survive in environments that preclude their growth. While some tolerance to environmental insults is adaptive, a wide range of protective mechanisms is induced when cells enter a stationary phase or become starved. These phenomena are under the control of the *rpoS* gene, which codes for a stationary-phase–specific sigma factor, expression of which triggers the development of a semidormant state in which bacteria can better resist multiple physical challenges (36). This factor and the gene products whose expression it controls are of vital significance to food microbiology; they form the basis for a global stress response in which one stress can confer protection to a wide range of other stresses. Under the influence of this factor, bacterial cells respond very quickly to unfavorable changes in their environment. Often these responses are phenotypic and remain in place only during stress (37).

Low pH Tolerance

Brown et al. (37) demonstrated "acid habituation" (38), a phenotypic response to an environmental insult, for five strains of *Escherichia coli*. These strains showed a wide range of intrinsic acid tolerance, which for each strain was enhanced by exposure to nonlethal acidity (pH 5) before exposure to a lethal acid challenge (pH 3). Neutralization of the growth medium partially reversed tolerance to acid stress, underlining that acid habituation is a phenotypic response. Furthermore, acid tolerance was correlated with changes in the fatty acid composition of the cell membrane. During acid habituation, monounsaturated fatty acids (16:1w7c and 18:1w7c) in the phospholipids of *E. coli* were either converted to their cyclopropane derivatives (cy17:0 and cy19:0) or replaced by saturated fatty acids. The degree of acid tolerance of the five strains of *E. coli* was highly correlated with the membrane cyclopropane fatty acid content, which may enhance the survival of cells exposed to low pH.

Low Water Activity Tolerance

Bacterial cells, when confronted by lowered water activity, regulate the internal environment by rapidly accumulating compatible solutes such as glycine betaine or carnitine (39). The solutes, which may be scavenged by the cell, exert their influence at very low concentrations; the effect is demonstrated both in limits and rate growth. These compounds appear also to provide cryotolerance as well as osmotolerance (40; Figure 2).

Energy Diversion

Microbial responses to stressful conditions may constitute a drain on the energy resources of the cell, e.g., in relation to the accumulation of compatible solutes (41). Knochel and Gould (42) argued "that restriction of the availability of energy will interfere with a cell's reaction to

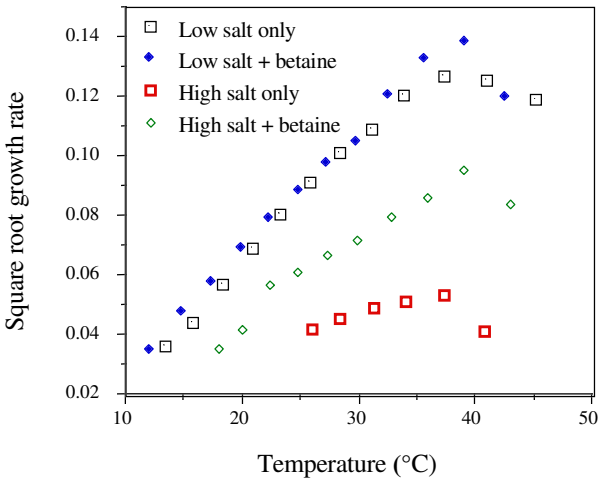

Figure 2. Effect of betaine on the growth of *Escherichia coli* in glucose-minimal medium. Without added NaCl the growth rate yield and minimum growth temperature are the same with and without betaine. With 4% NaCl the growth rate and yield are lower without betaine and the actual minimum temperature for growth is approximately 9°C lower than with betaine (K. Krist, unpub. data).

osmotic stress." The energy diversion hypothesis was supported by McMeekin et al. (9) on the basis of observations on the growth of *Staphylococcus xylosus* at nine different levels of water activity. Though T_{min}, the theoretic minimum temperature for growth, remained constant, the actual minimum temperature at which growth was observed increased with decreasing water activity, suggesting energy expenditure to cope with a_w stress.

Krist and Ross (unpub. data), however, challenged this explanation because of findings from growth rate and yield experiments on *E. coli* growing in a glucose-limited minimal minerals medium. With both decreasing temperature and water activity, the growth rate declined gradually, but the yield was not greatly affected until close to the point where growth ceased. As the substrate was converted to the same amount of biomass, this suggests that the stresses imposed by suboptimal temperature or water activity are not a major drain on the cell's energy reserves. Compatible solutes likely ameliorate the effect of both factors by maintaining enzymes in an active configuration (39). With pH, the growth rate of many organisms is unaffected across a wide range of pH values. To maintain intracellular pH, the cell uses considerable energy to export protons (43), and thus it is anticipated that yield will be affected.

Increasing knowledge of the physiologic response of bacterial cells to individual constraints and combinations of constraints will provide greater precision in defining growth-limiting conditions and possibly allow development of novel protocols to ensure the microbial safety of foods. As an example, the remarkable effect of compatible solutes on the growth rate and growth range conditions is an obvious advantage for bacteria growing under stressful conditions (Figure 2). Compatible solutes, such as betaine and carnitine, are widely distributed in foods of plant and animal origin and are easily available to bacteria and rapidly taken up by specific transport mechanisms (39,40). It is unlikely that growth might be controlled by "creating a hostile environment devoid of osmolytes," as suggested by Smith (44). However, it might be possible to use the specific uptake mechanism to deliver a compatible solute analogue with lethal effects on the cell. Alternatively, if the cell is moved from an environment in which growth is possible to one where growth ceases, compatible solutes may also allow enzymic reactions to continue within the cell, depleting energy reserves and inducing a greatly extended lag phase or death. This hypothesis is supported by the observations of many authors that survival is better at low rather than ambient temperatures. For example, Clavero and Beuchat (45) state, "Regardless of the pH and a_w, survival of *E. coli* O157:H7 was better at 5°C than at 20°C or 30°C." Furthermore, preliminary experiments in this laboratory suggest that *E. coli* declines more rapidly in the presence of betaine than in its absence (Krist, unpub. data).

Application of Predictive Models

The incorporation of predictive models into devices such as temperature loggers has been described for *E. coli* (46) and *Pseudomonas* (47), as has the development of expert systems from predictive modeling databases (48,49).

The current food poisoning crisis indicates that existing quantitative information on microbial growth, survival, and death, if properly applied, would have an immediate impact on the incidence of foodborne disease in the industrialized world. Even without the synthesis of data into mathematical models, simply logging the temperature history of food processing, distribu-

tion, and storage operations would provide much useful information. Loggers provide a hard copy of a temperature profile in real time and thus evidence of temperature abuse and the source of the abuse.

For loggers with appropriate software (46,47), the temperature profile may be interpreted in terms of microbial growth. However, the interpretation must be based on an informed analysis of the temperature history by a trained operator. The operator may, for example, be required to enter default values for initial bacterial numbers or provide an estimate of lag phase duration under specified conditions. Estimates of both imply general knowledge of food microbiology and specific knowledge of the process and products under consideration. The equivalence of an estimate of microbial growth derived from temperature profile to that obtained from conventional microbiologic criteria may also be necessary (15).

An alternative to the use of temperature loggers is the development of in- or on-package temperature tags as recommended in the U.S. Food Safety Initiative draft document Food Safety from Farm to Table (50). With temperature tags, informed interpretation is not required because abuse is indicated directly by the tag response. Therefore, the tag must indicate the significance of the environmental history for microbial behavior. The time/temperature tags available are based on physical or chemical changes that follow Arrhenius kinetics (9). While these may give a reasonable approximation of microbial growth in the normal range, the deviation of microbial responses becomes increasingly large as conditions move from normal to stressful. The intriguing possibility of a universal time/temperature indicator was flagged (51) on the basis of observations made of temperature effects on foodborne pathogens in this laboratory and by Snyder (52). The universal indicator is based on a relationship that describes the maximum specific growth rate of a continuum of organisms from psychrophiles to thermophiles in terms of Arrhenius kinetics with an apparent activation energy of ~80 kJ/mole. This value can be related to the activation energy/growth rate at any other temperature by a relative rate function derived from Belehradek (square root) kinetics.

Conclusions

We have argued that a thorough understanding of microbial ecology and physiology offers the best opportunity to control microbial populations in food and reverse the upward trend in the incidence of foodborne disease. Many food preservation strategies have their origin in empirical observations practiced for thousands of years. The systematic collection and collation of data on microbial behavior in foods spawned the discipline of food microbiology, within which predictive microbiology (quantitative microbial ecology) has accelerated our understanding of the microbial ecology of foodborne bacteria. Studies in microbial physiology will further enhance our knowledge and offer new possibilities for food preservation.

The most disturbing aspect of the current crisis is that simple application of existing knowledge would lead to a marked reduction in the incidence of foodborne disease. Education of food handlers and consumers in basic hygiene and the consequences of temperature abuse is urgently needed as is a greater depth of understanding for those in technical positions in the food industry or those with regulatory responsibilities. Furthermore, an appreciation of the need for shared responsibility for food safety within all sectors of the continuum from farm to table, including the consumer, has to be developed. The U.S. Food Safety Initiative draft document emphasizes this point, as does the structure of the Australian Meat Research Corporation's Microbial Food Safety Key Program (53).

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Strategies for Rapid Response to Emerging Foodborne Microbial Hazards[1]

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The foodborne outbreak paradigm has shifted. In the past, an outbreak affected a small local population, had a high attack rate, and involved locally prepared food products with limited distribution. Now outbreaks involve larger populations and may be multistate and even international; in many the pathogenic organism has a low infective dose and sometimes is never isolated from the food product. Delay in identifying the causative agent can allow the outbreak to spread, increasing the number of cases. Emergency intervention should be aimed at controlling the outbreak, stopping exposure, and perhaps more importantly, preventing future outbreaks. Using epidemiologic data and investigative techniques may be the answer. Even with clear statistical associations to a contaminated food, one must ensure that the implicated organism could logically and biologically have been responsible for the outbreak.

Mobilizing Resources

In the traditional paradigm of a foodborne disease outbreak, the cases were from a small local group, and the attack rate was high. Local health officials generally detected and investigated the outbreak. In the emerging paradigm, a diffuse outbreak may be spread over a very wide area, perhaps several counties or states, even with a low infective dose and a low attack rate. The outbreak may be registered only as an increase in sporadic cases and detected only because of specific laboratory-based subtyping surveillance. Whether *Salmonella* serotyping or another molecular method identifies a cluster of related cases, the investigations are more complex, and often no obvious food-handling error is found. Industry contamination may be involved, and implications may be industrywide or nationwide.

Detecting a widespread outbreak requires increased reliance on laboratory subtyping by state public health laboratories at a time when some states are considering eliminating or privatizing their laboratories. Surveillance data must be rapidly compared over increasingly broad regions, not just at the county level but at the state, regional, and national levels. Increased awareness is needed throughout the system that a local outbreak may herald a broader problem. Moreover, investigations must be conducted quickly to prevent future cases. Because of the low levels of microbiologic contamination, the right specimens and samples of food must be used. Available epidemiologic data should guide this selection so that the most likely vehicles are sampled. Traceback must extend beyond the immediate preparation of the implicated food to the whole chain of preparation, i.e., sources of ingredients, processing, storage, and transportation; cooperation at all levels of industry is required. The goals are to control the ongoing outbreak, remove the contaminated product from the market, and learn how to prevent similar outbreaks. Intervention in outbreaks must change. Emergency intervention must be based on solid epidemiologic data (appropriate study design and sample size and clear statistical association with logical and biologic plausibility) and cannot always wait for laboratory confirmation of a contaminated product. Illnesses will not wait for laboratory examination to yield the pathogen; the pathogen may not be detectable with current technologies, the food may not be available, and the delay can be critical.

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[1]The author has summarized the transcripts of panel discussions by Dennis Lang, Craig Hedberg, Eric Johnson, Suzanne Binder, and Philip Tarr.

The Human Side of Foodborne Disease

Public health officials in Washington state screened children with bloody diarrhea and hemolytic uremic syndrome (HUS) as the result of a large outbreak of *Escherichia coli* O157:H7 infection in 1993. One isolate of the organism caused perhaps 75% of the cases, not 100%. As a result, the source of the infection was identified, and regulatory action was taken to halt future cases. Hospitals reported fewer new cases after this action. However, 500 Washington state residents, two-thirds of them children under 15 years of age, became infected before the incriminating food could be removed from the market. The HUS attack rate was approximately 12% for children under the age of 16 years. The organism was recovered from the food product (hamburger), and DNA fingerprinting was initiated by several techniques. The number of colony-forming units of *E. coli* O157:H7 in the hamburger was relatively low.

This organism can cause severe life-threatening infection, even with an inoculum rate too low to be detected by testing. In the United States, the incidence of HUS is approximately 1.7 cases per 100,000 children under the age of 15 years. This figure is based on data from King County, Washington, in the early 1980s and indicates that there are 1,000 cases of HUS in the United States per year or an average of 2.8 cases per day in a population of 250 million. The Centers for Disease Control and Prevention has recently reported that only half of the country's microbiologists screen for this organism.

Clostridium botulinum, A Reemerging Pathogen

The outstanding property of *C. botulinum* is its ability to synthesize a neurotoxin of extraordinary potency (lethal dose is approximately one microgram). *C. botulinum* is an unusual foodborne pathogen in that it causes neuroparalytic rather than diarrheal disease. During illness, the first nerves affected are the cranial nerves in the head and eye; the paralysis can descend and affect every peripheral nerve in the body. The toxin can enter into foods, but in recent years, *C. botulinum* has also been found to colonize the intestinal tract of infants under 1 year of age and of adults that have undergone intestinal surgery or have been treated with antibiotics. The number of cases of adults with unusual clostridia that produce botulinum toxin is increasing.

Botulism occurs worldwide. The highest incidence is found in Poland and in Asia and is related to food handling (in Poland, home canned meats).

New food processes and packaging have been associated with the reemergence of botulism. A clam chowder outbreak in California involved a boxed food that was not properly stored. Because boxed foods generally do not require refrigeration, the food was kept at ambient temperature; however, it was not shelf stable and should have been refrigerated.

A large outbreak (30 cases) of botulism occurred in a restaurant in El Paso when potatoes were cooked in foil, left wrapped, and then used in potato salad. Baking had eliminated vegetative organisms, but the spores of botulinum were not killed. In this case, a low-acid food was held under anaerobic conditions.

Botulism outbreaks are probably the most reported type of foodborne illness. Changes in processing and ingredients in foods and formulations can inadvertently lead to the growth of *C. botulinum.* Failure to thoroughly heat a food product may allow the botulinum toxin to survive.

DNA Fingerprinting

Responding to the threat of emerging foodborne diseases requires public health surveillance that is based on epidemiologic methods and close collaboration between epidemiologists and public health laboratories. Surveillance for foodborne diseases should include pathogen-specific surveillance to identify clusters of cases caused by the same organism and epidemiologic investigations to identify the source.

The Minnesota State Department of Health is developing a new approach to foodborne disease surveillance. A *Salmonella* Enteritidis (SE) outbreak was first recognized by the public health laboratory when the number of SE isolates suddenly increased. Because many of the isolates came from clinical laboratories in southeastern Minnesota, the outbreak initially seemed regional. However, within 48 hours of initiating a case-control study, a nationally distributed food product was identified as the vehicle, and the nationwide scope of the outbreak was documented. This was an example of consequential epidemiology. When the association between food

consumption and SE was announced, the evidence implicating a particular brand was limited to a single case-control study of 15 matched pairs. Laboratory isolation of SE from official samples was not reported until 10 days later. This prompt action, based on epidemiologic data, prevented at least 10 days of potential exposure for thousands of consumers. Consequential epidemiology produces results that translate into disease prevention. We need to continue to develop models for how to rapidly evaluate and act on epidemiologic data and how to better coordinate activities regionally and nationally.

A critical element of the success of this type of foodborne disease surveillance is the specificity with which we can match isolates that may be epidemiologically linked. Molecular subtyping schemes such as pulsed-field gel electrophoresis can greatly improve identification of clusters of the same organisms such as particular *Salmonella* serotypes.

Another example involved analysis of a typical epidemiologic curve for a seemingly single outbreak. When isolates were analyzed, however, a cluster of small outbreaks was found. One was caused by infected food handlers at a restaurant. This outbreak would have continued, and the infected food handlers would have provided an ongoing source of infection to patrons had the cluster not been identified through subtype-specific surveillance. This incident serves as a model for how foodborne disease surveillance systems must be developed and used. Molecular subtyping has revolutionized our ability to conduct meaningful surveillance. We consider it an integral part of disease prevention and control and continue to explore its usefulness.

Parasites

Cyclospora cayetanensis is a protozoan coccidian parasite. A one-celled organism, it is related to other organisms such as *Toxoplasma* and *Cryptosporidium*. It is a prototypical emerging pathogen. *C. cayetanensis* is unusual in that it is not immediately infectious when excreted. Under optimal conditions, it matures in days to weeks, so direct person-to-person spread is very unlikely. An outbreak following a meal is probably not caused by the food handler. The organism appears to be seasonal, and in most places where it has been studied, it occurs in the spring or summer and causes little or no disease during the fall or winter. Infection has been reported throughout the world, and the key studies have been conducted in Peru and Nepal. Disease caused by *C. cayetanensis* is characterized by watery stools, nausea, weight loss, low-grade fever, fatigue, or any combination of these symptoms. The disease (which is easily treatable) can be quite protracted, and without treatment, relapse can occur. The mean incubation period of 1 week complicates the epidemiology; cases may not be recognized until 2 weeks after people have been exposed.

In 1996, more than 1,450 cases of *Cyclospora* were reported in the United States (87%) and Canada (13%). Approximately half of them were in clusters; the other half were sporadic (not epidemiologically linked to other cases). More than 65% of the 1,450 cases were laboratory confirmed; 22 infected patients were hospitalized. Fifty-five clusters were reported, 47 in the United States and 8 in Canada. An average of 28 attendees per event and a very high attack rate were reported. The attack rate was 56% for attendees, not for people who ate the implicated food. At least one type of fresh berry was served at every event and, despite other types of exposures, no other food was implicated in any cluster investigation. The berry did not always achieve statistical significance, largely because of the small number of attendees at a specific event. The berry most likely linked to the cases was later determined. That type of berry was served at 50% to 91% of the 55 events; it was the only kind served at 10 or 11 of the events.

We had overwhelming epidemiologic evidence, but we never identified *Cyclospora* on any raspberries. Two factors, at least, contributed to this. The test for *C. cayetanensis* did not exist when this outbreak occurred; therefore, no implicated raspberries were tested. The question in this investigation as in others is how much epidemiologic evidence is needed to implicate a food as the vehicle of disease? A review team may be needed to look at the epidemiologic data and determine if they are adequate to warrant informing the public about a hazardous food. We are seeing new pathogens, new species. As outbreaks cross into other states, the need for coordination between health officials in the states and in the federal government becomes more urgent.

Conclusions

Early identification of the outbreak and the organism can prevent future cases. Recent

investigations have found that the presence of an organism even at low levels can cause serious consequences. Fingerprinting organisms for identification during outbreaks is extremely important. In some instances, fingerprinting has helped identify several small outbreaks that initially appeared to be one large outbreak. We can no longer afford to wait for all the evidence and laboratory results to be collected and reported; we must use epidemiologic data. Once the outbreak is identified, DNA fingerprinting is needed to identify whether other outbreaks are occurring simultaneously.

A rapid and coordinated response is needed among state officials and federal agencies. Interventions should stop outbreaks and identify products causing illness so they can be removed from the market. Then health officials need to take the next step—investigate what happened and determine the cause so that similar outbreaks can be prevented.

Foodborne Illness: Implications for the Future

Richard L. Hall

Many outbreaks of foodborne illness, even those involving newly recognized pathogens, could have been avoided if certain precautions had been taken. This article will draw on existing information to suggest realistic measures that, if implemented, are most likely to avert or diminish the impact of new foodborne disease outbreaks.

I do not see microbiologic infections as totally different from other food hazards, or microorganisms as totally different from other life forms. We are all hosts and prey, parasites and predators. Even though we differ in size, complexity, and weaponry, we employ many similar strategies—the result of a shared planet, and a substantially shared genetic base. That is also why we have much to learn from animal disease surveillance.

Writing on emerging diseases, many argue forcefully for a broad and well-integrated view (1,2); however, aspects absolutely essential to understanding the problem are often omitted. This short article will doubtless make the same error. So, as Shakespeare had Prologue plead at the opening of Henry the Fifth, "Piece out our imperfections in your minds."

Factors Contributing to Emergence

Outbreaks occur whenever pathogenic agents in sufficient number or quantity encounter a susceptible population without effective interceptive measures. Then, if we did not expect it, we say "it emerged."

Genetic Variability

The large genetic variability of microorganisms is the principal reason why so often some survive after any unfavorable environmental change. Some strains are hypermutable, which reinforces the potential for survival, and have very short generation times, with bacterial minutes comparable to human years. As Dr. Lederberg notes, microorganisms are opponents with whom we cannot race—on their terms.

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Environment

Environmental factors also contribute to emergence. Hot, humid climates favor the growth of fungi and the production of mycotoxins. To borrow an example outside foodborne pathogens, an unusually wet season produced a sharp increase in the deer mice population and the consequent outbreak of hantavirus in the Four Corners area of the United States.

Behavior

Human actions and behavior directly affect food safety. People are vectors for disease, traveling far more often, farther, and more rapidly than ever before (3), and moving far more swiftly than rats, lice, and mosquitoes. Political boundaries frequently and perversely act as leaky sieves, letting diseases through unimpeded, while blocking measures for disease prevention, control, and treatment (1).

Urbanization

Urbanization is a major factor in emergence. Crowding increases human contact and opportunities for transmission. Particularly in developing countries, public health services lag far behind the rush from farm to city. Cities, especially in industrialized nations, are economic and governmental centers and harbor institutions of culture and learning. However, cities are also massive projects in the intensive monoculture of humans. With agriculture, it is entirely possible to carry out monoculture effectively and productively—esthetic considerations aside—if one provides for and tightly controls all essential inputs and conditions, monitors the process closely, and is prepared for prompt and effective intervention if something goes wrong. That hardly describes cities anywhere.

Raw Food Production

The effect of changes in raw food production and harvest practices on opportunities for foodborne outbreaks have been discussed. Centralized processing and wide distribution are the principal characteristics of the "new scenario," as compared to the "old scenario" of local production, home processing, and intrafamily consumption. A classic example is the recent Japanese outbreak of hemorrhagic *Escherichia coli.*

Denial

A behavior that encourages outbreaks is denying the existence of an epidemic—a practice more common in developing countries concerned about the effect of outbreak publicity on tourist trade and exports (4). One of our own attitudes is indifference to outbreaks perceived as commonplace and distant (5).

Economics

War and economic collapse provide unparalleled opportunities for disease outbreaks (e.g., cholera in central Africa). The infrastructure that provides clean water, community medicine, disease surveillance, and food control, even where it exists, is a fragile fabric, easily torn by economic, social, and physical disruption.

Technology

In spite of their benefits, technologies often bring new or enlarged risks. This is not an argument for returning to a state of nature. The invention of sausage doubtless increased the incidence of botulism; indeed, "botulus" is the Latin word for sausage. Without proper processing, any modern packaging that excludes oxygen can have the same effect.

Risk Factors

Factors such as age, illness, and medical treatment increase the risk for foodborne illness. Such increases also result from behavior that promotes the incidence of other diseases (e.g., AIDS).

Failure to Prevent and Control

The most common human action that adversely affects food safety is the avoidable lack of or failure to use effective prevention and control measures. That failure is why 85% of all outbreaks are traceable, about equally, to mishandling in homes or in food service establishments.

Interacting Factors

In much of the developing world, an interrelated and mutually reinforcing set of problems keeps foodborne disease at a high level (Figure). Approximately five million children under the age of 5 years living in the tropics die each year of malnutrition and diarrheal disease (6). Palliation is temporary; only economic and technical development can break through this net.

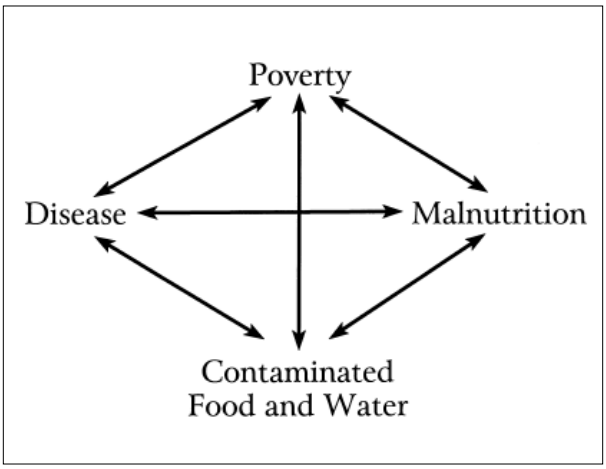

Figure. Problems causing foodborne disease in developing countries.

The contributing factors already mentioned would cause us problems, even acting singly. However, they interact, often synergistically. The combination of bacterial genetic variability and the ease and frequency of mutation of strains present a threat because the process enhances selection for new and more dangerous pathogens. Acid rain and recycling through ruminants may have encouraged the increased environmental durability of acid-tolerant *E. coli* O157:H7. The increasing popularity of marinades in the preparation of foods may have had the same effect.

The globalization of the food trade pulls together several of these contributing factors. One country's contaminated water leads to another country's contaminated raspberries. Refrigeration and controlled atmosphere can preserve pathogens, as well as foods, and spread them all over the world. The *Salmonella* Enteritidis outbreak from contaminated eggs in ice cream was a typically broad problem.

Technologic change combines many of these contributing factors. The intensive monoculture of plants and animals presents concentrated

opportunities. For example, an outbreak of southern corn blight was attributed to the narrow genetic base of a popular hybrid corn. Human-guided plant genetics was defeated by the much more rapid genetic adaptability of microorganisms.

Indiscriminate use of antibiotics and the ability of microorganisms to exchange genetic information has led to increasing resistance. Minimal processing of food, warmly received by natural food lovers, is an open invitation to slightly more durable pathogens to take over the food supply. Centralized processing and mass distribution push us even further in the race Dr. Lederberg reminded us we cannot win. Immunosuppression, due to disease or medication, combined with the failure to take simple sanitary or health precautions make the emergence of pathogens inevitable.

The Role of Public Health Measures

Achieving optimum public health requires many measures, each necessary but not sufficient in itself. The most basic measure is clean water, as recent incidents with strawberries and other fresh produce washed with contaminated water illustrate.

Effective food control structures (e.g., statutory and regulatory frameworks and inspection and enforcement agencies) are essential. A surveillance system has always been required and should be directed to populations at high risk. Newer methods of typing pathogens, faster response times, and enhanced analytical sensitivity are making those systems far more effective than they have been.

Family health programs and education are equally necessary. Food safety still depends heavily on how each of us chooses and uses (or abuses) our food, and each of us is not expert.

The Roles of Food Processing

We process food for the following main reasons: nutrition, safety, preservation, distribution, and esthetics. Cooking typically increases protein digestibility and destroys antinutrients. Fortification and enrichment add, restore, or increase essential nutrients. Cooking and canning destroy pathogenic organisms; cooking and steeping remove natural toxicants, such as cyanide from cassava. Canning, dehydration, salting, smoking, and preservatives, combined with proper packaging and storage, decrease spoilage. Canning, freezing, refrigeration, and controlled atmosphere storage provide variety and year-round availability. Flavors, colors, presale preparation, and stabilizers that prevent separation or crystallization increase consumer appeal and convenience.

Food Processing Technologies

Food processing technologies reduce our exposure to dietary pathogens in three ways, summarized by Professor E. M. Foster as "the three Ks": keep them out, kill all you can, keep the rest from growing.

One group of technologies removes or destroys microorganisms and naturally occurring toxicants by careful sanitation; heat treatment (e.g., cooking, retorting, pasteurizing, high temperature/short time treatment, ultrahigh temperature treatment, ohmic heating, and other newer techniques); radiation (widely useful and recently applied to all of our current methods); physical separation, (e.g., air separation, gravity tables, visual scanners, and other methods for reducing "natural and unavoidable defects" including gross contamination); dehydration; and new technologies (e.g., hydrostatic pressure, pulsed light).

A second group of technologies keeps contaminants well below dangerous levels. These include raw material quality control; careful sanitation; chemical preservation (i.e., fermentation, bacteriostats, pH control, water activity control, and controlled atmosphere storage); dehydration; and freezing and refrigeration.

The third group of technologies prevents recontamination through sanitation of the general environment and at the point of service, protective packaging, and proper handling to ensure packaging integrity.

Beyond these three groups of technologies are general principles governing the use of all such techniques. All foods require incoming quality control and careful sanitation. For nearly all foods except fresh produce and dried grains, one technology from each group listed above should be used. All technologies used must be applied effectively, or none is effective. These technologies must be used in combination, in a systems approach. Failure to apply properly a preceding technology can cause later technologies to increase microbiologic hazards. If oxygen is later excluded from improperly processed food by packaging (canning, foil, an

oil layer), anaerobic pathogens, such as *Botulinum*, are free to multiply.

Future Options

Food safety objectives must be established. There is a clear need for priorities and for employing the "principle of commensurate effort" (i.e., applying effort to risks in the order of their probable impact).

There is also the need for a broad view (1,2). The interaction of several factors, not all of which can be seen in advance, makes a team approach essential.

More effort at understanding the evolution of virulence (7) could provide us with insights on the genetic characteristics and the environmental conditions needed to minimize risk. The 1918-19 influenza epidemic can be used as an example; the present search for its genetic makeup suggests the value of prior knowledge. Knowing or estimating the probability of present or future virulence could prepare us for the more effective use of other measures. These include newer, faster, more sensitive methods of detection.

Improved detection would lead to more effective prevention and control measures. The need to establish priorities suggests here, as in cancer research, the value of biomarkers. Biomarkers are genetic or biochemical indicators of impending risk in the potential pathogen, preceding clinical or epidemiologic indications. Such biomarkers might indicate potential virulence, increased environmental durability, or increased resistance to heat, cold, unfavorable pH, preservatives, or antibiotics.

We need to apply available knowledge more effectively. Most outbreaks of foodborne disease are due to mishandling food in ways we already know how to avoid. The points that follow are not new, but as our food supply increases in complexity and geographic reach, these weapons must grow apace.

Clean water, public sanitation, disease surveillance, and food control are more important than ever. The value of effective disease surveillance has been demonstrated. But what we now have and do is not enough. A national response team must be created. Data should be shared more broadly (electronic linkage of laboratory and surveillance results) if we are to cope effectively with a global food supply, multistage processing by different firms at different locations, and national and international distribution. The effective application of these resources requires a Hazard Analysis and Critical Control Points (HACCP) approach (8).

Private health measures must grow in effectiveness and reach. We must make continuing and increasingly effective use of food processing technologies that reduce microbiologic risks. HACCP cannot be generic or static; it must be adapted to each specific product and processing facility and must be updated with continuing feedback on the hazards to be avoided. HACCP, fortified by the public health measures just described and by the results of the research discussed at this conference, will enable the food processing industry to meet the challenges of emerging pathogens.

Even with the steps just described, approximately 85% of all outbreaks occur as a result of food mishandling in food service establishments or homes. We need to extend HACCP principles to the food service sector as well. HACCP probably cannot reach into homes. If we are to communicate better, we need first to find out what consumers already know, what they want to know, and what they need to know. In short, if we are to educate effectively, we must have direct evidence of how well the information process is working.

Finally, we will never succeed in achieving desirable consumer risk-management practices until consumers understand the inevitability of some risks and minimize them. Food risks have been categorized into six groups in decreasing order of size: microbiologic, nutritional, natural toxicant, environmental contaminant, pesticide residues, and food additives (9). The take-home message on food safety for all of us, as consumers, can be summed up in three words: sanitation, variety, moderation. Sanitation deals effectively with microbiologic risks; variety and moderation deal with nutritional risks, minimizing the impact of the four remaining risks to the extent they are even potentially insignificant. Sanitation, variety, and moderation are within our own control as individual consumers. That is doubtless why we have done only a mediocre job of implementing them. Unless all of us can and do pursue these three objectives effectively, we are all at some unnecessarily increased risk.

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Vero Cytotoxin-Producing *Escherichia coli* O157 Outbreaks in England and Wales, 1995: Phenotypic Methods and Genotypic Subtyping

Vero cytotoxin-producing *Escherichia coli* O157 belonging to four phage types (PTs) caused 11 outbreaks of infection in England and Wales in 1995. Outbreak strains of different PTs were distinguishable by DNA-based methods. Pulsed-field gel electrophoresis best discriminated among strains belonging to the same PT, distinguishing six of the seven PT2 outbreak strains and both PT49 outbreak strains.

Vero cytotoxin-producing *Escherichia coli* O157 (O157 VTEC), first associated with outbreaks of human disease in North America in 1982, has since emerged as an important human pathogen; it causes mild nonbloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome, as well as less common manifestations such as thrombotic thrombocytopenic purpura with neurologic symptoms (1). Laboratory-confirmed infections with O157 VTEC in England and Wales increased from fewer than 10 in 1983 to 250 in 1990, 792 in 1995, and 660 in 1996 (2;3;Laboratory of Enteric Pathogens, unpub. data). The incidence of O157 VTEC infection in Scotland relative to its population is up to six times that in England and Wales, but wide geographic variations exist in England, Wales, and Scotland (4,5). The main reservoir for O157 VTEC appears to be healthy cattle, although recently organisms have been found in sheep (4,6,7). O157 VTEC infections are usually foodborne, associated with consumption of undercooked minced beef (most commonly as beefburgers), unpasteurized milk, and a variety of other vehicles such as salami, cheese, yogurt, water, salad vegetables, and fruit juice (7,8). Other routes of infection include animal contact and person-to-person spread, both in families and institutional settings (4).

O157 VTEC is differentiated by phage typing into more than 80 phage types (PTs) (9, R. Khakhria, pers. comm.). Polynucleotide DNA probes identify Vero cytotoxin (VT) genotypes and divide O157 VTEC into strains with VT1, VT2, or VT1+2 genes. However, certain phage and VT types predominate: thus in England and Wales approximately 50% of O157 VTEC are PT2 and produce VT2 (2,3). A range of DNA-based methods is available for further strain discrimination (4), and we have applied some of these to compare strains associated with outbreaks of O157 VTEC infection in England and Wales in 1995.

Eleven general outbreaks during 1995 affected members of more than one household or residents of an institution (Table), whereas 18 general outbreaks were reported during 1992 to 1994 (10). Most of the outbreaks in 1995 occurred in late summer and in the community (Table, outbreaks 5, 9, and 10); institutions (Table, outbreaks 1, 4, and 11); catering establishments (Table, outbreaks 3, 6, and 7); or a mixture of these (Table, outbreaks 2 and 8). Outbreaks occurred throughout England and Wales; of the 11, four were in the northern region. The incidence of hemolytic uremic syndrome was 0% to 36% in individual outbreaks and 8% overall. The case-fatality rate was 6%, mainly among the elderly. In six outbreaks, there was epidemiologic evidence for foodborne infection, but O157 VTEC was never isolated from food. Person-to-person spread was probably important in three outbreaks. The environmental and epidemiologic investigations of two of these incidents have been reported (11, 12).

For most outbreaks, epidemiologic investigation, phage typing of isolates, and further tests with VT1 and VT2 probes (Table) initially discriminated probable outbreak-associated cases from sporadic infections at the same time in the same area. Seven outbreaks were due to strains of PT2, VT2; two to PT49, VT2; and one to PT1, VT1+VT2. The other outbreak was associated

Table. Outbreaks of infection with O157 VTEC in England and Wales 1995, properties of outbreak strains

Out-break No.	Month	Region/setting (ref)[a]	Cases (HUS/ Fatal)	Phage type	VT probe[b]	VT2 subtype[c]	RFLP φ32511[d]	PFGE *Xba*I[e]	Likely transmission of infection
1	Jan	Northern/Nursing home	7 (0/2)	2	2	2+2c	PT2-A	PT2-1	Person-to-person
2	May	Wessex/Community; hospital	26 (2/0)	2	2	2	PT2-C	PT2-2	Foodborne
3	Jul	N.W. Thames/Hotel	5 (0/0)	1	1+2	2	PT1-A	PT1-1	Foodborne
4	Jul	N. Western/Residential home; hospital	3 (1/3)	2	2	2+2c	PT2-A	PT2-1	Person-to-person
5	Jul	Northern/Community (11)	12 (0/1)	2	2	2+2c	PT2-A	PT2-4	Foodborne
6	Jul	Northern/Restaurant	5 (1/0)	2	2	2+2c	PT2-A	PT2-1a	Foodborne
7	Jul	East Anglia/Holiday camp	4 (0/1)	49	2	2+2c	PT49-A	PT49-1	Foodborne
8	Aug	Wales/Day nursery; community	49 (2/0)	2	2	2+2c	PT2-B	PT2-3	Foodborne, person-to-person
9	Oct	W. Midlands/ Community (12)	11 (4/0)	2	2	2+2c	PT2-Avar	PT2-1b	Foodborne
10	Oct	Various/Community	3 (0/0)	RDNC[f]	1+2	2+2c	RDNC-A	RDNC-1	Unknown
11	Dec	Northern/Day nursery	2 (0/0)	49	2	2+2c	PT49-B	PT49-2	Unknown

HUS=hemolytic uremic syndrome; VT= Vero cytotoxin; RFLP=restriction fragment length polymorphisms; PFGE=pulsed-field gel electrophoresis

[a]Investigation of the epidemiology of two outbreaks has been reported previously (11,12)

[b]Determined by hybridization with digoxigenin-labeled polynucleotide probes for VT1 and VT2 genes (2,3).

[c]Based on polymerase chain reaction amplification with a sense primer specific for either the VT2 or VT2c sequence and a degenerate antisense primer that would anneal to known VT2 sequences (14).

[d]Hybridization with a probe comprising digoxigenin-labeled fragments of the VT2-encoding bacteriophage from strain E32511(15). Patterns were designated according to the phage type of the strain and a letter denoting a unique pattern type. The PT2-Avar pattern differed from PT2-A by the possession of a single extra hybridizing fragment.

[e]Profiles of *Xba*I digested genomic DNA. Patterns were designated according to the phage type of the strain and differentiated by number. Thus patterns PT2-1, PT2-2, PT2-3, and PT2-4 differed from each other by at least three fragment positions. Where there were single unique band differences from PT2-1 these were designated PT2-1a, etc.

[f]The designation RDNC indicates that the strain reacts with the typing phages but does not conform to a currently defined pattern.

with a VT1+VT2 strain that reacted with the typing phages but did not conform to a recognized type (reacts but does not conform [RDNC]). In outbreak 8, the O157 VTEC was resistant to sulphonamides and tetracyclines, whereas the other outbreak strains were sensitive to antimicrobial agents. In relation to community outbreaks 2, 5, and 8, several cases were infected with O157 VTEC that were similar to the outbreak strains, but the patients had no known epidemiologic link with the outbreak. Such strains were therefore included in the tests described below to provide evidence for the possible involvement of these cases. O157 VTEC that hybridize with the VT2 polynucleotide probe may carry different VT2 sequences (13). Strains from humans commonly possess VT2 or VT2c or both sequences. Polymerase chain reaction (14) showed that strains from all outbreaks except 2 and 3 possessed both types of VT2 sequences (Table). O157 VTEC belonging to PT1, such as those from outbreak 3, usually carry VT1 and only the VT2 sequence (13). The presence of the VT2, but not the VT2c, sequence differentiated the PT2 strain from outbreak 2 from all the other PT2-associated outbreaks. This property was exploited during the course of the outbreak to exclude patients infected with PT2 strains that had the VT2+VT2c genotype and not the outbreak VT2 genotype.

The O157 VTEC strains were further analyzed by Southern blot hybridization of *Eco*RI

restriction enzyme-digested genomic DNA with a probe comprising digoxigenin-labeled fragments of the VT2-encoding bacteriophage φ32511 (15). Strains of O157 VTEC give an array of up to 20 hybridizing fragments; these restriction fragment length polymorphisms (RFLPs) are different for strains of different PTs and can distinguish between strains of the same PT. The RFLP patterns of the PT1 and RDNC strains from outbreaks 3 and 10 distinguished them from each other and from all the remaining strains. The technique indicated that the PT49 strains from outbreaks 7 and 11 had distinct RFLP patterns (Table), but it did not differentiate the PT2 strains from outbreaks 1, 4, 5, and 6. These strains gave a single pattern (identical to that of a PT2 strain reported previously [15]) that we have found most commonly in strains of this PT (unpub. data). The PT2 strains from outbreaks 2 and 8 gave RFLP patterns that were different from this common pattern and from each other (Table). In both instances, the test was used to exclude those cases outside the outbreak.

Pulsed-field gel electrophoresis (PFGE) has been applied widely as a highly discriminatory method for fingerprinting bacterial pathogens. The method of Barrett et al. (16), modified as indicated in the Figure, identified between 15 and 20 *Xba*I-generated fragments of the O157 VTEC strains (Figure). PFGE patterns of PT1, PT49, and RDNC were reproducible and clearly distinct from each other; they differed from the most common PT2 pattern by at least six fragment positions. The PT49 strains associated with outbreaks 7 and 11 were distinguished from each other by their PFGE profile. Results of PFGE of O157 VTEC of PT2 from the outbreaks are shown in the Figure. The strains that caused outbreaks 1 and 4 were indistinguishable by all methods including PFGE (lanes 2 and 4). Two other strains, from outbreaks 6 and 9, were very similar to these two, differing at only one fragment position (lanes 6 and 8), but the patterns were distinct and reproducible. The PT2 strains from outbreaks 5 and 8 (lanes 5 and 7) differed from those associated with outbreaks 1 and 4 by at least 3 fragment positions and were distinguishable from each other. The strain from outbreak 2, which possessed only the VT2 gene, was clearly distinct from all the other PT2 strains by PFGE (lane 3). Criteria for the interpretation of patterns produced by PFGE have been published by Tenover et al. (17). Results of the analysis of O157 VTEC by PFGE suggest these criteria need modification for closely related organisms such as O157 VTEC (18).

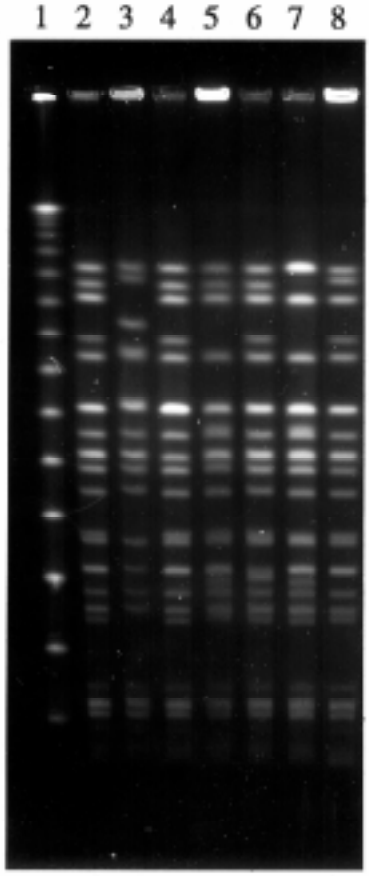

Figure. Pulsed-field gel electrophoresis of *Xba*I-digested genomic DNA of O157 VTEC PT2 isolated from outbreaks in 1995. Digests were separated on 1% agarose for 42h at a voltage gradient of 5.6 volts per cm with a pulse ramp time of 5 to 50 sec. Lane 1 contained a phage lambda DNA 48.5 kb ladder (Sigma). Lanes 2 to 8 contained digests of PT2 strains from outbreaks as follows: lane2, outbreak 1; lane3, outbreak 2; lane4, outbreak 4; lane5, outbreak 5; lane6, outbreak 6; lane7, outbreak 8; lane8, outbreak 9.

Although phage typing and polynucleotide probes for VT1 and VT2 genes rapidly characterized strains from the outbreaks in 1995, DNA-based methods were valuable in distinguishing outbreak cases from outlying ones. VT2 gene subtyping was rapid but not very discriminatory, whereas RFLP and PFGE techniques differentiated strains but were time-consuming. The highest level of discrimi-

nation (by PFGE) distinguished certain O157 VTEC that appeared identical by all other methods. Some of the differences detected by PFGE were minor, but for each outbreak, they were reproducible with all the strains examined in this study. The epidemiologic or evolutionary significance of these minor variations is difficult to evaluate.

Investigation of the epidemiology of outbreaks of O157 VTEC infection requires a combined use of typing and fingerprinting methods in a hierarchic manner consistent with practical and economic constraints. In outbreaks well defined by epidemiologic studies, phage typing and the identification of VT1 and VT2 genes, including VT2 subtyping, are likely to be sufficient. In outbreaks less clearly defined epidemiologically, DNA-based methods may assist in identifying those strains not associated with the outbreak; this is particularly helpful when the outbreak is due to a common phage type. DNA-based methods have been useful in linking human infections with associated foods (15) and animals (19).

Although PFGE gives a high level of discrimination between closely related O157 VTEC, it has certain disadvantages. It is time-consuming and may not be suitable for rapid identification of large numbers of strains. We recommend the use of a combination of phage typing, VT typing, and PFGE to provide good discrimination of O157 VTEC strains in epidemiologic investigations.

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Genetic Polymorphism Among *Cryptosporidium parvum* Isolates: Evidence of Two Distinct Human Transmission Cycles

We report the results of molecular analysis of 39 isolates of *Cryptosporidium parvum* from human and bovine sources in nine human outbreaks and from bovine sources from a wide geographic distribution. All 39 isolates could be divided into either of two genotypes, on the basis of genetic polymorphism observed at the thrombospondin-related adhesion protein (TRAP-C2) locus. Genotype 1 was observed only in isolates from humans. Genotype 2, however, was seen in calf isolates and in isolates from a subset of human patients who reported direct exposure to infected cattle or consumed items thought to be contaminated with cattle feces. Furthermore, experimental infection studies showed that genotype 2 isolates were infective to mice or calves under routine laboratory conditions, whereas genotype 1 isolates were not. These results support the occurrence of two distinct transmission cycles of *C. parvum* in humans.

Long considered a veterinary disease, cryptosporidiosis has emerged as an important infectious disease in humans (1). In immunocompetent persons, the disease is usually self-limiting; however, in the immunocompromised, it is frequently chronic, more severe, and sometimes fatal. Cryptosporidiosis is one of the major secondary diagnoses in people with AIDS and is associated with a twofold greater hazard of death than other AIDS-defining diagnoses (2).

A number of major waterborne outbreaks of cryptosporidiosis have occurred in urban settings (3); however, the disease also occurs sporadically. Since most *Cryptosporidium parvum* infections are self-limiting and symptomatically similar to other diarrheal diseases, the disease may often be undiagnosed or misdiagnosed in the absence of a recognized outbreak. Consequently, the actual incidence of cryptosporidiosis and the relative importance of each of its many modes of transmission are largely unknown. For these reasons, laboratory tools are needed for quantitative and qualitative environmental sampling and for strain analysis of *Cryptosporidium* isolates. These tools would be extremely valuable for source identification and outbreak investigations, for correlation with clinically important phenotypes, and for determining risk factors in nonepidemic settings.

A number of new nucleic acid-based approaches have been developed for detection, diagnosis, and typing of *C. parvum*, among them polymerase chain reaction (PCR)-based tests that focus either on random amplification of DNA polymorphisms or on specific polymorphic genetic loci (4-7). These PCR-based tests suggest the existence of strain variation and the possibility of two distinct transmission cycles among *C. parvum* isolates that infect humans. In this study, we examined genetic polymorphism among *C. parvum* isolates from human and nonhuman sources to identify strain-specific markers that could be correlated with epidemiologically important phenotypes.

Analytic Approach

Parasite Isolates

Thirty-nine isolates were examined from stool samples positive for *C. parvum:* 17 were obtained from humans or calves during outbreaks in the United States and Canada (Table 1); one was a calf isolate (Iowa calf) routinely passaged in neonatal Holstein calves in our laboratories; and 21 were obtained from cattle from Georgia, Alabama, Ohio, Oklahoma, Kansas, Iowa, Idaho, Utah, and Washington. All samples were collected and placed directly into a

Table 1. *Cryptosporidium parvum* isolates examined

Isolate	Implicated source	Host	Ref.
Maine, 1993	apple cider	human	16
Wisconsin, 1993	drinking water	human	3
Wisconsin, 1996	drinking water	human	*
Georgia (day care), 1995	person-to-person	human	*
Georgia (water-park), 1995	recreational water	human	17
Florida, 1995	drinking water	human	18
British Columbia, 1996	drinking water	human	15
Texas, 1996	unknown	human	*
Pennsylvania, 1997	bovine contact	human, calf	*

*Reference is this article.

2.5% potassium dichromate solution and were stored at 4°C. Oocysts were purified by using discontinuous sucrose and Percoll or cesium chloride gradients (8,9).

Isolation of Genomic DNA

Parasite DNA was isolated as described by Kim et al. (10). Briefly, oocysts were ruptured by using five freeze-thaw cycles (dry ice ethanol bath and 65°C) in a lysis buffer (120 mM NaCl, 10 mM EDTA, 25 mM Tris pH 7.5, 1% Sarkosyl) containing proteinase K. The samples were incubated for 1 hour at 55°C to inactivate nucleases. Then DNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1), precipitated with absolute ethanol, washed with 70% ethanol, and resuspended in TE buffer (10 mM Tris pH 8.0, 1 mM EDTA).

PCR Amplification and Sequencing and Analysis

The gene fragment of interest, a 369-bp region of the thrombospondin-related adhesive protein (TRAP-C2) of *C. parvum*, was amplified with the following primers: 5'-CAT ATT CCC TGT CCC TTG AGT TGT-3' and 5'-TGG ACA ACC CAA ATG CAG AC-3', which correspond, respectively, to positions 812 to 835 on the coding strand and positions 1,161 to 1,180 on the negative strand, of GenBank sequence X77586. The reactions were performed with Perkin-Elmer (Perkin-Elmer Corporation, Foster City, CA) PCR reagents, including 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 mM of each specific primer, and 2.5 U of Taq DNA polymerase. After a 1-minute hot start at 94°C, the reactions went through 35 to 40 cycles of denaturing at 94°C for 30 seconds, annealing at 45°C for 30 seconds, and extension at 72°C for 1 minute, followed by a 72°C incubation for strand completion.

An aliquot of each PCR product was examined by agarose gel electrophoresis; the remaining PCR product was purified by using the Wizard PCR Prep Kit (Promega Corporation, Madison, WI). Purified PCR fragments were sequenced directly on an ABI 377 automated sequencer by fluorescent cycle sequencing using dye-terminator chemistry with AmpliTaq FS (Perkin-Elmer-Applied Biosystems) according to the manufacturer's recommended procedure. The same primer sets used initially for PCR were used again for sequencing, diluted to a concentration of 10 pMoles in the final sequencing reaction. Downstream analysis of sequence data was accomplished by using the Sequence Navigator program (Perkin–Elmer-Applied Biosystems). Multiple sequence alignments were performed by using the Pileup program (11). Animal isolates were manually sequenced by the dideoxy chain-termination method (12), using the Sequenase Version 2.0 kit (J.T. Baker, Phillipsburg, NJ) with the sequences of a few isolates confirmed by automated sequencing.

Experimental Infection Studies

Purified oocysts ranged in age from 1 to 6 months at the time of inoculation of cell cultures or animals. This age range is well within the storage time that maintains oocyst viability and infectivity (e.g., laboratory-passaged isolates are 40% to 50% viable after storage for 6 months). Approximately 10^6 oocysts were administered orally to 2-day-old calves or to 4- to 6-day-old BALB/c or SCID mice by using established procedures (8,13). Beginning at day 5, stools were collected and examined daily by light microscopy or by immunofluorescent flow cytometry for *C. parvum* oocysts (14).

Findings

Sequence Determination and Analysis

A single specific band of 369 bp, corresponding to bases 812 to 1180 of the 1.1 kb *C. parvum* TRAP-C2 gene (GenBank accession number

X77586) was amplified from 39 different isolates (Figure 1). Although the sequence similarity was very high among all gene fragments, multiple alignments showed two primary genotypes. These genotypes could be established on the basis of nucleotide substitutions at five independent positions, three being silent changes and the other two resulting in amino acid changes (Figure 2). Of the five changes, four were transitions, and one was a transversion.

Genotype 1 included human isolates from Wisconsin, Georgia, Florida, and Texas. Genotype 2 contained human isolates from Maine and British Columbia, human and calf isolates from Pennsylvania, the Iowa calf laboratory strain, and 21 bovine isolates from various locations around the country. Two human isolates (one from Florida and the Texas isolate) appeared to represent a variant of genotype 1. In both cases, they shared the first four positions with the other genotype 1 isolates. The fifth position, however, was the same as that of genotype 2 isolates.

Experimental Infection Studies

The results of experimental infection studies are shown in Table 2. The genotype 2 isolates from human outbreaks in Maine and Pennsylvania and from a calf in Iowa all readily infect both mice and calves. The genotype 2 isolate from British Columbia was also reported to be infective to immunosuppressed C57BL/6 mice (15). None of the genotype 1 isolates from humans—from Wisconsin, Florida, a Georgia day-care facility, and a Georgia water park—could be established in either mouse or calf. A single sample, the Georgia day-care isolate, was examined for its ability to infect a neonatal pig. This isolate caused a brief moderate infection in a neonatal pig host (data not shown) but not in calves or mice. One of the Wisconsin isolates and the Georgia day-care isolate were tested for their infectivity to MDCK cell cultures; both successfully infected this cell line (data not shown).

	Position:	15	42	64	111	244
	Isolate:					
			Genotype 1			
Human Isolates	Milwaukee 93 /1	G	C	T	C	T
	Milwaukee 93 /2	G	C	T	C	T
	Milwaukee 93 /3	G	C	T	C	T
	Milwaukee 96	G	C	T	C	T
	Georgia-DC 95	G	C	T	C	T
	Georgia-WP 95 /1	G	C	T	C	T
	Georgia-WP 95 /2	G	C	T	C	T
	Florida 95 /1	G	C	T	C	T
	Florida 95 /2	G	C	T	C	T
	Florida 95 /3	G	C	T	C	T
	Florida 95 /4	G	C	T	C	T
	Florida 95 /5	G	C	T	C	C
	Texas 96	G	C	T	C	C
			Genotype 2			
	Maine Cider 93	A	T	G	T	C
	B.C. Canada 96	A	T	G	T	C
	Pennsylvania 97 (H)	A	T	G	T	C
Calf Isolates	Pennsylvania 97 (C)	A	T	G	T	C
	Iowa calf (CDC)	A	T	G	T	C
	Published calf	A	T	G	T	C
	Other bovine (n=21)	A	T	G	T	C

Figure 1. Alignment of TRAP-C2 nucleotide positions that show polymorphism among *Cryptosporidium parvum* isolates from human and nonhuman sources. Published calf sequence refers to Genbank accession number X77586. Other bovine (n=21) refers to 21 samples (from Georgia, Alabama, Ohio, Oklahoma, Kansas, Iowa, Idaho, Utah, and Washington) that had the same genotype.

Conclusions

All isolates examined in this study could be grouped easily into two distinct genotypes defined by nucleotide substitutions at five positions within the TRAP-C2 locus, with genotype 1 containing a variant at the fifth position that was represented by two isolates. All isolates in genotype 1 were from human stool. The isolates in genotype 2, however, were from both human and bovine sources. In the limited number of isolates that were tested in experimental infection studies, all genotype 2 isolates could be established readily in mice and calves. None of the genotype 1 isolates, however, could be shown to be infective to either of these hosts. The genotype and experimental infection data suggest the possibility of two distinct populations of *C. parvum* cycling in humans. One population appears to involve zoonotic transmission from calf-to-human with subsequent human-to-human and human-to-calf transmission. The other population appears to involve an anthroponotic transmission cycle, exclusively in humans. This hypothesis is consistent with the data from the epidemiologic investigations from which the isolates were obtained.

Genotype 2 characteristics were identified in human isolates from the Maine 1993, British Columbia 1996, and Pennsylvania 1997 outbreaks, and in all isolates from bovine sources. Both the Maine and Pennsylvania outbreaks could be directly linked to a calf source of *C. parvum*. The Maine outbreak was associated with contaminated apple cider (16). Interestingly, *C. parvum* oocysts were isolated directly from apple cider, the press used for preparing the

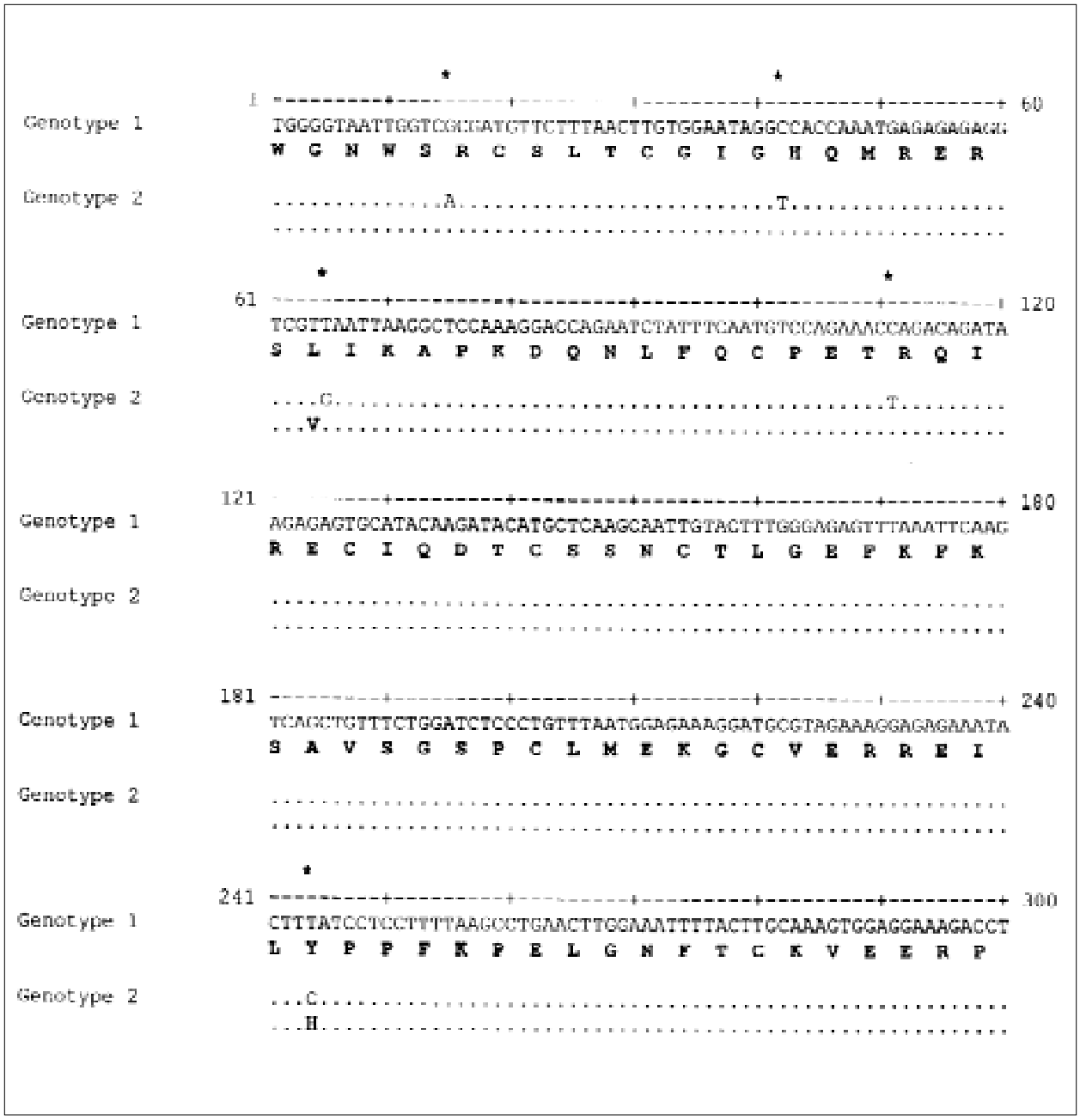

Figure 2. DNA and putative amino acid sequences of *Cryptosporidium parvum* TRAP-C2 genotypes 1 and 2.

cider, and a calf stool specimen from the farm that supplied the apples.

The Pennsylvania focus involved three families that together purchased three young calves that subsequently developed scours. Nine members of three families had diarrhea, and two were hospitalized. *C. parvum* oocysts were isolated from two calves and five humans; all isolates examined demonstrated the genotype 2 pattern.

The British Columbia isolate came from a human patient infected in an outbreak (approximately 2,000 cases) that occurred in the small rural community of Cranbrook (15). During the outbreak investigation, *Cryptosporidium* oocysts were identified in human fecal specimens, in cattle manure specimens found near the watershed, and in water samples from the reservoir intake.

Table 2. Experimental infection studies with *Cryptosporidium parvum* isolates from various sources

Isolate	Experimental host	Infection
Maine, 1993	mouse	+
	calf	+
Wisconsin, 1993	mouse	-
	calf	-
Georgia (day care), 1995	mouse	-
	calf	ND
Georgia (water park), 1995	mouse	-
	calf	-
Florida, 1995	mouse	-
	calf	ND
Iowa (bovine), 1984	mouse	+
	calf	+

ND = not done

Of the genotype 1 isolates examined, epidemiologic investigations were conducted for the Georgia water park 1995, Florida 1995, and Wisconsin 1993 outbreaks. In the Georgia water park outbreak, approximately 2,900 persons met the case definition for clinical cryptosporidiosis. In a sample of these patients, the following risk factors were evaluated in telephone interviews: swimming in lakes or pools, exposure to day care or to persons with diarrhea, contact with young animals, drinking water from various sources, chronic illness, and water park attendance. The only factor independently associated with diarrheal disease was water park attendance (17).

The Florida 1995 outbreak occurred at a day camp in central Florida and had approximately 70 cases (18). Risk factors examined included participating in camp activities, eating lunches provided at the camp, and drinking water from various specified sources. *C. parvum* oocysts were observed in the stools of 16 persons and in water from an outside tap. Fecal contamination (of unknown origin) of the tap was the suspected source of the outbreak. Five specimens were examined from this outbreak, all of which belonged to the genotype 1 grouping; one displayed additional polymorphism at nucleotide position 244 (Figure 1). The Texas 1995 isolate showed this same polymorphism, which we think is most accurately described as a subset of genotype 1.

The Wisconsin 1993 outbreak, which affected more than 403,000 people, is the largest waterborne disease outbreak ever recorded in the United States. Four isolates were examined, three isolated during the original outbreak and a fourth isolated in 1996 from an AIDS patient with a chronic infection who had initially been infected in the 1993 outbreak.

During the Wisconsin outbreak, possible sources of contamination of Lake Michigan with *Cryptosporidium* oocysts included cattle along two rivers that fed Milwaukee Harbor, slaughterhouses, and human feces (3,19). The genotypic and experimental infection data from the four isolates we examined suggest a human rather than bovine source. However, these results come from the analysis of only four samples from a massive outbreak, and the degree to which these samples are representative of the entire outbreak remains uncertain.

All genotype 2 isolates examined in this study came from persons that had direct links or potential exposure to *C. parvum* from an infected animal. All samples tested in experimental infection studies were also infective to both mice and calves. In the genotype 1 isolates, however, while the initial source of the cases was never directly determined experimentally, no confirmed links to bovine sources were found, but exposure to water contaminated with human feces could have occurred. Furthermore, of the isolates tested in experimental infection studies, none could successfully infect laboratory animals. These results lead us to suggest the possibility of a second transmission cycle that is anthroponotic and maintained through person-to-person contact or through human sewage contamination of the water supply.

The observations reported here with respect to genotypic variation among *C. parvum* isolates from humans and animals are very similar to those reported by other groups. These studies generally reported one allozyme pattern or genotype associated with human isolates and a second genotype or allozyme in bovine samples and a subset of human samples. The specific genes or regions examined differed in each study but included electromorphs of phosphoglucomutase and hexokinase (20), random amplified polymorphic DNA (RAPD) analysis of an unspecified region (4), a repetitive DNA sequence (6), the 18S rRNA gene and adjacent internal transcribed spacer (ITS) region I (5), the *Cryptosporidium* oocyst wall protein (COWP) locus (7), and the dihydrofolate reductase-thymidylate synthase (DHFR-TS) gene (21). At least two additional studies suggest the possibilities of two transmission cycles, on the basis of epidemiologic or

experimental observations (22,23).

The TRAP-C2 protein is a member of a class of proteins present in all apicomplexans examined to date (24-26). This protein is associated with the cell surface and micronemal complex of these parasites and is thought to be involved in surface attachment; consequently, changes in this protein could affect attachment specificity and the resultant host range. Were this the case, such a mechanism could explain why the host range of one genotype might be different from that of a second genotype, resulting in distinct transmission cycles. In the two isolates that make up the variant of genotype 1, the T-to-C transition results in a change in the amino acid sequence from tyrosine to histidine. If variations in this protein affect host preference, the histidine-to-tyrosine change would have to be inconsequential with respect to protein function and host specificity. Observations in *Plasmodium* suggest that the WCSP motif in the TRAP gene is the functional domain involved in surface attachment; however, the polymorphism we observed in *C. parvum* did not involve this region. Additional studies are needed to clarify the relationship, if any, of polymorphism in this gene to host range.

The conclusion that two transmission cycles exist for *C. parvum* is now supported by the results of independent groups, using markers at six different genetic loci. This conclusion, if valid, may have important implications for the prevention and control of cryptosporidiosis in urban settings. Cattle have been the most commonly implicated source of water contamination in outbreaks outside the United States but not conclusively within the United States. Measures for preventing water contamination have in some cases included the removal of cattle from watershed areas in or around municipalities. If, however, sewer overflows and inadequate sewage treatment are the primary source of water contamination in urban settings where anthroponotic cycles are being maintained, focusing solely on cattle could fail to eliminate a very important source of infection.

The results of this study suggest the need to 1) combine the typing approaches of various groups into a multilocus approach for genetic typing of *C. parvum* that would result in a reliable and robust method for strain typing, 2) apply multilocus typing to a large number of *C. parvum* isolates both from epidemic and isolated cases and from a large geographic distribution to determine the prevalence of these two genotypes and their quantitative importance as indicators of specific risk factors, and 3) identify additional genetic loci that will allow more precise determination of strain variation and linkage of genotypic variation to specific clinical and epidemiologically important outcomes.

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Irradiation Pasteurization of Solid Foods: Taking Food Safety to the Next Level

In the 19th century, milk from diseased cattle produced in unsanitary surroundings and distributed under filthy conditions to an increasingly urbanized population sickened and killed consumers by the thousands (1,2). Wide acceptance of the germ theory and the sanitary awakening that followed led to vast improvements in animal health and hygiene, and the safety of dairy products improved substantially. Dairy farmers in northern Europe discovered that heating milk fed to their calves further reduced the risk for tuberculosis in their herds, and after overcoming concerns that the new thermal pasteurization technology would corrupt the dairy industry, destroy the nutritional value of milk, and lead to serious public health problems, the same level of protection was offered to human consumers a few decades later (3,4). More recently, thermal pasteurization has been suggested for eliminating low level contamination of juice by foodborne pathogens (5,6). However, for the safety of solid foods that enter kitchens as raw agricultural commodities, including meat, poultry, and seafood, we continue to rely solely on animal health programs and sanitation. Therefore, as we approach the 21st century, preventable illness and death caused by vegetative bacterial and parasitic foodborne pathogens remain substantial public health problems (7,8).

Irradiation pasteurization of solid foods with low doses of gamma rays, X-rays, and electrons will effectively control vegetative bacterial and parasitic foodborne pathogens (9-11). Public concerns, similar to those raised against thermal pasteurization of milk, have been advanced in opposition to irradiation pasteurization, and it has been claimed that if we but paid more attention to sanitation and proper cooking, these products could be safely consumed without introducing new technologies.

Perhaps. However, the residual risk for infection that remains after state-of-practice sanitation during production, harvest, processing, distribution, and preparation yields an unacceptable level of illness and death. In addition, the admonition to properly cook works only if culturally acceptable food preferences do not include undercooked and raw foods. Increased interest (encouraged by the public health and nutrition community) in fresh produce as part of a high fiber, low fat diet, further reduces the effectiveness of proper cooking as a disease control strategy (12).

Recent outbreaks of foodborne illness associated with undercooked meat and uncooked fresh produce, and the emergence of the previously unrecognized foodborne hazards that spawned the conference whose proceedings are reported in this issue of Emerging Infectious Diseases, have stimulated interest in methods of pasteurizing solid food without altering its raw appearance and characteristics. Research is under way on a variety of promising approaches, including pulsed energy, bright light, high pressure, and other nonthermal technologies, but few are ready for immediate application (13,14; Fed Reg 61:42381-83, 1997). Irradiation pasteurization, on the other hand, is a well-established process with clearly documented safety and efficacy that can be put into widespread use as quickly as facilities can be sited and built (15).

Good practice guidelines and Hazard Analysis and Critical Control Points programs (HACCP) can result in raw meat, poultry, seafood, and produce with sufficiently low levels of pathogen contamination that irradiation doses as low as 1 to 3 kGy yield adequate margins of safety for common foodborne pathogens such as *Campylobacter, Cryptosporidium, Escherichia coli, Listeria, Salmonella*, and *Toxoplasma* (9). No other control for *Campylobacter* contamination of poultry meat is apparent, and other approaches to ground beef safety have proven inadequate to prevent intermittent low level contamination with *E. coli* O157:H7. Likewise, no other solutions are immediately available to control pathogen contamination of produce intended for raw consumption, and irradiation doses used appear adequate for the bacterial and parasitic pathogens involved in recent outbreaks (Donald Thayer, pers. comm.)

The food industry appears reluctant to fully embrace irradiation pasteurization despite the obvious and painful failure of alternative approaches to prevent foodborne infections. Much of this reluctance stems from the perception that consumers reject the process and will refuse to buy irradiated food. Indeed, surveys have shown considerable consumer confusion and ignorance about food irradiation (16), and

reports on public antipathy toward things radioactive abound. However, consumer surveys also demonstrate profound and growing public concern about microbial food safety, and decreasing concerns about the safety of irradiated food (17). Knowing as little about it as they appear to, approximately half of the consumers surveyed have expressed willingness to try irradiated food if it will decrease their risk for illness (16). In addition, when educated about food irradiation, 90% of survey participants expressed interest in purchasing irradiated foods; sampling such food increased interest to 99% (18).

Irradiation pasteurization is not the cure for all food safety ills. Pasteurization of any sort is no match for bad sanitation and substandard practices, and irradiation pasteurization can be overwhelmed by large numbers of pathogens. Just as obviously, foods produced and processed under appropriate conditions that are then properly packaged and irradiated are subject to postpasteurization contamination. In addition, the doses of irradiation used to pasteurize fresh meat and poultry are not sufficient to kill bacterial spores. Thus, if anaerobic packaging is the method used to protect irradiated foods from postpasteurization contamination, *Clostridium botulinum* could pose a risk if the cold chain is disrupted.

Vibrio infections associated with consumption of raw molluscan shellfish can be prevented with irradiation pasteurization, but the Norwalk-like viruses also frequently associated with raw shellfish appear to be more radioresistant than vegetative bacterial pathogens. Levels of irradiation an order of magnitude greater than pasteurizing doses for meat and poultry also are necessary to inactivate hepatitis A virus. To reduce the risk for foodborne hepatitis A and Norwalk virus infections, it will be necessary to reduce the level of exposure of food to human feces. This is true regardless of whether or not foods are to be pasteurized. Although irradiation pasteurization will not eliminate all seafood-borne pathogens, it will reduce the potential of seafood to cause illness. Seafood HACCP and advances in viral diagnostics and environmental virology will help ensure that prepasteurization conditions are sufficient to yield seafood appropriate for irradiation pasteurization (19,20). Just as thermal pasteurization works well for liquid foods like milk and juice, but not for solid foods for which raw characteristics are desired, irradiation pasteurization works well for meat, poultry, seafood, and soft fruit, but wilts leafy vegetables and sprouts. That irradiation pasteurization does not work for every food and every pathogen is poor justification for not applying it for those food/pathogen combinations for which it has been shown to work so well.

Consumer surveys have demonstrated public concerns over worker and environmental safety that have also contributed to the reluctance of some to build and use food irradiation facilities. These concerns are appropriate and addressable. Because food irradiation and irradiation sterilization of nonfood items like medical supplies are so well established, proper facilities design and operating characteristics are well known. The relatively short half-life of Cobalt 60 and its insolubility in water reduce environmental concerns, which can be eliminated altogether by using electricity-generated X-rays and electron beams instead of a radioactive source. Proper education and training has protected employees of irradiation sterilization facilities; employees of food irradiation facilities should not be qualitatively different from other employees in similar industries.

Thus, a broadly applicable solution to many of our food safety problems exists and has existed for a number of decades. It is disappointing that the public health community has been so silent for so long on this issue. Faced with the liability of marketing hazardous foods, it is puzzling why the food industry has not stepped into the vacuum created by this lack of leadership from public health. Presentations at the Conference on Emerging Foodborne Pathogens make it clear that new foodborne hazards are being stacked on top of old, unresolved food safety problems—broadly applicable solutions are desperately needed. Just as thermal pasteurization of milk protected us from *E. coli* O157:H7 before we knew it was in raw milk, irradiation pasteurization can protect us from tomorrow's emerging foodborne pathogen.

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Non-O157 Shiga Toxin–Producing *Escherichia coli* Infections in Europe

To the Editor: Shiga toxin–producing *Escherichia coli* (STEC) infections are an important cause of severe human disease. Although most infections are caused by strains of serogroup O157, STEC pathogenic to humans may belong to other serogroups usually referred to as non-O157 STEC.

Recently, Tarr et al. (1) and Acheson et al. (2) described infections attributable to STEC O103 and expressed concern that non-O157 STEC may pose an underestimated threat to public health in the United States. In fact, non-O157 STEC is often overlooked in clinical microbiology laboratories because the toxigenic phenotype is not exploited to identify such pathogens. Rather, most laboratories use sorbitol MacConkey agar and serotyping (which cannot detect most non-O157 STEC) to identify *E. coli* O157:H7.

Since the end of the 1980s, non-O157 STEC infections have caused as many as 10% to 30% of sporadic cases of hemolytic uremic syndrome (HUS) in Germany (3), Italy (4), and the United Kingdom (5). Moreover, HUS outbreaks have been associated with STEC O111:H- in Italy (6) and France (7).

During 1996, we observed a sudden increase in infections attributable to STEC O103 and O26 in Germany and Italy. In our laboratory in Germany, *E. coli* O103:H2 was not identified among 345 non-O157 STEC isolated between 1985 and 1995 but represented 12 (18.2%) of 66 of the non-O157 STEC isolated during 1996. HUS developed in two infected patients.

Among cases reported to Italy's nationwide HUS surveillance system from 1988 to 1995, evidence of infection with STEC O103 or O26 was found in two (1.5%) and nine (6.6%) of 135 cases, respectively. Since 1996, infection with STEC O103 and O26 has been diagnosed in three (11%) and nine (33%) of 27 HUS cases, respectively.

These observations indicate that identification of non-O157 STEC should be considered by clinical laboratories. Immunoenzymatic tests (based on either toxin antibodies or receptors) that detect Shiga toxins produced by fecal bacterial isolates or present in stool specimens are now available (8,9). Use of these tests should be considered in analyzing the stools of patients with HUS, bloody diarrhea, or painful nonbloody diarrhea, if classic microbiologic analysis fails to yield *E. coli* O157:H7 or another standard enteric pathogen, such as Campylobacter, Salmonella, or Shigella.

The sudden appearance or increase of rare non-O157 STEC in our populations is worrisome. Most non-O157 STEC, as well as the sorbitol fermenting O157:H- strains (10) associated with HUS in several European countries, would be missed by laboratories using standard microbiologic detection methods, such as sorbitol MacConkey agar screening. Because of the considerable clinical and epidemiologic urgency, clinical microbiologists and physicians should seek out these such pathogens in appropriate clinical situations.

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The Taxonomy of *Cyclospora*

To the Editor: In the article by N.J. Pieniazek and B.L. Herwaldt (1) on the rRNA gene of *Cyclospora cayetanensis,* the authors suggest that *Cyclospora* should be placed in the genus *Eimeria* because the rRNA genes of the two genera have similar sequences. The article refers to Norman D. Levine's chapter on the Apicomplexa in the Illustrated Guide to the Protozoa (2). Regrettably, the authors failed to read the whole chapter and to recognize that the initial characteristics for placing the oocyst of a coccidium in its proper genus are the number of sporocysts and then the number of sporozoites in each sporocyst. The genus *Eimeria* has four sporocysts and two sporozoites in each sporocyst. The genus *Cyclospora* has two sporocysts, each of which has two sporozoites.

The original taxonomists (3) of *C. cayetanensis* recognized that it should be placed in the taxonomic family Eimeriidae, close to *Eimeria*, but they adhered to the traditional designation for genera of coccidia. Pieniazek and Herwaldt should be cognizant of the rules of zoologic nomenclature as well as the fact that certain morphologic characteristics of protists have served us well for many decades and continue to be useful. There are serious consequences to changing the classification of an organism, and it should not be thought that one can make such a change casually. I encourage the editors of Emerging Infectious Diseases to seek the advice of those who understand what should be done with respect to the classification and nomenclature of organisms.

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Reply to W.C. Marquardt: Dr. Marquardt's advocacy for reliance on morphologic characteristics even if phylogenetic data become available that lead to a different conclusion runs counter to that expressed in an article he coauthored, which supports the importance of molecular data (1). The introduction of that paper states the following:

"Early systematists relied largely on light microscopic structures and life cycle patterns to separate protozoa taxonomically.... Apicomplexans display enormous variations in life cycle patterns, physiology, cytology, and biochemistry. There is no consensus on which characteristics should be relied upon to infer phylogenetic relationships. Developmental and ultrastructural features have been used to infer evolutionary relationships among representative genera in the class Sporozoea. However, comparisons of phenotypic characters are qualitative and lack objective quantitative assessment to infer genetic relationships. Sequence similarities between proteins or genes which share a common evolutionary history can be used to infer quantitative phylogenetic relationships. The small subunit (16S-like) rRNAs and their coding regions are especially useful for estimating the extent of genetic relatedness over broad evolutionary ranges."

That paper concludes with the statement that "ribosomal RNA sequence analyses of other apicomplexans are required in order to test the validity of relationships inferred from structures and life cycle patterns." Similarly, we concluded our paper as follows: "Reports based on morphologic features alone may suffer from poor resolution of features needed for classification of closely related

organisms. To improve our understanding of the taxonomy of human-associated *Cyclospora*, molecular evaluation of isolates of additional *Cyclospora* and *Eimeria* species, especially other mammalian species, is needed."

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Foodborne Diseases Active Surveillance Network (FoodNet)

The Foodborne Diseases Active Surveillance Network (FoodNet) is the foodborne disease component of the Emerging Infections Program (EIP) of the Centers for Disease Control and Prevention (CDC). A collaborative project of CDC, the seven EIP sites, the U.S. Department of Agriculture (USDA), and the U.S. Food and Drug Administration (FDA), FoodNet consists of active surveillance for foodborne diseases and related epidemiologic studies designed to help public health officials better understand the epidemiology of foodborne diseases in the United States. FoodNet was established in 1995 in five locations: Minnesota, Oregon, and selected counties in Georgia, California, and Connecticut. The total population of these sites, or catchment areas, is 14.7 million, or 6% of the population of the United States. FoodNet was expanded to selected counties in Maryland and New York in 1997. The goals of FoodNet are to describe the epidemiology of new and reemerging bacterial, parasitic, and viral foodborne pathogens; estimate the frequency and severity of foodborne diseases that occur in the United States per year; and determine how much foodborne illness results from eating specific foods, such as meat, poultry, and eggs.

Foodborne diseases are common; an estimated 6 to 33 million cases occur each year in the United States. Although most of these infections cause mild illness, severe infections and serious complications do occur. The public health challenges of foodborne diseases are changing rapidly; in recent years, new and reemerging foodborne pathogens have been described, and changes in food production have led to new food safety concerns. Foodborne diseases have been associated with many different foods, including some previously thought to be safe, such as eggs and fruit juice, both of which have transmitted *Salmonella* during recent outbreaks. Public health officials in the seven EIP sites are monitoring foodborne diseases, conducting epidemiologic and laboratory studies of these diseases, and responding to new challenges from these diseases. Information gained through this network will lead to new interventions and prevention strategies for addressing the public health problem of foodborne diseases.

Current "passive" surveillance systems rely upon reporting of foodborne diseases by clinical microbiology laboratories to state health departments, which in turn report to CDC. Although foodborne diseases are extremely common, only a fraction of them are routinely reported to CDC through these surveillance systems. Inadequate reporting results from a complex chain of events that must occur before a case is reported, and a break at any linkage along the chain results in a case not being reported (Figure). FoodNet is an "active" surveillance system, meaning public health officials frequently contact microbiology laboratory directors to find new cases of foodborne diseases and report these cases electronically to CDC. In addition, FoodNet is designed to monitor each of the events that occurs along the foodborne diseases pyramid and thereby allow more accurate and precise estimates and interpretation of the prevalence of foodborne diseases over time. Because most foodborne infections cause diarrheal illness, FoodNet focuses these efforts on persons who have a diarrheal illness.

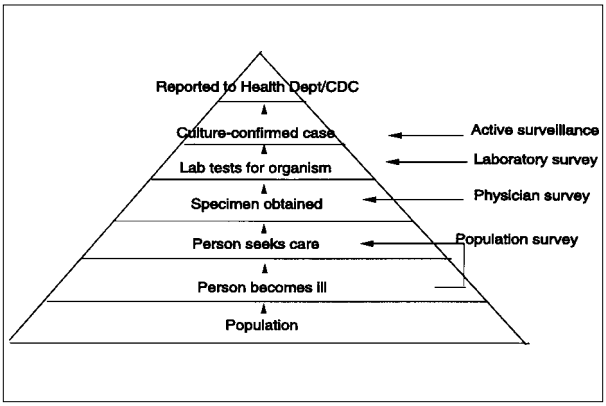

Figure. The prevalence of illness pyramid. Passive surveillance data represent only the tip of the iceberg. For a bacterial infection to be included in the passive surveillance system, it must pass through the following steps: a person becomes ill with a diarrheal disease, the patient must go to a doctor, the doctor must order a bacterial stool culture, the assigned microbiology laboratory must culture for this organism and report the infection to the state health department, and the state health department in turn must report the infection to CDC. This passive surveillance system is the means by which the number of cases of foodborne illness is currently determined at CDC; if any step does not occur, foodborne illness is not reported. FoodNet is designed to collect information along each step of this pyramid.

FoodNet Components

Active Laboratory-Based Surveillance

The core of FoodNet is population-based active surveillance at over 300 clinical microbiology laboratories that test stool samples in the seven participating sites. In active surveillance, the laboratories in the catchment areas are contacted regularly by collaborative FoodNet investigators to collect information on all laboratory-confirmed cases of diarrheal illness. Since January 1996, information has been collected on every laboratory-diagnosed case of *Salmonella*, *Shigella*, *Campylobacter*, *Escherichia coli* O157, *Listeria*, *Yersinia*, and *Vibrio* infection among residents of the catchment areas of the five original sites; this information is transmitted electronically to CDC. The result is a comprehensive and timely database of foodborne illness in a well-defined population.

Survey of Clinical Laboratories

In October 1995, collaborative FoodNet investigators conducted a baseline laboratory survey of all microbiology laboratories in the five original catchment areas to determine which pathogens are included in routine bacterial stool cultures, which tests must be specifically requested by the physician, and what specific techniques are used to isolate the pathogens. A baseline survey will be conducted in the two new sites, and a follow-up survey to assess any recent changes in laboratory practices was conducted in the original sites in 1997. Practices in clinical laboratories have been found to vary; some laboratories look for a wider variety of bacteria than others. The methods used to collect and examine specimens are being investigated because these can influence whether the laboratory finds disease-causing bacteria.

Survey of Physicians

To obtain information on physician stool-culturing practices, collaborative FoodNet investigators mailed a survey questionnaire to 5,000 physicians during 1996. Analysis of these data is ongoing. Because laboratories test stool specimens from a patient only upon the request of a physician or other health-care provider, it is important to measure how often and under what circumstances physicians order these tests. As changes occur in the way health care is provided in the United States, stool-culturing practices may also change. The practices of physicians who send stool samples to laboratories within the catchment areas will be monitored by surveys and validation studies.

Survey of the Population

Collaborative FoodNet investigators contact randomly selected residents of a catchment area and ask whether the person has had a recent diarrheal illness, whether the person sought treatment for the illness, and whether the person had consumed certain foods known to have caused outbreaks of foodborne illness. During 1996, 750 residents of the catchment areas were interviewed by telephone each month (9,000/year). Because many who become ill with diarrhea do not see a physician, little is known about the number of cases of diarrhea in the general population and how often persons with diarrhea seek medical care. The population survey is an essential part of active surveillance for foodborne illness because it allows for an estimate of the population who seeks medical care when affected by diarrheal illness.

Case-Control Studies

In 1996, the FoodNet began case-control studies of *E. coli* O157 and *Salmonella* serogroup B and D infections. More than 60% of *Salmonella* infections in the United States are caused by serogroup B and D *Salmonella*. These large case-control studies will provide new and more precise information about which food items or other exposures may cause these diseases. To allow the most precise classification of the isolates from the patients in these studies, the *Salmonella* and *E. coli* O157:H7 laboratory specimens from these patients are sent from FoodNet sites to CDC for further study, including antibiotic resistance testing, phage typing, and molecular subtyping.

Accomplishments

Since becoming operational on January 1, 1996, FoodNet has tracked the rates of foodborne diseases. Even in the first year of data collection, numerous interesting patterns and outbreaks were detected. Surprisingly high isolation rates for *Y. enterocolitica* in Georgia and *Campylobacter* in California were detected. An outbreak of *Salmonella* infections caused by contaminated alfalfa sprouts was detected in Oregon. Two outbreaks of *E. coli* O157:H7 infections were detected in Connecticut, one due to lettuce and

one to apple cider. FoodNet has also provided the infrastructure for conducting active surveillance for new and reemerging diseases. When an association between bovine spongiform encephalopathy in cattle and variant–Creutzfeldt-Jakob disease in humans was suspected in the United Kingdom, EIP personnel conducted surveillance for this rare human disease. EIP personnel also collaborated in the investigation of a multistate outbreak of *Cyclospora* infections associated with consumption of raspberries from Guatemala.

Future FoodNet Projects

Several projects in 1997 will focus on *Campylobacter*, including a case-control study to determine the risk factors for infection and determination of antibiotic resistance patterns among *Campylobacter* strains.

Collaborative FoodNet investigators will establish active surveillance for hemolytic uremic syndrome, a serious complication of *E. coli* O157:H7 infection.

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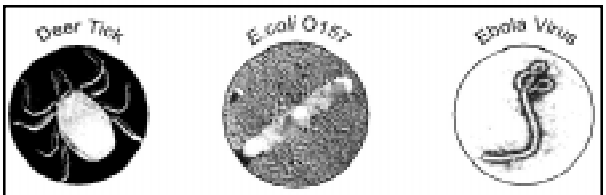

International Conference on Emerging Infectious Diseases

March 8-11, 1998
Atlanta, Georgia

Abstract deadline: October 31, 1997

The Centers for Disease Control and Prevention, the Council of State and Territorial Epidemiologists, the American Society for Microbiology, and the National Foundation for CDC, along with more than 50 agencies and organizations are cosponsoring the 1998 International Conference on Emerging Infectious Diseases. This conference will offer new opportunities for education, collaboration, and partnership with colleagues worldwide to explore the most current research, surveillance, and prevention and control programs addressing all aspects of emerging infectious diseases. Attendance is limited to 2,500 participants.

The meeting will consist of general and plenary sessions, symposia, and roundtables with invited speakers, presentations on emerging infections activities, oral and poster presentations based on submission of an accepted abstract, and exhibits. Conference topics will include work on surveillance, epidemiology, research, communication, training, and prevention and control of emerging infectious diseases, as well as emergency preparedness and response.

Abstracts should address new, reemerging, and drug-resistant infectious diseases that affect human health. Deadline for abstract submission is October 31, 1997. Information on later submission of abstracts for consideration as late breakers is included in the program materials. For additional information, access announcements at www.cdc.gov/ncidod/EID/eid.htm or the National Center for Infectious Diseases (www.cdc.gov/ncidod/whatsnew.htm), send an e-mail to meetinginfo@asmusa.org, or call 202-942-9248. Proceedings of the conference will be published in the Emerging Infectious Diseases journal.

Errata

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In "Fluoroquinolone Resistance in *Neisseria gonorrhoeae*" by J.S. Knapp et al., in the Table, p. 35, under the column R of Equivalent MIC (mg/ml), and on the row Ofloxacin, 400 mg subrow Ofx, 5, which cited reference 6, the number should be ≥2.0, not ≥1.0. The reference for this criterion (reference 6 on p. 3) should read "Detection of quinolone-resistant *Neisseria gonorrhoeae*."

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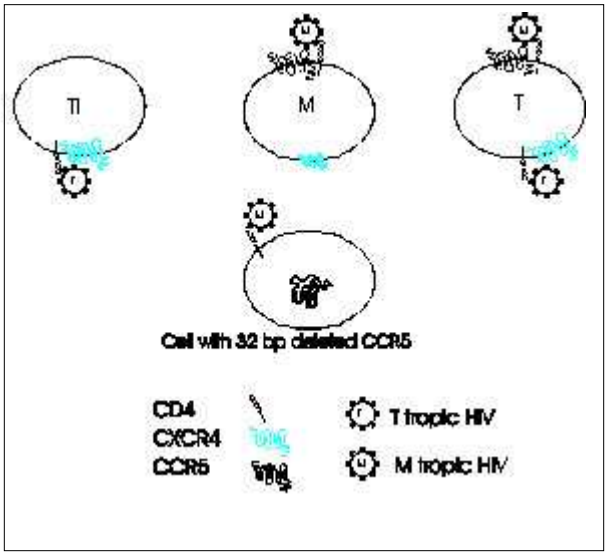
In "Host Genes and HIV: The Role of the Chemokine Receptor Gene CCR5 and Its Allele (ΔCCR5) by J.M. McNicholl et al., the following figure should have been printed as Figure 2, p. 264. The figure legend is correct.



In "Emerging Foodborne Diseases" by S.F. Altekruse et al., in Table 1, p. 285, the number of *Escherichia coli* O157:H7 cases per year in the United States should be 25×10^3, not 725×10^3. The legend to Figure 2, p. 288 should read "Percentage of U.S. population over 65 years of age, 1900-2040 (projected)."

In "Molecular Epidemiologic Investigations of *Mycoplasma gallisepticum* Conjunctivitis in Songbirds by Random Amplified Polymorphic DNA Analyses" by D.H. Ley et al., in Figure 1, p. 376, the photographs of the house finch and American goldfinch were inadvertently reversed.

We apologize to our readers for these errors.

Editorial Policy and Call for Articles

Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal has an international scope and is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, demography, sociology, and other disciplines. Inquiries about the suitability of proposed articles may be directed to the Editor at 404-639-3967 (tel), 404-639-3075 (fax), or eideditor@cdc.gov (e-mail).

Emerging Infectious Diseases is published in English and features three types of articles: Perspectives, Synopses, and Dispatches. The purpose and requirements of each type of article are described in detail below. To expedite publication of information, we post journal articles on the Internet as soon as they are cleared and edited.

Spanish translations of some articles can be accessed at ftp://fcv.medvet.unlp.edu.ar/pub/EID. Articles by authors from non-English-speaking countries can be made simultaneously available in English and in the author's native language (electronic version of the journal only). Articles published in this way are translated from English into the author's native language and appear in the same issue of the journal.

Instructions to Authors

Manuscripts should be prepared according to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" [Ann Intern Med 1997:126[1]:36-47].

Begin each of the following sections on a new page and in this order: title page, abstract, text, acknowledgments, references, each table, figure legends, and figures. On the title page, give complete information about each author. Give current mailing address for correspondence (include fax number and e-mail address). Follow Uniform Requirements style for references. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations. Tables and figures should be numbered separately (each beginning with 1) in the order of mention in the text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Italicize scientific names of organisms from species names all the way up, except for vernacular names (viruses that have not really been speciated, such as coxsackievirus and hepatitis B; bacterial organisms, such as pseudomonads, salmonellae, and brucellae).

All articles are reviewed by independent reviewers. The Editor reserves the right to edit articles for clarity and to modify the format to fit the publication style of Emerging Infectious Diseases.

Documents sent in hardcopy should also be sent on diskette, or by e-mail. Acceptable electronic formats for text are ASCII, WordPerfect, AmiPro, DisplayWrite, MSWord, MultiMate, Office Writer, WordStar, or Xywrite. Send graphics documents in Corel Draw, Harvard Graphics, Freelance, or save as .TIF (TIFF), .GIF (CompuServe), .WMF (Windows Metafile), .EPS (Encapsulated Postscript), or .CGM (Computer Graphics Metafile). The preferred font for graphics files is Helvetica. If possible, convert Macintosh files into one of the suggested formats. Submit photographs as glossy, camera-ready photographic prints.

Send all manuscripts and correspondence to the Editor, Emerging Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, Mailstop C-12, Atlanta, GA 30333, USA, or by e-mail to eideditor@cdc.gov.

Perspectives: Contributions to the Perspectives section should provide insightful analysis and commentary about new and reemerging infectious diseases or related issues. Perspectives may also address factors known to influence the emergence of infectious diseases, including microbial adaptation and change; human demographics and behavior; technology and industry; economic development and land use; international travel and commerce; and the breakdown of public health measures. Articles should be approximately 3,500 words and should include references, not to exceed 40. Use of additional subheadings in the main body of the text is recommended. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch.

Synopses: Submit concise reviews of infectious diseases or closely related topics. Preference will be given to reviews of emerging and reemerging infectious diseases; however, timely updates of other diseases or topics are also welcome. Synopses should be approximately 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch.

Dispatches: Provide brief updates on trends in infectious diseases or infectious disease research. Include descriptions of new methods for detecting, characterizing, or subtyping emerging or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome. Dispatches (1,000 to 1,500 words of text) should not be divided into sections. Provide a short abstract (50 words); references, not to exceed 10; and figures or illustrations, not to exceed two.