EMERGING INFECTIOUS DISEASES

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

A peer-reviewed journal published by the National Center for Infectious Diseases Vol. 4 No. 3, July–Sept 1998

New Agents The Global Threat Foodborne Diseases Vector-borne Issues

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

In Index Medicus/Medline, Current Contents, Excerpta Medica, and other databases

Electronic Access

Retrieve the journal electronically on the World Wide Web (WWW), through file transfer protocol (FTP), or by electronic mail (e-mail). WWW and FTP: Access the journal at http://www.cdc.gov/eid or from the CDC home page (http://www.cdc.gov), or download it through anonymous FTP at ftp.cdc.gov. The files can be found in the directory pub/EID. LISTSERVer (e-mail lists): To subscribe to a list, send an e-mail to listserv@cdc.gov with the following in the body of your message: subscribe listname (e.g., subscribe EID-TOC). EID-TOC will send announcements of new articles and the table of contents automatically to your e-mail box. Obtain individual articles on WWW or through FTP.

Emerging Infectious Diseases

Emerging Infectious Diseases is published four times a year by the National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC), 1600 Clifton Road, Mailstop C-12, Atllanta, GA 30333, USA. Telephone 404-639-3967, fax 404-639-3075, e-mail eideditor@cdc.gov.

EMERGING INFECTIOUS DISEASES

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

A peer-reviewed journal published by the National Center for Infectious Diseases

The journal is distributed electronically and in hard copy and is available **at no charge**.

☐ YES, I would like to receive Emerging Infectious Diseases.

Please print your name and business address in the box and return by fax to 404-639-3075 or mail to
EID Editor
CDC/NCID/MS C12
1600 Clifton Road, NE
Atlanta, GA 30333

Moving? Please give us your new address (in the box) and print the number of your old mailing label here_____

Contents

EMERGING INFECTIOUS DISEASES

Volume 4 • Number 3 *July–September 1998*

David Satcher, U.S. Surgeon General; Donna Shalala, U.S. Secretary of Health and Human Services.

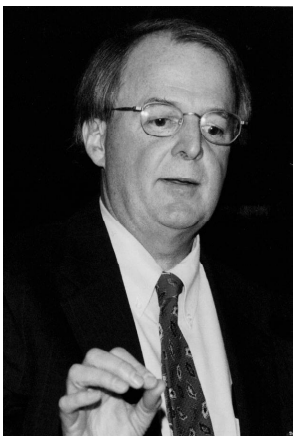
Cover: T. Moore (1941), Phantasmagoria, oil on board. From the collection of Abdu Azad.

Conference photos by Jim Gathany.

Contents

EMERGING INFECTIOUS DISEASES

Volume 4 • Number 3 *July–September 1998*



James M. Hughes, Director, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

About the International Conference on Emerging Infectious Diseases

Stephen A. Morse
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

More than 2,500 researchers, clinicians, laboratorians, veterinarians, and other public health professionals from all 50 states and more than 70 countries convened in Atlanta on March 8-11, 1998, for the International Conference on Emerging Infectious Diseases. The conference, organized by the Centers for Disease Control and Prevention (CDC), the Council of State and Territorial Epidemiologists, the American Society for Microbiology, and the National Foundation for CDC along with 62 other cosponsors,[1] provided a forum for the exchange of ideas and possible solutions to the problems of new and reemerging infectious diseases, including potential threats presented by bioterrorism. Several agencies and organizations sponsored satellite partnership meetings on March 8 and March 12.

More than 85 sessions (12 plenary sessions, 17 invited panels, 35 poster sessions, and late-breaking abstracts) were presented on surveillance, epidemiology, prevention, and control of emerging infectious diseases, as well as emergency preparedness and response and reemerging or drug-resistant infectious diseases. Topics included foodborne diseases, infectious diseases transmitted by animals and insects, nosocomial infections, infections in immunocompromised patients and persons outside the health-care system, infectious causes of chronic disease, blood safety, host genetics, vaccines, global climate change, and immigration and travel.

In delivering the keynote address, Nobel laureate Joshua Lederberg reviewed the scientific basis for the emergence of infectious diseases. U.S. Health and Human Services Secretary Donna Shalala and Assistant Secretary for Health and Surgeon General David Satcher, along with representatives from the World Health Organization, the Pan American Health Organization, and the U.S. Agency for International Development, and representatives from academia and industry addressed the national and international ramifications of emerging infections. In closing the conference, James Hughes, director, National Center for Infectious Diseases, CDC, stressed the importance of building bridges and forging new partnerships to prevent and control the emergence of infections into the next millennium.

In publishing the conference presentations and discussions in this journal, the organizers hope to capture the energy expressed by all participants, further disseminate new information on emerging infections, and stimulate more research and other initiatives against this important public health threat.

---

[1]Alliance for the Prudent Use of Antibiotics, American Academy of Pediatrics, American Association of Blood Banks, American Association of Health Plans, American Cancer Society, American College of Preventive Medicine, American Hospital Association, American Medical Association, American Mosquito Control Association, American Public Health Association, American Sexually Transmitted Diseases Association, American Society of Clinical Pathologists, American Society of Tropical Medicine and Hygiene, American Veterinary Medical Association, Association of American Veterinary Medical Colleges, Association of Schools of Public Health, Association of State and Territorial Directors of Health Promotion and Public Health Education, Association of State and Territorial Health Officials, Association of State and Territorial Public Health Laboratory Directors, Association of Teachers of Preventive Medicine, Burroughs Wellcome Fund, Emory University School of Medicine, Fogarty International Center, Food and Drug Administration, Indian Health Service, Infectious Diseases Society of America, International Life Sciences Institute, International Society for Infectious Diseases, International Society of Travel Medicine, International Union for Health Promotion and Education, International Union of Microbiological Societies, Minority Health Professions Foundation, Morehouse School of Medicine, National Aeronautics & Space Administration, National Association of City and County Health Officials, National Association of State Public Health Veterinarians, National Council for International Health, National Foundation for Infectious Diseases, National Hispanic Medical Association, National Institute of Allergy and Infectious Diseases, National Medical Association, National Oceanographic & Atmospheric Administration, Office of Science and Technology Policy, Pan American Health Organization, Rollins School of Public Health of Emory University, Society for Healthcare Epidemiology of America, Society for Occupational and Environmental Health, Society for Public Health Education, The Carter Center, The Henry J. Kaiser Family Foundation, The HMO Group, The Robert Wood Johnson Foundation, The Rockefeller Foundation, The World Bank, U.S. Agency for International Development, U.S. Department of Agriculture, U.S. Department of Defense, U.S. Department of State, U.S. Department of Justice (INS), U.S. Department of Veterans Affairs, U.S. Environmental Protection Agency, World Health Organization.

Collaboration in the Fight Against Infectious Diseases

Donna E. Shalala
U.S. Secretary of Health and Human Services

Two hundred years ago, the U.S. Public Health Service, of which the Centers for Disease Control and Prevention (CDC) is an essential part, began as a humble maritime hospital in New York City. Its mission was simply to stop infectious disease from coming in on ships and spreading across our country. Today, as we celebrate the anniversary of the Public Health Service, another historic event has occurred. One of the great detective hunts of the 20th century came to an end. Scientists at the U.S. Department of Defense confirmed that tissue from a woman's body buried near the Bering Strait contains genetic material from the 1918 Spanish flu virus—the virus that caused the worst pandemic the world has ever known. This discovery will help us map the genetic structure of the microbe that sent a wave of death crashing around the globe 80 years ago.

It is hard to believe today that flu could be so nearly apocalyptic. In just 11 months, at least 24 million people died, and most of humanity was infected. The infected often never knew what hit them; in the morning they felt fine; by night they could be dead—drowned as the lungs filled with fluid. There was no explanation, no protection, no cure. The pandemic produced scenes from a gothic horror novel—but it was all too real. In Philadelphia alone, 11,000 died of the flu in a single month. The dead were left in gutters, and death carts roamed the city in a surreal scene from medieval times. As the deaths mounted all over the world, orderly life began to break down. Schools and churches closed; farms and factories shut down; homeless children wandered the streets; their parents vanished. The acting U.S. Army Surgeon General, Victor Vaughn, calculated that if the pandemic continued its mathematical rate of acceleration, it soon could spell the end of humankind.

But then, as silently, as mysteriously, as quickly as it came, the terror began to fade away. People stopped dying. The victims were buried. Life returned to normal. The great flu was soon pushed off the front pages and out of the public eye. When avian flu first appeared last year, we wondered if perhaps another pandemic had begun. An influenza subtype that had never before produced illnesses or deaths in humans now did. While it appears that the spread of avian flu has halted without the appearance of human-to-human transmission, the danger is far from over because the critical period may be just beginning—this is the start of the traditional flu season in Hong Kong.

The emergence of avian flu points up a broader concern: complacency over infectious disease. It is easy to assume that modern medicine has defeated this enemy once and for all. Our comfort is a natural byproduct of our progress and success—the remarkable breakthroughs in antibiotics and vaccines, thanks to the work of scientists and researchers worldwide. We eradicated smallpox—consigning one of history's deadliest killers from the medical books to the history books. But infectious disease remains the leading cause of death worldwide and the third leading cause in the United States. While we may be winning some old battles, we are struggling with some new adversaries—emerging infectious diseases such as Ebola, hantavirus infection, new strains of tuberculosis (TB), AIDS, and Lassa fever, to name a few. In fact, the World Health Organization (WHO) has labeled the growing threat of infectious disease a global crisis.

The time has come to replace complacency with a new sense of urgency—to launch a renewed, unified, global effort against infectious disease. Nature may have the power to create a pandemic—but together we have the power to prevent it, to stop it, to overcome it, to cure it. And there is no time like the millennium. For today, history and human progress have created an "ironic contradiction" in the fight against infectious disease: some of the same forces that

invite pandemics can also be harnessed to fight pandemics. With the globalization of travel and trade, immigration, communication, and industrialization, we have a smaller world with porous borders. Nations are more interconnected, people are more interdependent, and humanity is less divided by what the Indian poet Tagore called our "narrow domestic walls." So the bad news is that we have fewer barriers against the spread of infectious disease; yet the good news is that those fewer barriers mean new avenues to progress and the potential for sharing information and efforts to stop infectious disease.

We now have the power to push infectious diseases off the world stage but only if governments, world health organizations, the private sector, scientists, and researchers work together with a global strategy. How do we successfully wage this global battle against infectious disease? The answer lies in what we can learn from the 1918 pandemic; it provides three important lessons—challenges for all of us.

The first lesson is that we must assume it could happen again. Influenza pandemics have regularly swept the world every 10 to 40 years, and it has been 30 years since the last influenza pandemic, Hong Kong flu, killed 700,000. Nature is creative, and the flu has great potential for mutating. If a strain changes dramatically, we could suddenly have a virus for which we may have no immunity, no vaccine, and no cure. The threat is not just the flu—the spectrum of new infectious diseases is constantly expanding, while old diseases, such as TB, have evolved into entirely new killers because they developed antibiotic resistance.

The advent of antibiotics in the 1940s was one of the chief reasons we began to defeat infectious disease. However, almost as soon as antibiotics were available, microbes mutated and developed resistance. In the 1950s to 1970s, we produced so many new antibiotics that there was always an alternative medication; today, the flood of new antibiotics has diminished to a trickle, while the microbes have continued to grow resistant. Antibiotic-resistant bacteria are becoming more common in hospitals and among patients with depressed immune systems. In Japan in 1996 and in the United States last year, we started to see a strain of staphylococcus infection, the most common hospital-acquired infection, which could sometimes withstand vancomycin—our most potent treatment. But almost simultaneously, the first antibiotic to fight a new generation of "super bugs," Synercid, won limited approval from a Food and Drug Administration (FDA) advisory panel. If it wins full approval, it will be the first drug in a new arsenal of weapons. FDA continues to work with drug manufacturers to bring new antibiotics to market as safely and rapidly as possible.

Antibiotic resistance is not just a medical problem; it is also a behavioral problem. Patients too often demand antibiotics for every illness—even for viral infections (like the flu) that do not respond; patients often do not finish the course of medication, allowing the remaining bacteria to develop resistance; many doctors overprescribe; and the pharmaceutical industry has limited its antibiotic development because of cost. The widespread use of antibiotics in farm animals may also be helping the spread of drug-resistant genes. Given the consequences, we must act now to combat the diminishing effectiveness of antibiotics. That is why CDC is strengthening surveillance and implementing education campaigns about the problem, why the National Institutes of Health (NIH) is studying resistance, and why FDA is promoting judicious antibiotic use. But this is not a job for government agencies alone. Each and every one of us who understands the risks needs to spread the message that antibiotics are being misused, abused, and overused.

The next pandemic could also result not from a mutating bug or ineffective antibiotics but from an act of bioterrorism. Whether bioterrorism is state sponsored or undertaken by a lone terrorist, it is not just a problem for the military or law enforcement; it is also a challenge for the entire public health community. If a specific threat is issued—perhaps someone claims to have released a toxic agent in a public place—trained public health officials must first verify that an incident has occurred. They may need to decontaminate the area, identify exposed populations, and deliver preventive measures and treatments. Too often, a threat is not issued, no warning is given. In such a situation, public health officials must first quickly determine the deadly agent, the route of exposure, and the likely source.

The U.S. Department of Health and Human Services (DHHS) is coordinating with our partners in other agencies and the military to ensure the proper training of state and local health officials, the availability of vaccines and drugs, and the enhancement of our surveillance

capacity and expertise. There is also an administrationwide effort to train emergency response teams and health-care providers in 120 cities. We must enhance our ability now to address the growing threat of bioterrorism.

The second lesson concerns preparation for a potential pandemic. We cannot wait until the next deadly microbe appears on the world stage. Therefore, since 1993, HHS has been leading a federal, state, and local effort to develop a "pandemic influenza plan." As a result of the avian flu episode, we have sped up the process to complete the plan and pursue its full implementation. Meanwhile, CDC is studying the impact of antiviral medications and alternative ways to produce vaccines. NIH is working with the pharmaceutical industry to develop and test innovative vaccines, including a nasal spray that delivers an inoculation dose of the virus. FDA is issuing new drug permits for experimental influenza vaccines. With new viruses knocking at the door, we cannot afford to be caught unprepared. Because only in the movies can we save the world from a deadly disease in just 24 hours.

We need commitment in responding to all emerging infectious disease. We need a worldwide "surveillance and response network" that can quickly identify and stop an outbreak. We have already laid the groundwork for such a system with bilateral and multilateral talks on disease monitoring with our partners in Europe, Japan, Asia, and Africa. For example, at the Denver Summit in 1997, the group of eight industrialized nations, including the United States, pledged to help develop a global disease surveillance network and coordinate an international response to infectious disease. Working through the Trans-Atlantic Agenda with the European Union (EU), the United States and EU countries have begun to share surveillance data on Salmonella infections. Additionally, through the U.S.-South Africa Bilateral Commission, our two countries are training health personnel in South Africa in surveillance and applied epidemiology. I look forward to working closely with WHO to further globalize our approach to surveillance and response.

U.S. agencies are already supporting the efforts of WHO to improve communications networks and to build regional centers for monitoring disease. CDC and WHO jointly run 12 world monitoring stations for the flu alone. Perhaps the best example of the kind of monitoring and surveillance system needed worldwide is the excellent system that stopped the avian flu outbreak in Hong Kong. On a routine basis, officials collect throat swabs from people with flulike symptoms. The samples are analyzed, and if suspicious, they are immediately sent to CDC, which functions as one of the WHO International Reference Laboratories for East Asia. When the first known case of avian flu was diagnosed in a 3-year-old boy, warning bells went off immediately. When a second case appeared in November, health officials around the world went on alert, and a team from CDC left for Hong Kong. Over the next 2 months, work continued to define the extent of the outbreak, including who was becoming ill, why they were becoming ill, and whether the virus could spread from person to person and cause a pandemic. The slaughter of more than one million chickens seems to have halted the virus at least for now.

Hong Kong's surveillance system proved that early detection of infectious diseases can prevent their spread. David Heymann of WHO once asked a provocative question: What would have happened if we had had an excellent surveillance system in place in Africa when the AIDS outbreak first occurred? Perhaps we could also have halted that virus in its tracks. Perhaps we would have spared ourselves the second great pandemic of the 20th century. AIDS taught us that regardless of a person's sexual orientation, color, wealth, or home, if we hesitate in our fight against infectious diseases and fail to detect and track them early, they will eventually affect us all.

We cannot simply deal with each potential pandemic as it arises. We must also look over the horizon and seize new possibilities to head off infectious diseases before they can occur. We must fully harness this golden age of global telecommunications (from satellites to the Internet) to create a truly global surveillance and monitoring network and find new ways to prevent, stop, overcome, and cure infectious disease. That is one of the reasons that President Clinton proposed the 21st Century Research Fund—a historic national effort to spur the best minds of this generation to unlock scientific discoveries, unravel scientific mysteries, and uncover scientific advances. Today, the pace of medical discovery is not limited by science or imagination or intellect but by resources. Thus, the research fund will provide a US$1.1 billion budget increase for NIH next year. It is the first

down payment on an unprecedented 50% expansion of NIH over the next 5 years. This funding will enable NIH to do more to develop new ways to diagnose, treat, and prevent disease. We are also seeking a boost in CDC funding to step up our ability to identify and investigate infectious disease outbreaks, including foodborne outbreaks. CDC will play a key role in a new initiative by the U.S. Agency for International Development to develop programs in targeted countries to fight the growing threat of bacterial resistance, TB, and malaria. This new American investment in fighting infectious disease will not only pay off in America, because in this world without borders, a discovery by any one nation will benefit us all and brings us a little closer to preventing the next pandemic.

The third lesson of the great pandemic of 1918 is that we have the power to prevent the next pandemic and defeat emerging infectious diseases, but only if our nations step up the fight together. Because diseases recognize no borders, in our fight against them, neither can we. Or as Dr. Bruntland of WHO has stated, when it comes to public health, "solutions, like the problems, have to be global in scope." That is why U.S. and Japanese scientists have held three international conferences together on infectious diseases and research. It is why some members of the Asian-Pacific Economic Cooperation Area, including Thailand, Indonesia, and the Philippines, have developed a communications network to track cases of multidrug-resistant TB. And it is why CDC, FDA, and other U.S. agencies are providing assistance to the Russian Federation and the Newly Independent States, which have faced a large increase in infectious disease in the post-Soviet era.

If we truly want to end the threat of infectious diseases, we must do even more together. We must inject into global gatherings—no matter where they are, no matter what the subject—the urgency of working together to defeat infectious disease. We must never let research into infectious disease become a forgotten step-child. We must continue to invest in vaccine research and development and ensure that preventive vaccines are available, affordable, and effective everywhere. We must work with all our partners in the private sector to ensure that drugs, vaccines, and tests are available during an infectious disease emergency. We must ensure that all urban populations have access to essential facilities, especially clean water, because vaccines and medicines can do little if water is unclean. We must work together to deal with urban overcrowding, poverty, and poor sanitation, which are spreading infectious disease in many parts of the world. Finally, we must pool our greatest resources—our imagination and intellect—to fight this collective fight. For as Joshua Lederberg once noted, "Pitted against microbial genes, we have mainly our wits."

Let us pit our wits (and our will) to this battle, together, to heed the lessons of the great pandemic and so ensure that it does not happen again, that we are prepared, and that we always work together. If we do, our children—the children of the millennium—will remember the 21st century as a time of health and hope, a time of promise and possibility, a time of medical miracles and scientific marvels. I have absolutely no doubt that we can do it, that we must do it, that we will do it.

Effective Global Response to Emerging Infectious Diseases

Claire V. Broome
Centers for Disease Control and Prevention, Atlanta, GA, USA

To discuss the global efforts needed to detect and control emerging infections, I will begin with a personal experience. In 1987, a large epidemic of meningococcal meningitis occurred during the haj, the annual pilgrimage of Moslems to Mecca. The Centers for Disease Control and Prevention (CDC) sent a team of epidemiologists and laboratorians to Kennedy Airport to meet the thousands of pilgrims returning to the United States. Returning pilgrims were given chemoprophylaxis; nasopharyngeal cultures showed that 11% of the pilgrims carried the epidemic strain of group A *Neisseria meningitidis*, the causative agent. Only 25% of the returning pilgrims were intercepted and treated; thousands of others dispersed throughout the country (presumably with the same 11% carriage rate of this highly virulent strain). Were U.S. surveillance systems adequate to rapidly detect any subsequent outbreaks? We were completely dependent on local physicians to diagnose cases, on laboratories to isolate and serotype the organism, on the notification systems to inform the state and federal agencies. In this instance, the United States was fortunate and did not see any secondary outbreaks. Other countries were not so fortunate; large epidemics occurred in Chad, Kenya, and Tanzania as a result of the same virulent clone of *N. meningitidis*. The importation of this epidemic clone illustrates the central importance of local capacity to diagnose, report, and control emerging infectious diseases.

A more recent example is the 1997 influenza H5N1 outbreak in Hong Kong: the outbreak illustrates what systems are needed to detect a new organism and to respond appropriately. First, the Hong Kong public health system had to have the capacity to isolate the organism and to recognize that it was not an ordinary influenza strain. Because infections emerge at the local level, the capacity to detect new threats when they arise should be available throughout the world. Secondly, the specialized diagnostic reagents had to be available and the reference laboratories had to be able to make a definitive identification, not just of that initial strain, but of the hundreds of other strains evaluated. In this case, H5 reagents (the result of National Institutes of Health [NIH] research) had been distributed (by CDC) to reference laboratories internationally. The capacity to respond to potential outbreaks with expert epidemiologic investigation also had to be in place. The team that went to Hong Kong consisted of epidemiologists, laboratorians, a public affairs specialist, and an expert in animal influenza. The team worked closely with Hong Kong colleagues to detect new cases by implementing an enhanced surveillance system. They targeted not only hospitals but also outpatient settings. Most importantly, they designed studies to rapidly determine whether the strain could be transmitted from human to human. Would the H5N1 isolates share the pathogenic potential of human influenza, which is so readily transmissible from human to human, or was this strain relatively limited in its ability to spread? The kind of rapid but rigorous epidemiologic studies undertaken by the outbreak response team were invaluable in answering this question; fortunately, the strain had limited potential for human-to-human transmission. Still, we cannot become complacent; given the genetic recombination potential of influenza viruses, we need to maintain and enhance our surveillance systems worldwide.

Through the U.S. emerging infections initiative, the number of laboratory surveillance sites supported to look for new influenza strains has been increased. In China, sites had been expanded from 6 to 12, which improved the ability of the World Health Organization (WHO) system to monitor evidence of dissemination of this strain on the Chinese mainland. Through the CDC WHO Collaborating Center on Influenza, we made diagnostic kits based on the NIH H5 reagents available to reference

laboratories around the world so that many different areas can detect H5N1 should it emerge. At the same time, the WHO Collaborating Center was actively engaged in training activities.

The H5N1 example shows that we are somewhat better able to deal with emerging infections in 1997 and 1998 than we were in 1987. The example also underscores what is needed: dramatically strengthened local surveillance, including both laboratory and epidemiologic capacity; commitment on the part of local governments; and a strong collaborative international research and response system.

Two other areas of international capacity development contribute to effective response to emerging infections. The first is Field Epidemiology Training Programs. These programs operate on the assumption that the best way to develop epidemiologic capacity in a country is to train local professionals who are committed to continuing to work with the government in surveillance, outbreak response, epidemiology, and other aspects of public health management. Field Epidemiology Training Programs have been developed in 17 countries. These programs are now planning to create an executive secretariat to facilitate collaboration and provide regional expertise. WHO and CDC are working with these countries to ensure necessary support and coordination with international surveillance. The second area is communication systems. The Internet globally facilitates our ability to share technical and surveillance information.

We are better able in 1998 to address the threats of emerging infections, but we are by no means fully prepared. We must have the capacity to identify new or reemerging threats and to respond successfully. We need to be creative and efficient in identifying necessary resources; for example, the polio eradication program has developed a global network of laboratories and is strengthening the surveillance systems needed to identify poliomyelitis cases. Eradication activities also contribute to health capacity development, and the laboratory and surveillance capacities created for polio eradication should also be useful in detection of and response to emerging infectious diseases. Many other creative approaches and collaborations are needed for an effective global response to whatever our microbial adversaries may produce.

Anthony S. Fauci
National Institutes of Health,
Bethesda, Maryland, USA

Karen Hein
National Academy of Sciences,
Washington, D.C., USA

Claire V. Broome
Centers for Disease
Control and Prevention,
Atlanta, Georgia, USA

Addressing Emerging Infectious Disease Threats—Accomplishments and Future Plans

James M. Hughes
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

In 1962, Sir McFarland Burnet wrote, "One can think of the middle of the 20th century as the end of one of the most important social revolutions in history—the virtual elimination of the infectious disease as a significant factor in social life" (1). This statement is at the core of many years of neglect of infectious diseases—it represents complacency with a capital "C," and we are now paying the price.

Infectious diseases, the leading cause of death worldwide (2) and the third leading cause of death in the United States, have returned with a vengeance (3). Between 1980 and 1992, infectious disease deaths increased by 58% (39% after age adjustment); the major contributors were HIV infection and AIDS, respiratory disease (primarily pneumonia), and bloodstream infection.

In 1994, the Institute of Medicine published Emerging Infections: Microbial Threats to Health in the United States (4). This report broadly defined as emerging "new, reemerging, or drug-resistant infections whose incidence in humans has increased within the past two decades or whose incidence threatens to increase in the near future." This report, which detailed the factors involved in emergence, reminds us that we live in a global village.

Spurred on by the Institute of Medicine's report and by outbreaks of *Escherichia coli* O157 (January 1993), cryptosporidiosis (April 1993), and hantavirus pulmonary syndrome (May 1993), the Centers for Disease Control and Prevention and its partners produced a strategic plan for addressing emerging infectious diseases (5). The plan focused on increasing surveillance and response capacity; addressing applied research priorities; strengthening prevention and control programs; and repairing the public health infrastructure at local, state, regional, national, and global levels. Incremental implementation of this plan is ongoing. An update plan will be published in the fall of 1998.

Addressing Emerging Infections in the United States: Implementation of CDC's Plan

Emerging Infections Programs

Seven Emerging Infections Programs have been established through cooperative agreement awards (California, Connecticut, Georgia, Maryland, Minnesota, New York, and Oregon). These programs share core projects on invasive bacterial and foodborne diseases. The California program is focused on the San Francisco Bay Area. Four of the seven programs also focus on identifying the causes of unexplained deaths and severe illnesses in previously healthy persons ages 1 to 49 years.

Epidemiology and Laboratory Capacity Cooperative Agreements

Thirty awards established cooperative agreements with 28 states and two large cities (Los Angeles and New York) (Figure). Funds are used in different ways in different locales, but each recipient works toward strengthening infectious disease surveillance capacity and improving laboratory capacity and the reporting and analysis of infectious disease surveillance data. In addition, CDC has established three new

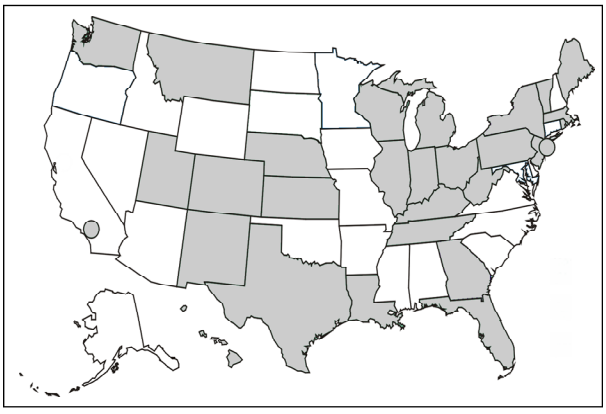


Figure. Epidemiology and laboratory capacity cooperative agreements (shown in gray).

provider-based sentinel surveillance systems with several partners. One network is based in emergency departments in academic medical centers (Emergency ID Network); a second, involving infectious disease clinicians, is in collaboration with the Infectious Diseases Society of America; and the third involves collaboration with the International Society of Travel Medicine (Geo-Sentinel), which involves travel medicine clinics in the United States and other countries.

The National Food Safety Initiative

Because of inadequate foodborne disease surveillance in the United States, the safety of the food supply could not adequately be assessed. Six million to 81 million cases have been estimated (M. Osterholm, unpub. data). Food Safety from Farm to Table (6), released in 1997, underlines the Clinton Administration's commitment to improving food safety.

The National Molecular Subtyping Network

The national molecular subtyping network (7) for foodborne disease surveillance (PulseNet) represents a model of disease surveillance that takes into account the globalization of the world's food supply. During the summer of 1997, the state public health laboratory in Colorado using molecular fingerprinting techniques (pulsed-field gel electrophoresis) recognized a cluster of 15 cases of *E. coli* O157:H7 infections from widely scattered areas in the state (8). Rapid epidemiologic investigation implicated undercooked ground beef from a single company, resulting in the recall of 25 million pounds of ground beef and the closing of the plant that produced it. This outbreak illustrates the critical role of public health laboratory capacity and rapid public health action in outbreak detection and response. Before the recent advances, this outbreak probably would not have been detected.

The Emerging Infectious Diseases Laboratory Fellowship Program

In an effort to strengthen public health laboratory capacity, CDC in collaboration with the Association of State and Territorial Public Health Laboratory Directors will be providing opportunities for training state public health laboratory workers (9). Forty-five fellows have participated in this program. An international track will be inaugurated in the summer of 1998 with the support of the CDC Foundation and Eli Lilly and Company.

The Emerging Infectious Diseases Journal

To better track trends and analyze new and reemerging infectious disease issues around the world, CDC established a quarterly, peer-reviewed international journal (www.cdc.gov/eid/). The journal, a part of the communications component of the strategy against emerging infections, has facilitated the exchange and dissemination of scientific information about these infections.

Future Plans

Antimicrobial resistance, new and reemerging infections, and a strong public interest in health will demand vigilance, renewed efforts, and strengthened partnerships in infectious diseases. An update of CDC's strategic plan along with cooperative efforts across government and private organizations all over the world will drive future efforts for the control of new and reemerging infections.

References

1. Burnet M, White DO. Natural history of infectious disease. London: Cambridge University Press; 1962.
2. World Health Organization. The World Health Report 1997: conquering suffering, enriching humanity. Report of the Director-General. Geneva, Switzerland: The Organization; 1997.
3. Pinner RW, Teutsch SM, Simonsen L, Klug LA, Graber JM, Clarke MJ, Berkelman RL. Trends in infectious diseases mortality in the United States. JAMA 1996;275:189-93.
4. Institute of Medicine. Emerging infections: microbial threats to health in the United States. Washington: National Academy Press; 1992.
5. Centers for Disease Control and Prevention. Addressing emerging infectious disease threats: a prevention strategy for the United States. Atlanta (GA): U.S. Department of Health and Human Services, Public Health Service; 1994.
6. U.S. Department of Health and Human Services, U.S. Department of Agriculture, U.S. Environmental Protection Agency. Food safety: from farm to table. A national food-safety initiative. A Report to the President, May 1997. Washington: Government Printing Office; 1997.
7. Stephenson J. New approaches for detecting and curtailing foodborne microbial infections. JAMA 1997;277:1337-40.
8. Centers for Disease Control and Prevention. *Escherichia coli* O157:H7 infections associated with eating a nationally distributed commercial brand of frozen ground beef patties and burgers—Colorado, 1997. MMWR Morb Mortal Wkly Rep 1997;46:777-8.
9. Emerging Infectious Diseases Fellowship Program. Emerg Infect Dis 1995;1:105.

Global Surveillance of Communicable Diseases

David L. Heymann and Guénaël R. Rodier
World Health Organization, Geneva, Switzerland

Globalization and Health: The Need for Global Surveillance

A recent report of A/Sydney/05/97-like (H3N2) influenza on a cruise ship from New York to Montreal demonstrates the ease with which communicable diseases can be transferred across international borders (1). In this outbreak 2.7% of passengers and 0.5% of crew had acute febrile respiratory illness during or after the cruise and introduced this antigenic variant of influenza A into both Canada and the United States.

Other viral infections and parasitic diseases are also associated with population movements. During 1996, fatal yellow fever infections were imported into the United States and Switzerland by tourists who traveled to yellow fever–endemic areas without yellow fever vaccination (2,3). During the same year approximately 10,000 cases of malaria were imported into the European Community, one fourth of them from the United Kingdom (4). Had mosquito vectors been present, these diseases could have set up endemic cycles. Misdiagnosed by an unsuspecting health worker, they could have been fatal.

Bacterial infections such as meningococcal meningitis and cholera are also spread with ease by international travelers. Among the pilgrims for the Haj in 1987, 7.7 per 100,000 returned to their countries of origin with meningitis (5). Cholera, often associated with religious pilgrimage and movement of refugees, resulted in 70,000 cases and a 22% fatality rate in 1995 among recently arrived Rwandese refugees in Goma, Democratic Republic of the Congo (formerly Zaire) (6). Rickettsial diseases such as louse-borne typhus have also recently caused illness and death among refugee and prison populations of Burundi and Rwanda (7,8).

Population movement is only part of the globalization fallout. Expansion in international travel and commerce in food and medicinal biologic products provides another potential source of communicable diseases such as hepatitis and other bloodborne infections. Social and environmental changes linked to urbanization, mobility, and deforestation have created new opportunities for infection, while rapid adaptation of microorganisms has facilitated the return of old communicable diseases and the emergence of new ones. With the rapid evolution of antimicrobial resistance, treatments for a wide range of parasitic, bacterial, and viral infections have become less effective. Today, a communicable disease in one country is a global concern.

In industrialized countries, where deaths due to communicable diseases have greatly decreased over the past century, the concern is to prevent diseases from entering and causing an outbreak or reemergence. In developing countries, the concern is to detect communicable disease outbreaks early and to stop their mortality, spread, and potential harm to trade and tourism. When cholera entered Peru in 1991, it spread through the existing sanitation and water systems, causing more than 3,000 deaths (9). Seafood export embargoes and decreased tourism cost an estimated loss of US$770 million to the Peruvian economy in 1 year. Negative economic impact can also occur in the more robust industrialized economies, the most recent example being bovine spongiform encephalopathy and the new variant of Creutzfeldt-Jakob disease in the United Kingdom.

Concerns about communicable diseases in both industrialized and developing countries can best be addressed through strong surveillance systems, renewed commitment to public health, and strong international partnerships to strengthen national and international cooperation in communicable disease prevention and control. In view of the disparity among national surveillance systems, partnerships in global surveillance are a logical starting point in this area of common commitment.

Global Surveillance: An Essential Public Health Instrument

With globalization, strengthened communicable disease surveillance at the global level has become an essential public health instrument. In addition to providing necessary information for monitoring communicable diseases and evaluating control measures, global surveillance serves as an early warning system for epidemics and provides the rationale for public health intervention. Early detection of communicable diseases and immediate public health intervention can curtail the numbers of communicable illnesses and deaths and negative effects on international travel and trade. At the close of the 20th century, which has seen the affairs of all countries become ever more intertwined, global communicable disease surveillance and response is a decisive element in controlling communicable disease.

Global surveillance provides health advice for international travelers and guidance to those involved in international transport and trade, including the food, plant, animal, and animal products industries. At the same time, it supplies crucial data to support the Biological Weapons Convention and to prevent or anticipate bioterrorism. To be effective, global surveillance must be free of, and be perceived as free of, political bias. Global surveillance requires a neutral reporting and response environment, and the World Health Organization (WHO) is strengthening the framework within which it can be fostered.

Global Networking

Formal Sources of Information

Government and university centers such as the U.S. Centers for Disease Control and Prevention, the U.K. Public Health Laboratory Service, the French Instituts Pasteur, the global network of schools of public health, and the Training in Epidemiology and Public Health Intervention Network (TEPHINET) provide confirmed reports of communicable diseases. Most of these sites are or will become part of the WHO Collaborating Centre network. This network, as well as the WHO Regional Offices, WHO country representatives, and other WHO and UNAIDS reporting sites, contributes to global surveillance along with reporting networks of other United Nations agencies such as the United Nations High Commissioner for Refugees and the United Nations Children's Fund. International military networks such as the U.S. Department of Defense Global Emerging Infections System, private clinics, individual scientists, and public health practitioners complete the network of formal information sources.

Geographic gaps and deficiencies in expertise in these networks must be rectified. These networks must develop means of including the private sector as well as other sources of valid information such as military and research laboratories. They must represent both human and animal infections and provide information on antimicrobial resistance and the environment, including water, insect vectors, and animal reservoirs.

Informal Sources of Information

Telecommunications, media and Internet access, and rapid information exchange across the globe permit public health professionals around the world to communicate more effectively. Many groups, including health professionals, nongovernmental organizations, and the general public, have access to reports on disease outbreaks, challenging national disease surveillance authorities, which were once the sole source of such information. Public Internet sites are dedicated to disease news and include sites for medicine and biology as well as major news agencies and wire services.

Such electronic discussion sites, accessible through free and unrestricted subscription, are valuable sources of information. Their scope may be worldwide (ProMed, TravelMed), regional (PACNET in the Pacific region), or national (Sentiweb in France). They exemplify unprecedented potential for increasing public awareness on public health issues.

The Global Public Health Information Network is a second generation electronic surveillance system developed and maintained by Health Canada. Its powerful search engines actively crawl the World-Wide Web looking for reports of communicable diseases and communicable disease syndromes in electronic discussion groups, news wires, and elsewhere. Searches are in English and French and will eventually expand to all official languages of the WHO, to which it has created close links for verification.

Other network sources for communicable disease reporting include nongovernmental organizations such as the Red Cross and Crescent

societies, Médecins sans Frontières, and Medical Emergency Relief International (Merlin), and Christian religious organizations such as the Catholic and Protestant mission networks.

Legally Mandated Sources of Information

The International Health Regulations (IHR) are a legal instrument that requires WHO member states to report diseases of international importance: currently plague, cholera, and yellow fever. Countries have not uniformly complied, often fearing unwarranted reactions that affect travel and trade. In addition, the official international reporting mechanism has not evolved with the new communications environment and does not include many communicable diseases of importance to international public health. A revision of IHR is therefore being directed toward a stronger role in global communicable disease surveillance and control. Currently being evaluated in a pilot study in 21 countries, the revised IHR emphasizes immediate notification of all disease outbreaks of urgent international importance. Electronic reporting of specific clinical syndromes of importance to public health will help countries report immediately, facilitating rapid alert and appropriate international response while awaiting laboratory verification. Once the diagnosis is confirmed, it will also be fed into the system, permitting any necessary adjustments to the international response. When the revision is complete, IHR will constitute an important public health tool as a source of information linked to an appropriate international response.

Pulling the Networks Together: Exchange and Verification of Global Surveillance Information

A neutral environment, internationally accepted surveillance standards and norms, and wider use of modern communication tools is required to bring all these networks into a global surveillance system—a true "network of networks." The network has been developed together with the 191 WHO member states and other partners, including the European Union-U.S. Task Force on Emerging Communicable Diseases and the U.S.-Japan Common Agenda and has been cited as an area of collaboration by the G-7/G-8 member countries at both the Lyon (1996) and the Denver (1997) Summit Meetings. Requirements for monitoring the intentional use of pathogenic microbes have also been addressed in the network, specifically in the revision of the IHR, in collaboration with the ad hoc Group of States Parties to the Biological Weapons Convention.

Nonverified information about communicable diseases coming from within the networks, including that from IHR, requires rapid verification from multiple sources other than the originator. Such "disease intelligence" requires information management skills, knowledge of field conditions, and commonly used, standardized medical language compatible with modern communication technology. WHO has therefore created an electronic verification system based on its internationally accepted norms and standards. This user-friendly system consists of an electronic repository for ready information access, regular electronic communication with network members, and a tracking and follow-up mechanism to verify each piece of information.

The power of the verification system is its network of contributors, which includes official government channels and all participating networks. Electronic mail provides immediate follow-up with easy-to-archive responses at low cost. Communications keep the focus on diseases with international implications to avoid information overload. The criteria used to determine international implication include suddenness of onset, illness and death, potential for international spread, and likely effects on international travel and trade. Timely sharing of relevant information strengthens networking and contributes to common awareness of current events, thus increasing international preparedness.

Epidemic Preparedness and Response

Once a communicable disease outbreak has been confirmed, pertinent information is placed on the World Wide Web, available to the general public. At the same time, an international response including technical and humanitarian partners is mounted if required. A WHO team arrives on site within 24 hours of outbreak confirmation to make an initial assessment and begin immediate control measures and prepare the ground for the larger international response if needed. By linking the international response to systematic global surveillance, a worldwide "network of networks" is available from which to

solicit support, thus ensuring that no one country, technical, or humanitarian partner must bear the entire burden.

How It Works in Practice: Global Influenza Surveillance

Influenza surveillance, one of the most developed global surveillance and monitoring systems of WHO, started in 1948 and developed over the years into a highly successful global partnership. The network now involves 110 collaborating laboratories in 82 countries, constantly monitoring locally isolated influenza viruses and providing information on true emergence and spread of different strains.

National case detection systems and laboratories have been strengthened using internationally accepted norms; virus isolates from national laboratories are analyzed in more detail in one of the four WHO Collaborating Centers for Influenza. The data are then used by experts associated with the surveillance system to make recommendations on the three virus strains to be included in the next season's influenza vaccine. Thus, information generated from global surveillance results in an important and unified public health response each year. The annual design of the vaccine also represents outstandingly successful collaboration between the public and private sectors.

In parallel to the surveillance program, national and global plans are being developed to systematically address the next influenza pandemic. Both the surveillance system and the elements of the global pandemic plan were tested during the outbreak of the avian influenza A(H5N1) virus in humans in Hong Kong in late 1997. The rapid identification of the virus strain in one of the collaborating laboratories in the Netherlands, mobilization and coordination of an investigating team from WHO Collaborating Centers in the United States, extensive epidemiologic and laboratory studies, prompt dissemination of public information, development of diagnostic test kits for international distribution, and identification of a virus line suitable for vaccine development, all contributed to a timely, ordered, and effective response to the outbreak.

WHO will celebrate the 50th anniversary of global influenza surveillance with a meeting bringing together participants from the national influenza laboratories and WHO Collaborating Centers and other experts. Participants will look back over past successes and lessons learned and ahead to needs for improved surveillance and control of influenza in the 21st century, including research priorities. The success of the global influenza program can serve as a model for the continued development and strengthening of international collaboration in the surveillance and control of other communicable diseases.

References

1. Centers for Disease Control and Prevention. Update: influenza activity—United States, 1997-98 season. MMWR Morb Mortal Wkly Rep 1997;46:1094-8.
2. World Health Organization. Yellow fever in a traveller. Wkly Epidemiol Rec 1996;30:342-3.
3. Office Fédéral de la Santé Publique. Information EPI. Berne, Switzerland; 1996; Bulletin 28:5.
4. Commission of the European Communities. Imported malaria. European Communicable Disease Bulletin 1998;3(4):35-42.
5. Moore PS, Reeves MW, Schwartz B, Gellin EG, Broome CV. Intercontinental spread of an epidemic group of *Neisseria meningitidis* strain. Lancet 1989;8657:260-3.
6. Goma epidemiology group. Public health impact of Rwandan refugee crisis: what happened in Goma, Zaire. Lancet 1995:345:339-44.
7. Bise G, Cominx R. Epidemic typhus in a prison in Burundi. Trans Royal Soc Trop Med Hyg 1997;91:133-4.
8. A large outbreak of epidemic louse-borne typhus in Burundi. Wkly Epidemiol Rec 1997;21:152-3.
9. World Health Organization. Cholera in Peru. Update. Wkly Epidemiol Rec 1991;20-141-6.

Emerging Infections: An Evolutionary Perspective

Joshua Lederberg
The Rockefeller University, New York, New York, USA

Our relationship to infectious pathogens is part of an evolutionary drama (1). Here we are; here are the bugs. They are looking for food; we are their meat. How do we compete? They reproduce so quickly, and there are so many of them. They tolerate vast fluctuations of population size as part of their natural history; a fluctuation of 1% in our population size is a major catastrophe. Microbes have enormous potential mechanisms of genetic diversity. We are different from them in every respect. Their numbers, rapid fluctuations, and amenability to genetic change give them tools for adaptation that far outpace what we can generate on any short-term basis.

So why are we still here? With very rare exceptions, our microbial adversaries have a shared interest in our survival. With very few exceptions (none among the viruses, a few among the bacteria, perhaps the clostridial spore-forming toxin producers), almost any pathogen reaches a dead end when its host is dead. Truly severe host-pathogen interactions historically have resulted in elimination of both species. We are the contingent survivors of such encounters because of this shared interest.

Microbial Resources

Intraclonal Processes

DNA Replication

Microbial intraclonal methods of variation are legion. DNA replication is error prone, and often the constraints of precise replication are turned off in the presence of DNA damage or other injury. Microbes often live in a sea of mutagens, chemical and physical. If we go out in the sun, our skin is damaged; in microbes, UV irradiation goes unimpeded to the very core of their DNA. Those that are not killed are rapidly mutated.

RNA Replication

RNA replication is particularly error prone. There are no editing mechanisms for examining the fidelity of replication; therefore, the concept of the quasispecies swarm was recently generated. For many RNA viruses, retroviruses in particular, the rates of mutation are so high that to a close approximation, every particle is genetically different (in at least one nucleotide) from every other particle. They are rapidly evolving as swarms of genotypes, no single genotype being totally representative. Natural selection plays a substantial role. The role of cooperativity in infection of these viruses, particularly among retroviruses and HIV, has not been adequately investigated. Rous sarcoma virus is a case in point. It may be difficult for a single particle, many generations removed from the original competent infector, to consummate an infection by itself, but it can be complemented by other helper viruses present in the same cell.

Haploid Organisms

Most of the organisms we are dealing with are haploid, so they have no delay in expressing new genetic factors. The prompt expression may potentially augment cumulative genetic alterations, but in the short run, a resistance mutation will manifest itself almost immediately and will be subject to natural selection very promptly. Multicopy plasmids, which would behave differently, are exceptions.

Phase Variation

Phase variation occurs in almost every pathogenic bacterium, in malaria parasites, in trypanosomes, and in Borrelia. Changes that appear to be mutational, on closer examination turn out to be microbial access to an archive of genetic information, much of which has been silenced and then reappears as an adaptive change. The flagellar antigens of salmonella provide the historic example; they can exist in either so-called specific phase or group phase, going back to H1 or H2 loci. We now know that they are the result of silencing one of these loci by

the position of a piece of DNA that can be inverted to move the promoter from one locus to another and give a very sudden transformation of the serotype from type 1 to type 2. This is a completely reversible phenomenon; the same event can reinvert that DNA. Many species of site-specific recombinases are capable of scrambling and rescrambling the bacterial genome in order to silence and unsilence genes that may be then carried in an archival state. I pondered why bugs use this mechanism for keeping genes in a cryptic state when gene expression can be (and often is) regulated in other ways. The simplest speculation is that phase variation very often entails controlled antigenic factors. A bug does not want to telegraph to its host in advance that it is carrying even a tiny relic of an alternative epitope because that will provoke immunity on the part of the host even before it has undergone that phase variation.

Genetic factors also control the rates of mutability; whether these factors do or do not directly influence adaptability to virulence is controversial. Preliminary reports suggested that virulent bacteria had a higher incidence of mutators. We now realize that mutators are quite prevalent, and therefore bacteria are constantly facing environmental challenges.

Interclonal Processes

Recombination mechanisms are quite promiscuous. Conjugation, which can occur between bacteria of widely varying kinds, is most often recognized by plasmid transfer and every now and then by mobilization of chromosomes. Conjugation can even occur across kingdoms, between a bacterium and a yeast, or between a bacterium and a plant. In the case of the rhizobium-like parasite, the crown gall bacterium, genetic material is transferred from the bacterium into the chromosomes of the host plant. Similar phenomena probably occur in eukaryotic infections. Some genes in viruses and bacteria almost certainly were of eukaryotic origin. Some bacteria can deliver DNA intercellularly to their host animals.

Plasmid interchange (movement of tiny bits of DNA from one species to another) is not just a laboratory curiosity; it is the mechanism for rapid spread of antibiotic resistance from widely different species, one to another. It adds even greater cogency to our concerns about the less than optimally advantageous use of antibiotics (e.g., in animal husbandry). The mechanisms exist to make it easy not only for single antibiotic resistance but whole blocks of resistance to be moved from one bacterium to another.

Host-Parasite Coevolution

Microbes' shared interest in our survival will dominate the overall picture of their evolution. Can this help us predict the outcome of the balance between the host and the pathogen? The possible outcomes are so divergent that it is very difficult to predict in detail what is going to happen in any particular confrontation.

The long-term trend is coadaptation, in which the host acquires factors for resistance and the parasite acquires factors for mitigation and longer survival of (and thereby in) the host. These factors may be genetic mutations, which will certainly be selected.

Other factors include human cultural changes, such as hygienic procedures. The human species outdoes all other species in adopting behavior that is self-destructive rather than self-protective. I am not convinced that every nuance of human behavior has been specifically evolved. Most of our behavior, even the maladaptive self-destructive kind, is learned: the pity and the hope of our species.

Pathogens find it to their advantage to mitigate their virulence, provided they can do so without compromising their livelihood. That is the tightrope they walk. Rhinovirus, the agent of the common cold, is an extremely successful pathogen. We do little to get rid of it. We go to work and school with our runny noses. The virus has a number of adaptations (including the very moderation of its disease process) that tend to facilitate its spread. I worry that a rhinovirus may some day mutate into a somewhat more virulent form, given that it is capable of very rapid spread.

Evolutionary Strategies

The parasite's dilemma is that if it proliferates rapidly, it may kill the host; that would be a winning strategy if transmission were easy, vectors readily available, the host's behavior obliging, and mosquitoes abundant for high-density spread. Such circumstances are present in northwest Thailand where *Plasmodium falciparum* would be unlikely to survive for very long (because of its profound effects on its host) if the density of spread to new hosts were

not favorable. In modern hospitals, the mosquitoes are health-care attendants who inadvertently facilitate the transfer of infection from one patient to another.

Toxins

It is a wonder that the inexhaustible reservoir of potent toxins has not spread much further. Botulinum toxin, one of the deadliest compounds, is produced in abundance by *Clostridium botulinum*, whose spread to other organisms and potential for becoming a major public health threat can easily be imagined. Why is this toxin so confined? The underlying biologic mechanisms are not confining it; rather, its lethality keeps it under control. The microbe kills its host rapidly, and if it cannot continue to multiply even in the dead host, it reaches a dead end.

In specific physiologic circumstances, these rules of natural selection might not apply. *Escherichia coli* O157 is a case in point. O157 has little to do with *E. coli*; it is a shigella with a little cloak of *E. coli* antigens. O157 should not be used as the sole diagnostic criterion for the spread of shigelloid disease. The toxin genes can inhabit other vectors. The ecologic implications of its human and bovine virulence are not clear. Perhaps polymorphism (changes in bacterial genotype) alters its virulence in human and bovine species. The human loop is quite incidental to its overall survival, as far as we know. The attack rate in humans is only 1%. How has *E. coli* O157 evolved? We understand that as poorly as we understand the sporadic emergence of Legionella from the soil into our air-conditioner ducts.

Proliferation Rate

If the parasite adopts another strategy and proliferates slowly, we have an evolutionary mechanism in which our own immune system is looking for deviants; this mechanism will be presenting new epitope receptors waiting to be stimulated. Most acute infections produce a full immune response at a humoral and a cellular level within a week or 10 days. So the microbe that proliferates slowly is laying the groundwork for its own vulnerability unless it adopts some further tactics (e.g., phase variation, stealth tactics, antigenic mimicry, exploiting the autotolerance that the host needs to survive its own immune system). Parasites also compete with commensals, with probiotic organisms. This is where HIV runs into severe trouble. Left to its own devices, HIV would not kill its host; but by knocking down the host's immune system, the virus opens the door for other organisms, including commensals, opportunists that can thrive only when the immune defenses are attenuated.

Symptoms

Vectors are rarely symptomatic, almost never severely symptomatic. The plasmodium would not benefit from killing the mosquitoes that transmit it. If a rabid dog can be considered a vector, its behavioral anomaly illustrates another adaptation that serves the purposes of the parasite.

This line of thinking, what some people have called evolutionary medicine—call it common sense—leads us to look at symptoms. To what extent should we be treating them? Some we treat because they are life-threatening. But is fever, for example, a host defense? Is it a mode of bacterial attack? Is the bacterium or virus producing pyrogens because a higher temperature will promote its own replication? Are pyrogens just side effects of other evolutionary adaptations that have not come to equilibrium? It is hard to avoid models that assume equilibrium; few complex physiologic systems are so obliging. We should question symptoms from an evolutionary perspective. How did they come to be there? This approach may open the door to new avenues of thought in examining the disease process. Cough, diarrhea, or hemorrhage may serve the purposes of the parasite; even so, we may still have to treat hemorrhage, but how far should we go in treating cough? On the one hand, if not too severe, cough may eliminate some of the infectious load from the body; on the other hand, cough generates an aerosol that further disseminates the organism. Cough may have to be treated as a public health measure as much as a therapeutic measure. Diarrhea is another example; it may be a way of eliminating the parasite or a special adaptation enhancing dissemination.

Other symptoms (malaise, headache, pain, itching) probably have different answers. Pain is a puzzling symptom, which plays an indispensable role by drawing attention to a disease. Once the disease is acknowledged, there is no reason in the world not to treat pain. Yet I know of no infection (other than chronic leprosy) that induces anesthesia. It would seem to me that a microbe bent on thriving would impart a sense of

euphoria (rather than pain) to its host; we would welcome it and infect ourselves with it. Analgesia may be the eventual moral hazard of biotechnology, the internalized moonshine still or poppy patch.

The ultimate symptom, death of the host, is almost never to the advantage of the parasite. Death signals a breakdown in the equilibrium (the contract between parasite and host) that could have had a better outcome had both sides been more witting.

Zoonotic Interactions

Many lessons of evolutionary relationships come from zoonotic interactions. Infections that break out of their host of origin often have a very severe impact on their new host. Hantavirus is an outstanding recent example. The pathologic processes in the rodent carriers hardly compare with those in humans. Most zoonotic transfers simply do not work. They are host specific; many are neutral. Every now and then, a zoonotic transfer has enormously larger pathologic implications for the host; these are the transfers we focus on. We presume that the filoviruses and perhaps HIV are in that category. Many, not all, simian immunodeficiency viruses are perceptibly less virulent in their natural host than HIV is in humans, perhaps another example of equilibrium breakdown.

How could the zoonoses be pathogenic when they require so many subtle adaptations to come into a host and really cause disease? Dozens, if not hundreds, of bacterial genes would have to work in concert to be pathogens. Microbes make proteins and carbohydrates, familiar to our systems of immunity. Therefore, if the parasite does not know how to live in the earthly host and the host cannot cope with totally alien parasites, we end up with a wash.

Consider tsutsugamushi fever, scrub typhus. Bangkok is reporting intermediate levels of drug resistance in Orientalia in tsutsugamushi in central and eastern Thailand. The life cycle is one of essentially a hereditary symbiont; the tick is transmitted transovarially and can be communicated from tick to microbe or humans, where it rapidly proliferates. Reinfection back to the tick is not of consequence, which must be a fairly recent spillover of pathogenicity for which there is not ongoing selection. Nothing in the life history of Orientalia would sustain its pathogenicity to maintain its high infectivity.

Years ago planetary quarantine became a policy consideration, beginning with Sputnik in the late 1950s and the early planning of our space program. Would it be permissible to move contaminated spacecraft from one planet to another? Certainly proliferating organisms on Earth could be easily carried to Mars. What would happen if we brought back Mars samples? These considerations resulted in an international convention for the conservation of the microbial virginity of celestial bodies. Sterilization protocols were applied to the Viking Mars spacecraft and by the Russians in the 1970s.

Maternal Immunity

One mechanism of accommodation is not genetic but physiologic: maternal immunity. The recent outbreak of canine distemper in the lions of the Serengeti (1) demonstrates a quasihereditary cycle that does not involve the genes at all but rather is the propagation of maternal immunity, partial immunity on the part of the offspring, easier adaptation to infection by the host.

Mitochondria—the Ultimate Pathogens

What are the ultimate pathogens, the ultimate symbionts? The mitochondria. A bacterial invader probably 2.5 billion years ago got into the first eukaryotic cells and conferred oxidative machinery. Who is serving whom? We generally think mitochondria are to our advantage, but think how hard we work to shovel the coal into the furnace that the mitochondria have provided in every cell of our body. Symbiosis is a fact of life, not always friendly or mutually accommodating. In bacteria, plasmids confer great advantages for some functions, but many plasmids also convey a "leave me and you die" message. The plasmid encodes simultaneously for a toxin and an antitoxin but makes sure that the toxin has a longer lifespan. So a bacterium careless enough to drop its plasmid will suffer. The plasmid has the long-term advantage of ensuring that only cells able to continue to proliferate will continue to have the plasmid. So knowing who is serving whom in these kinds of relationships is very complicated.

Patterns of Evolution

Thanks to the wonders of genomics and DNA analysis, we have a good overall model of the tree of life and the overall patterns of evolution. By the criterion of 16S RNA, extraordinary evolutionary changes have occurred within the

multicellular branch, but these changes are not at the level of fundamental housekeeping machinery; they have to do with growing brains, eyes, branches, and flowers, incidental items not at the level of cellular physiology.

Viruses

Where do viruses come from? Certainly in the world of eukaryotic viruses, no one can say with confidence what the evolutionary provenance is. We believe that viruses originated from some kind of cellular organelle, perhaps ultimately from the nuclear DNA, perhaps from the other organelles. Many of them would have to have undergone enormous changes, and we cannot say which came from where in any tangible example. This complexity can be illustrated (in the prokaryotic systems) by the ease with which viral genomes can be integrated into bacterial chromosomes. These are all double-stranded DNA bacterial viruses, so they have the same fundamental structure as bacterial chromosomes. They go in and out with ease and can be integrated and mobilized, sometimes as viruses, sometimes as bacterial genes. It is impossible to say which came first. If one could point to an evolutionary progression of clusters of genes in a bacterium on the way to generation of a new virus, it would be of some help, but how would one know it was not the relic of a very old one coming back again? Our most fundamental knowledge is very primitive.

Prions

Prions offer a new paradigm, much of which we do not understand. Stan Prusiner has argued that prions are pure proteins. Trying to understand how a pure protein can propagate confounds our conceptions of the transmission of biological information. So let us say that prion protein (e.g., scrapie prion protein) is a conformational modification of a normal protein, prp-c, coded for by an endogenous gene, a part of the normal genome, not an essential gene. Infected mice show some functional disorders but can survive. One might argue that we do worse with this gene than without it as long as we are susceptible to this modification.

Not much new sequence information is imparted to the normal prion to convert it to the infective agent. The change may be merely in the prion's conformation. We must consider other mechanisms that might cause that same conversion.

The rare nonfamilia incidence of sporadic Creutzfeldt-Jakob disease (CJD) poses a possible example, although it is difficult to exclude some contact with prions in individual cases. We might watch for CJD-like disease as an incident to other kinds of toxic insults. One implication of the protein-prion model, not discussed hitherto, is that conformer alterations may ensure from chemical or physical trauma to preexisting prp-c; heat, toxins, side effects of other infections are candidates (2). Let us carefully label this as wild speculation, pending badly needed assays for this conformer-altering capacity. Other protein-aggregate or amyloid-based diseases (like Alzheimer's) likely have a nucleating episode in their pathogenesis, even if there is no means of contagion from one person to another. At least in the pancreas, amyloid aggregation is a side effect of protein injury by glycation (3).

Emerging Pathogens

What are we going to do about new, mutant, and recombinant pathogen strains? What can we anticipate about new major outbreaks? How should we be defending ourselves? The good news of course is the wonderful technology in the offing, one marvelous innovation after another in every field of prophylaxis, vaccines, understanding of pathogenic phenomena. The genomics work on bacteria is paying off and may even justify the overall project of human genomics all by itself with its insights into microbial evolution and potential targets for new discoveries in disease management.

At a very high strategic level, we have the basic knowledge to control foodborne epidemics, waterborne epidemics, and fecal-borne diseases. At a technologic level, even sexually transmitted diseases can be controlled. One neglected medium is air. Can we do as well in preventing airborne transmission? Effective control may come down to something as elementary as a face mask like that worn by police in 1918. Control of even a vicious airborne epidemic like influenza should not be above our technical capability. Tens or even hundreds of millions of lives might be at stake over such elementary matters.

The introduction of a new hemolysin into existing anthrax strains in a demonstration of their pathogenicity in golden hamsters (4) required additional epitopes to vaccinate those hamsters against this anthrax. This first example of an artificially contrived new human pathogen

illustrates additional challenges in the fight against emerging infections.

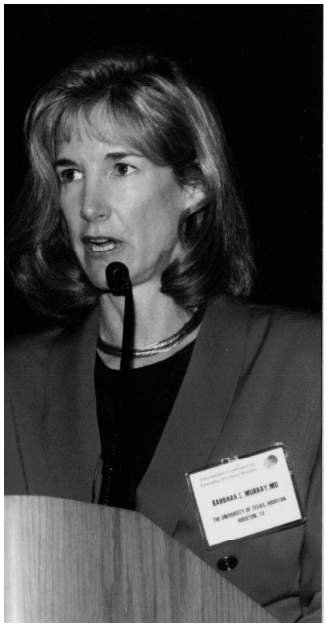
Natural infection and disease are enough of a challenge and should not be compounded by human-made agents of death. Biological warfare cannot be endured and must not be tolerated.

Dr. Lederberg, Nobel laureate in physiology or medicine, is a research geneticist, Sackler Foundation scholar, and president emeritus at the Rockefeller University. Dr. Lederberg currently conducts research on genetic exchange mechanisms in bacteria.

References

1. Lederberg J. Infectious disease as an evolutionary paradigm. Emerg Infect Dis 1997;3:417-23.
2. Causette M, Planche H, Delepine S, Monsan P, Gaunand A., Lindet B. The self catalytic enzyme inactivation induced by solvent stirring: a new example of protein conformational change induction. Protein Eng 1997;10:1235-40.
3. Kapurniotu A, Bernhagen J, Greenfield N, Al-Abed Y, Teichberg S, Frank RW, et al. Contribution of advanced glycosylation to the amyloidogenicity of islet amyloid polypeptide. Eur J Biochem 1998;251:208-16.
4. Pomerantsev AP, Staritsin NA, Mockov YV, Marinin LI. Expression of cereolysine AB genes in *Bacillus anthracis* vaccine strain ensures protection against experimental hemolytic anthrax infection. Vaccine 1997;15(17-18):1846-50.

Gail H. Cassell (L)
Lilly Research Laboratories, Indianapolis, Indiana, USA



Helene Gayle (L)
Centers for Disease Control and Prevention, Atlanta, Georgia, USA
Barbara Murray
University of Texas Medical School, Houston, Texas, USA

Emerging Infectious Diseases: A Brief Biographical Heritage

D. Peter Drotman
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

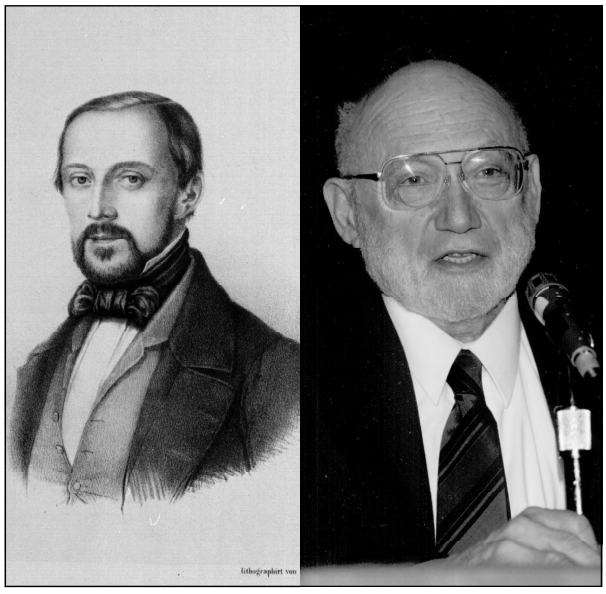
The concept that infectious (and other) diseases emerge and reemerge is not new, and neither is the search for causes of disease emergence. However, societies frequently overlook or forget that microbes evolve, adapt, and emerge in response to nonmicrobial and even nonbiologic changes in the physical and social environment. Sometimes we need to be rudely reminded of this lesson. Two scientists who have delivered such reminders, both in the form of landmark reports, are Rudolf Virchow, a 19th century German pathologist, statesman, and anthropologist, and Joshua Lederberg, the American microbiologist who coined the phrase "emerging infectious diseases" within the last decade (Photo). We owe much to the pioneering vision of these scientists.

Infectious diseases have been emerging for at least as long as humans have inhabited the earth. Every student of microbiology, medicine, and public health learns about the triangle of host, environment, and agent; what is not clear is how the three change over time, often in response to changes in another side of the triangle. Factors that influence such changes do evolve, but many are surprisingly constant. How easily and often some of these factors are overlooked is often both consequential and tragic; a historical example illustrates this point.

Rudolf Virchow, the founder of cellular pathology, wrote the first textbook in that field and established the principle that disease results from disturbed cellular function. As a young physician and anatomic pathologist in Berlin, he was assigned by the central government to investigate an epidemic in Upper Silesia, a sector of the Prussian Empire populated by a Polish-speaking minority. He completed the field portion of his investigation on March 10, 1848 (exactly 150 years before the International Conference on Emerging Infectious Diseases). The report he wrote was remarkable.

Even though Virchow was working before the germ theory of disease was accepted, at a time when disease causation was highly debated and microbes were not well described, he seems to have correctly diagnosed typhus (or possibly relapsing fever) as the cause of the Silesian epidemic (1). Even though Virchow's diagnosis cannot be confirmed, it is consistent with clinical descriptions and epidemiologic inference. He clearly demonstrated that the conditions and vectors for typhus and relapsing fever (famine and malnutrition, humid climate, poor housing, poverty) were present in Upper Silesia in 1847 to 1848. The agents that cause epidemic louse-borne typhus fever *(Rickettsia prowazekii)* and relapsing fever *(Borrelia recurrentis*) were not described until many years later.

Virchow's report was a scathing criticism of the Prussian government, which he squarely blamed for the epidemic. Virchow considered the Silesian outbreak investigation a defining



Rudolf Virchow and Joshua Lederberg.

episode in his life and career, so when the government largely ignored the report and his recommendations (Table 1), he became a passionate voice in politics, albeit in a minority role. He died in 1902, a revered scientist with a lifetime of magnificent achievements, but also with desires to have done more to improve public health and social conditions. We still have a lot to learn from Virchow's life and work.

Joshua Lederberg was awarded the Nobel Prize for medicine in 1958 for his discoveries concerning genetic recombination and the organization of the genetic material of bacteria. He is President Emeritus of The Rockefeller University in New York, a member of the Institute of Medicine, an advisor to presidents, and a 20th century Rudolf Virchow. Like Virchow, Lederberg recognized that microscopic changes make much larger differences, particularly when viewed in the context of global changes. Like Virchow, he coauthored a prescient report that associated a pressing health emergency with larger social, political, and environmental changes. The similarities between the two reports are striking (Tables 1, 2). Each regarded control of diseases as primarily social, political, and environmental. We overlook this common theme at our collective peril.

Unlike Virchow's report, the words of Joshua Lederberg are being translated into actions. Those actions can be spurred by disseminating information and building partnerships to effectively address the ongoing threat of emerging infectious diseases.

Table 1. Virchow's recommendations to the Prussian government regarding the typhus epidemic in Upper Silesia, 1848 (2)

Political reform and local self-government, including local coordination of relief efforts
"Education, with its daughters, liberty and prosperity" (3)
Economic reform
Agricultural reforms, including development of cooperatives
Building of roads
Acceptance of Polish as an official language (while most Silesians spoke Polish, nearly all the physicians and school teachers assigned by the central government spoke only German)
Separation of church and state (he criticized the Catholic hierarchy) (4)

Table 2. Factors in disease emergence—The Institute of Medicine's 1992 report on emerging infections (5)

Human demographics and behavior
Technology and industry
Economic development and land use
International travel and commerce
Microbial adaptation and change
Breakdown of public health measures

References

1. Eisenberg L. Rudolf Ludwig Karl Virchow: Where are you now that we need you? Am J Med 1984;77:524-32.
2. Silver GA. Virchow, the heroic model in medicine: Health policy by accolade. Am J Public Health 1987;77:82-8.
3. Virchow RL. Report on the Typhus Epidemic in Upper Silesia. Translated in: Rather LJ, editor. Rudolf Virchow: Collected Essays on Public Health and Epidemiology, 2 vols. Canton (MA): Science History Publications 1985:311.
4. Taylor R, Rieger A. Medicine as a social science: Rudolf Virchow on the typhus epidemic in Upper Silesia. Int J Health Services 1985;15:547-59.
5. Lederberg J, Shope RE, Oaks SC, editors. Emerging Infections: Microbial Threats to the United States. Washington: National Academy Press, 1992.

New and Reemerging Diseases: The Importance of Biomedical Research

Anthony S. Fauci
National Institutes of Health, Bethesda, Maryland, USA

A generation ago, it was suggested that the threat of infectious diseases would soon become an artifact of history. Today, as we approach the new millennium, the folly of this position is increasingly clear. My 87-year-old father recently reminded me of this. In the course of his lifetime, spent almost entirely in New York City, he has witnessed two pandemics of extraordinary impact: the global influenza pandemic of 1918–1919, which killed more than 20 million people worldwide, and the HIV/AIDS pandemic, which began to accelerate in the early 1980s and continues unabated in some parts of the world. In addition, at least 30 other new and reemerging diseases and syndromes have been recognized since the 1970s, including liver disease due to hepatitis C virus, Lyme disease, foodborne illness caused by *Escherichia coli* O157:H7 and *Cyclospora*, waterborne disease due to *Cryptosporidium*, hantavirus pulmonary syndrome, and human disease caused by the avian H5N1 influenza virus (Figure 1). Clearly, we remain vulnerable to new and reemerging diseases.

New diseases are superimposed on endemic diseases such as diarrheal diseases, malaria, tuberculosis (TB), and measles, which continue to exact a huge toll. Indeed, malaria and TB, among others, are reemerging in a drug-resistant form. Today, infectious diseases remain the leading cause of death worldwide and the third leading cause of death in the United States. Many pathogens are becoming increasingly resistant to standard antimicrobial drugs, making treatment difficult and in some cases impossible. Moreover, chronic conditions generally considered noninfectious actually have been found to have a microbial etiology.

Awareness of Emerging Infections

The challenges posed by infectious diseases are recognized by the public and the media, as well as by political leaders and policy makers at the highest levels of government. There is a growing awareness that we live a global community, that diseases do not recognize borders, and that the U.S. public health community has an important role to play in fostering global health.

The Importance of Research

The infectious diseases community faces a difficult challenge: coping with ongoing problems such as malaria and TB while preparing for the inevitable emergence of diseases that are unknown or are recognized but will reemerge in a more threatening form. Available resources must be maximized by sustaining and increasing collaboration between federal agencies, academia, industry, and nongovernmental agencies, all of which play important roles in the fight against infectious diseases.

Within the federal government, the Centers for Disease Control and Prevention's (CDC) work in detecting and tracking pathogens is critical, especially with regard to diseases that have recently emerged or have the potential for emergence. Equally important, and complementary to CDC's efforts, is basic and clinical research supported by the National Institutes of Health (NIH) and other agencies. Historically, basic research has led to important, often serendipitous, advances that have illuminated the etiology of sometimes mysterious diseases and facilitated the development of diagnostics, therapies, and vaccines (Figure 2).

At the National Institute of Allergy and Infectious Diseases (NIAID) at NIH, we have increased funding for emerging diseases from $39.3 million in fiscal year 1993 to an estimated (president's budget) $85.0 million in fiscal 1999 (Figure 3). Approximately 21% of the NIAID non-AIDS infectious diseases budget is devoted to emerging infectious diseases.

With the help of our advisory committees, we have defined five priorities in emerging and reemerging diseases research: 1) supporting the application of relevant scientific knowledge and

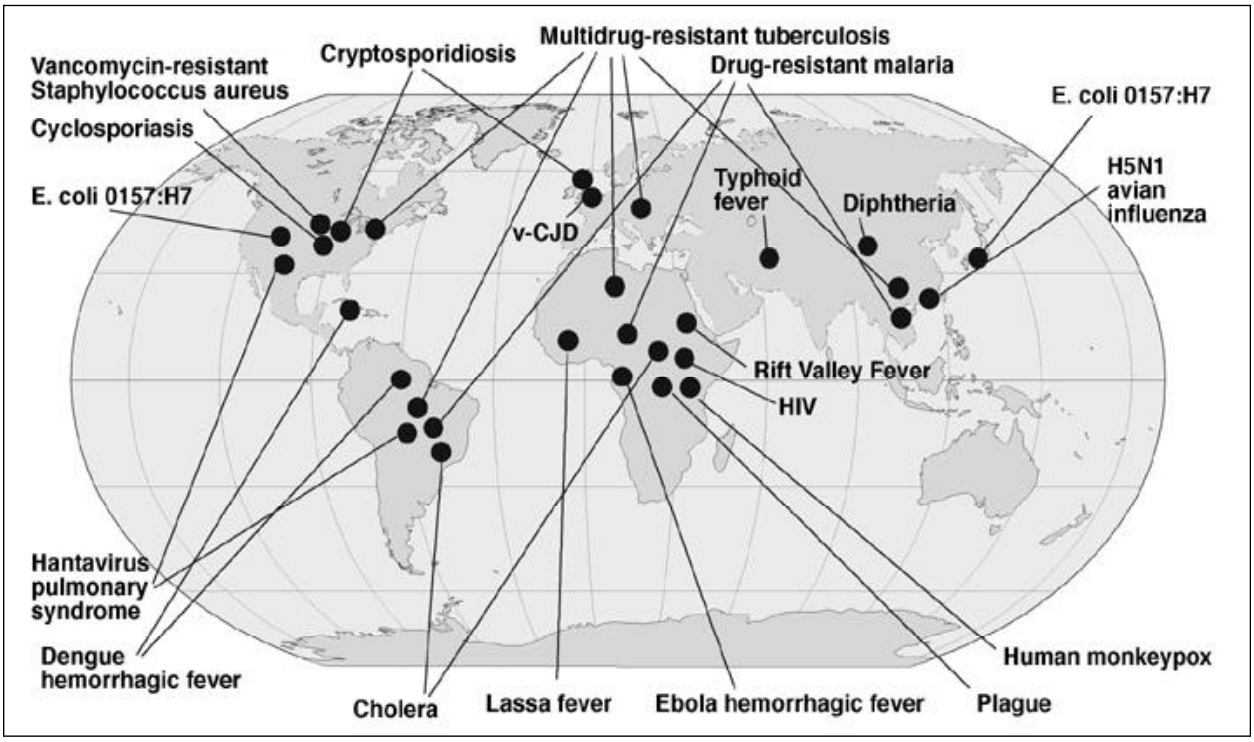


Figure 1. Examples of new and reemerging diseases.

new technologies to the detection, identification, and interdiction of emerging diseases, by expanding research on ecologic and environmental factors influencing disease emergence and transmission; 2) supporting the application of recent discoveries and new biomedical technologies to the identification, management, and control of emerging diseases, by expanding research on microbial changes and adaptations that influence disease emergence; 3) providing fundamental information for developing prevention and treatment strategies that can be employed to ameliorate disease impact, by expanding research on host susceptibility to emerging or reemerging pathogens; 4) supporting the development and validation of vaccines, therapeutics, and other control strategies for specific diseases with the potential to emerge or reemerge; and 5) strengthening the current U.S. research and training infrastructure for detecting and responding to outbreaks of infectious diseases.

Among many studies domestically and internationally, NIAID sponsors five international programs in tropical infectious diseases, most of which have components both in the United States and in the countries where the incidence of these diseases is greatest. It is essential to engage scientists in host countries and work with them collaboratively, both to tap their expertise as well as to help them build research infrastructure on their home soil.

Successful Partnerships

The public and private sectors, including government, academia, and industry, bring complementary skills and perspectives to the research endeavor. Cross-sector collaboration can yield extraordinary dividends. A cogent example is the development of protease inhibitors for the treatment of HIV disease.

After HIV was identified in 1983, researchers funded by NIH and others began to intensively study the structural and regulatory genes of HIV and the role these genes and their products play in the replication cycle of the virus. This work led to an understanding of the importance of the HIV protease enzyme and methods to express, purify, and crystallize the enzyme. Building on these findings, researchers in the private sector designed and produced specific inhibitors of HIV protease and worked closely with the Food and Drug Administration,

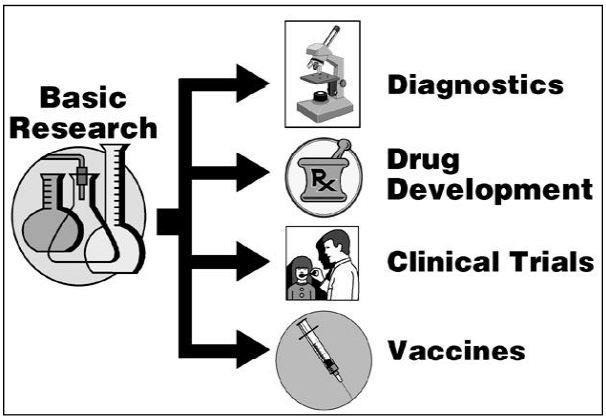


Figure 2. Emerging infectious diseases: a research approach.

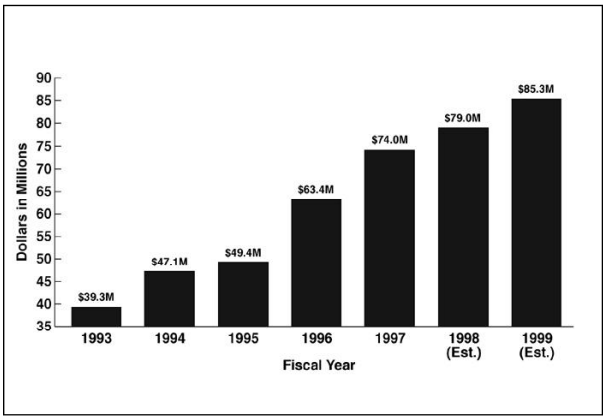


Figure 3. Emerging diseases funding (National Institute of Allergy and Infectious Diseases).

NIH, and others to assess protease inhibitors in clinical trials.

The first of four licensed protease inhibitors reached the market in December 1995. Given in combination with at least two other antiretroviral drugs, protease inhibitors dramatically reduce levels of plasma viremia in a substantial proportion of patients. Both controlled and observational studies show that these potent regimens can provide a substantial clinical benefit.

Although drug combinations that include protease inhibitors have helped many patients, it is far too soon to become complacent or declare victory. Many patients have not benefited from the new drugs or cannot tolerate their side effects, and drug resistance will inevitably become more widespread. The development of the next generation of antiretroviral agents is crucial and will require the skills of investigators in both the public and private sectors. However, the cost of antiretroviral drugs will probably keep them beyond the reach of much of the developing world; therefore, the development of an HIV vaccine is of paramount importance.

Malaria Initiatives at NIH

Until relatively recently, AIDS was virtually the only emerging disease with global impact that was widely discussed in the United States; however, other diseases such as malaria and TB have actually caused more illnesses and deaths over the past 2 decades.

Malaria kills up to three million persons each year, most of them children in sub-Saharan Africa. In the past year, NIH has worked with research organizations and donor agencies from around the world to form a coalition called the Multilateral Initiative on Malaria. This unprecedented initiative will enhance international collaborations, encourage the involvement in malaria research of scientists from malaria-endemic countries, and identify additional malaria research resources. In addition, NIH has bolstered its long-term commitment to malaria research. NIH-supported malaria projects—many in collaboration with other government and international agencies—include 1) a new repository of materials available to researchers worldwide; 2) basic, field-based, and clinical research on all phases of malaria research; and 3) projects to determine the genetic sequences of important malaria species.

Responding to Avian H5N1 Influenza

An outbreak of avian H5N1 influenza in Hong Kong recently alarmed the medical community and the world. The multinational response to this outbreak has involved the close collaboration of many organizations (Figure 4). As part of NIH's long-standing research into respiratory viruses, we had in our reagent repository the specific antisera needed to quickly develop test kits that were used effectively by CDC and others for detecting and tracking the virus. We also have supported the rapid production of a recombinant vaccine against avian influenza virus for use in laboratory and health-care personnel at risk. Without a strong research base, the rapid response to this emergency would not have been possible.

Vaccine Development

With avian flu, malaria, AIDS, and other new and reemerging diseases, an important goal of NIH is the development of vaccines. If just four recently developed vaccines (hepatitis B, rotavirus, *Haemophilus influenzae* type b, and acellular pertussis) were universally administered, more than three million deaths could be prevented each year.

Historically, scientific advances in microbiology and related disciplines have driven the development of new vaccines. For example, the identification of microbial toxins, as well as methods to inactivate them, allowed the development of some of our earliest vaccines, including those for diphtheria and tetanus. In the 1950s, new tissue culture techniques ushered in a new generation of vaccines, including measles, mumps, and rubella. In recent years we have seen rapid advances in our understanding of the immune system and host-pathogen interactions, as well as technical advances such as recombinant DNA technology, peptide synthesis, and gene sequencing. Each of these has facilitated the development of new vaccines and vaccine candidates for important pathogens.

Sequence information can be used in many ways and promises to be useful in identifying antigens to incorporate into vaccines, as well as determining the factors that influence the antigenicity or virulence of a microbe. The complete genetic sequences of more than 13 microorganisms have now been published. More than 60 other sequencing projects for medically important pathogens, such as *Plasmodium* spp., *Mycobacterium* spp., *Chlamydia trachomatis*, *Vibrio cholerae,* and *Neisseria gonorrhoeae,* are under way.

Conclusion

The importance of basic research to the control of emerging and reemerging diseases cannot be overemphasized. Emerging diseases research encompasses many disciplines, and research advances that fall under the rubric of emerging diseases will be relevant not only to specific diseases being studied but to a broad range of disciplines such as vaccinology, immunology, and drug development (Figure 5). In turn, research in these areas is critical to advances in emerging and reemerging diseases. With a sustained commitment to basic research and cross-sector collaboration, important scientific findings and technological advances can be translated into improved global health and reduced susceptibility to new microbial threats.

Acknowledgment

The author thanks Greg Folkers for helpful discussion related to the preparation of this manuscript.

References

1. Institute of Medicine, Board on International Health. America's vital interest in global health. Washington: National Academy Press; 1997.
2. Centers for Disease Control and Prevention. *Staphylococcus aureus* with reduced susceptibility to vancomycin—United States, 1997. MMWR Morb Mortal Wkly Rep 1997;46:765-6.

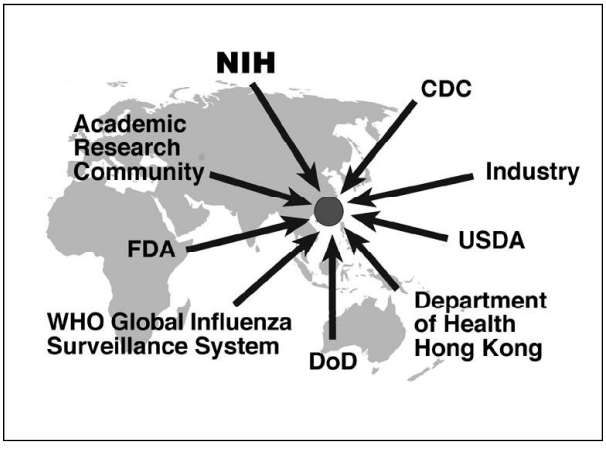


Figure 4. Response to H5N1 avian influenza outbreak in Hong Kong.

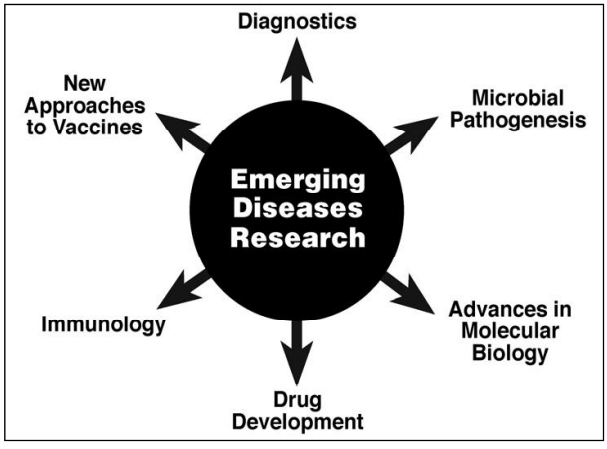


Figure 5. Benefits of emerging diseases research.

3. Centers for Disease Control and Prevention. Update: isolation of avian influenza A (H5N1) viruses from humans—Hong Kong, 1997-1998. MMWR Morb Mortal Wkly Rep 1998;26:1245-7.
4. The CVI strategic plan: managing opportunity and change: a vision of vaccination for the 21st century. Geneva: Children's Vaccine Initiative, 1997. Sponsored by UNICEF, United Nations Development Program, World Health Organization, World Bank, Rockefeller Foundation.
5. Two cheers for the multilateral malaria initiative. [editorial]. Nature 1997;388:211.
6. Fauci AS. Biomedical research in an era of unlimited aspirations and limited resources. Lancet 1996;348:1002-3.
7. The Institute for Genomic Research. TIGR Microbial Database [database online] [cited 1998 Apr 1]. Available from: URL: http://www.tigr.org.
8. World Health Organization. World Health Report 1997—conquering suffering, enriching humanity. Geneva: The Organization; 1997.

Health Policy Implications of Emerging Infections

Karen Hein
Institute of Medicine, National Academy of Sciences, Washington, D.C., USA

The solutions to emerging disease problems involve politics and policy issues, as well as solid science. The National Academy of Sciences' Institute of Medicine (IOM), whose mission is to "improve the health of people of the nation and the world," draws upon the expertise of elected members as well as others in the United States and other nations to make policy recommendations. Groups convene to debate contentious issues and publish evidence-based reports with recommendations to government, academia, industry, and the public.

Evidence-based reports are the foundation upon which policy can be built. In this last decade, IOM has produced several documents that have focused on emerging infections and provided a springboard for policy on a local, nationwide, and international scale. The U.S. Capacity to Address Tropical Infectious Disease Problems (1987) (1) concluded that U.S. capacity was barely adequate and that improvement in policies and modest additional funding could make a substantially stronger contribution to the field. Required efforts included sustained support for basic and applied research; accelerated development and testing of new preventive, therapeutic, and diagnostic technologies; sustainable career structures for tropical disease professionals; increased capacity to train U.S. tropical disease professionals and those from developing countries in research and public health service; development of disease surveillance capabilities; strengthened institutional capabilities in developing countries; and flexible, responsive administration of programs.

The Future of Public Health (1988) (2) report made three basic recommendations regarding the mission of public health and defined its core functions to be assessment, policy development, and assurance. It also included guidance for the government's role in fulfilling the public health mission and the responsibilities unique to each level of government. The report has been a useful blueprint for the past decade.

Emerging Infections: Microbial Threats to Health in the United States (1992)(3) identified significant emerging infectious diseases, determined what might be done to deal with them, and recommended how similar future threats might be confronted to lessen their impact on public health. The document focused on factors contributing to disease emergence, not the diseases themselves: human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, microbial adaptation and change, and the breakdown of public health measures.

Sexually Transmitted Diseases: The Hidden Epidemic (1997) (4) focused on the need for a new social norm of healthy sexual behavior. The small investment in prevention efforts was contrasted with the very high costs of care for treating sexually transmitted diseases (STDs) (Figure 1). The report also examined the obstacles and opportunities presented by managed care. Limitations include the low priority for STD prevention, emphasis on short-term cost savings,

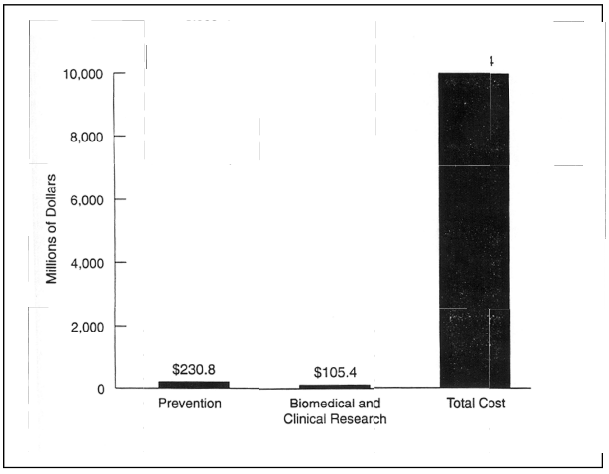


Figure 1. Estimated annual direct and indirect costs for selected sexually transmitted diseases (STDs) and their complications in 1994 versus national public investment in STD prevention and research in federal fiscal year 1995 (4).

varying technical capabilities for diagnosis and treatment, and patient concerns about confidentiality and treatment of partners not enrolled in the same health plan. Lastly, opportunities for training and continuing education in STD control and prevention are not built into most managed care settings. The report called for several steps including a national campaign to heighten awareness of the human and financial costs of STDs and to promote the use of social marketing techniques for their prevention. A recent innovative informational campaign used a niche approach and a social marketing strategy with the spot video Hittin' the Skins and the public service announcement Knockin' Boots (D. Futterman, pers. comm.), geared toward alerting 16- to 21-year-olds of the need for HIV testing.

Many related activities, in addition to the IOM reports, have underscored the danger of emerging infectious diseases and reiterated the warnings about the overall erosion of the U.S. public health system during the 1990s. The reports also provided specific, detailed recommendations for action by individual agencies. In 1994, the Centers for Disease Control and Prevention (CDC) published Addressing Emerging Infectious Disease Threats: A Prevention Strategy for the United States. In the same year, the U.S. National Science and Technology Council's Committee on International Science, Engineering, and Technology (an interagency working group) was convened to consider the global threat of emerging and reemerging infectious diseases and in 1995 published the report Infectious Disease—A Global Health Threat. In 1995, the National Security Council asked the federal government to examine its preparedness to respond to global epidemics.

In 1995, the Food Safety and Inspection Service, CDC, and the Food and Drug Administration (FDA) developed the Sentinel Site Study, which evolved into FoodNet and now includes collection of more precise information on the incidence of foodborne disease in the United States. In 1996, President Clinton's administration set out a new policy to establish a worldwide infectious disease surveillance and response system and expand certain federal agency mandates to better protect American citizens.

In the 1996 NIAID Research Agenda for Emerging Infectious Diseases, the National Institutes of Health described research and training issues relevant to the national strategy for confronting the threat of emerging and reemerging infections and related its approach to addressing these issues.

In 1996, the Department of State established an Emerging Infectious Diseases and HIV/AIDS Program to serve as a focal point for the development and implementation of U.S. foreign policy objectives to improve the health of U.S. citizens and to stem the spread of infectious diseases worldwide through various international bilateral and multilateral negotiations. This program has received $50 million in funding. Other government agencies, including FDA, U.S. Agency for International Development, Department of Defense, National Oceanic and Atmospheric Administration, National Aeronautics and Space Administration, and U.S. Department of Agriculture, have also examined the issue of U.S. vulnerability to epidemics and resurgence of infectious disease threats.

The IOM's Forum on Emerging Infections is the most recent activity within the National Academy of Sciences to keep sustained attention on these issues. The forum was established in 1996 to provide a structured opportunity for discussion and to scrutinize critical, and possibly contentious, scientific and policy issues related to research on and the prevention, detection, and management of new and reemerging infections. The forum has organized a series of workshops to be conducted over 30 months. Workshop topics include costs of infectious diseases, surveillance, antimicrobial resistance, effects of health-care restructuring on public health and basic research related to infectious diseases, capacity for emergency response to emerging and reemerging infectious diseases, education and training needs, predicting the future, and behavioral interventions.

Orphans and Incentives (5), a 1998 report, is the first publication of the forum; it focused on constraints that have left an undefined group of "urgently needed medical products in an orphaned condition which demands special attention." The authors examined these products across the product cycle and then classified them into categories for which incentives might be developed to bolster the competitiveness of such products in industrial portfolios.

The 1998 report Antimicrobial Resistance (6), the second publication of the forum, examined increases in the number of pathogens, multidrug-

resistant strains, compromised persons (including HIV-infected patients), deaths from infection with resistant organisms, speed of the global spread, and costs of health care. The report also examined decreases in the antimicrobial armamentarium, amount of research and development expended when resistance was not seen as a major threat, and funding for public health infrastructure and addressed the following topics: expansion, coordination, and improvement of the diverse elements of surveillance; need for relatively small but thoughtful investments in research, clinical management and practice, and policy; use of antibiotics in food production; ways to prolong the effectiveness of existing antibiotics; basic research and incentives for new antibiotics; and legal and regulatory mechanisms in key areas of need.

A soon-to-be-published report on a March 1998 workshop on managed care will examine the implications of managed care systems on emerging infections by reviewing basic and clinical research, clinical practice guidelines, surveillance and monitoring, prevention, education and outreach, and product development.

These reports and events have examined research on emerging infectious diseases and crafted a series of policy recommendations. They put forth a rationale for why the United States should invest in global health. The 1997 report, entitled America's Vital Interest in Global Health (7), provided a new framework for thinking about the benefits to the United States, as well as to the rest of the world, of our increased participation. The movement of two million people each day across national borders and the growth of international commerce are inevitably associated with transfers of health risks (e.g., infectious diseases, contaminated food, terrorism, and legal or banned toxic substances). U.S. commitment to global health serves to protect our people, enhance our economy, and advance our international interests. Moreover, governments are no longer the sole agents in the global health arena (Figure 2).

The United States can contribute not only with funding, but also with the scientific and technical expertise in its health sector. The United States should lead from its strengths (medical science and technology) in the areas of research and development, surveillance, education and training, global partnerships, and coordination and leadership. In this way, the United States "can do well by doing good."

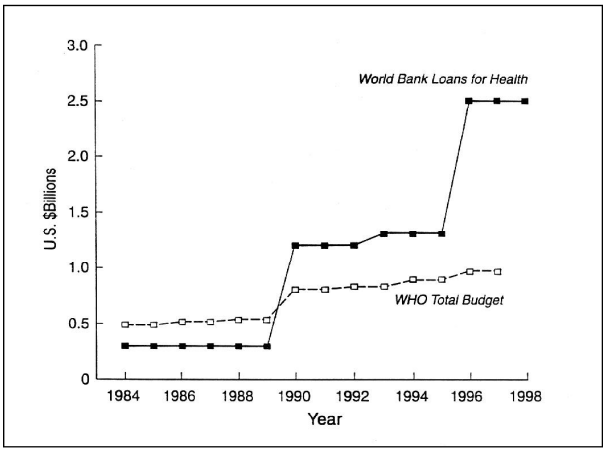


Figure 2. The growing role of the World Bank in health (7).

Acknowledgments

The author thanks Carol Bock for her assistance in preparing this manuscript and Jonathan Davis for collecting background materials.

References

1. The U.S. capacity to address tropical infectious disease problems. Washington: National Academy Press; 1987. p. 88. Sponsored by the Board on Science and Technology for International Development, Office of International Affairs, National Research Council and Institute of Medicine, National Academy of Sciences.
2. The future of public health. Washington: National Academy Press; October 1988. p. 240. Sponsored by the Institute of Medicine, Division of Health Care Services.
3. Lederberg J, Shope RE, Oaks SC Jr, editors. Emerging infections: microbial threats to health in the United States. Washington: National Academy Press; October 1992. p. 312. Sponsored by the Institute of Medicine, Division of Health Sciences Policy and Division of International Health.
4. Eng TR, Butler WT, editors. The hidden epidemic: confronting sexually transmitted diseases. Washington: National Academy Press; 1997. p. 392. Sponsored by the Institute of Medicine, Board on Health Promotion and Disease Prevention.
5. Harrison PF, Lederberg J, editors. Orphans and incentives: developing technology to address emerging infections, Workshop Report. Washington: National Academy Press; 1997. Sponsored by the Institute of Medicine.
6. Antimicrobial resistance: issues and options. Workshop Report. Washington: National Academy Press; 1998. Sponsored by the Institute of Medicine, Forum on Emerging Infections.
7. America's vital interest in global health, protecting our people, enhancing our economy, and advancing our international interests. Washington: National Academy Press; 1997. Sponsored by the Institute of Medicine, Board on International Health.

Detection and Identification of Previously Unrecognized Microbial Pathogens

David A. Relman
Stanford University, Stanford, California, USA, and Veterans Affairs Palo Alto Health Care System, Palo Alto, California, USA

Features of a number of important but poorly explained human clinical syndromes strongly indicate a microbial etiology. In these syndromes, the failure of cultivation-dependent microbial detection methods reveals our ignorance of microbial growth requirements. Sequence-based molecular methods, however, offer alternative approaches for microbial identification directly from host specimens found in the setting of unexplained acute illnesses, chronic inflammatory disease, and from anatomic sites that contain commensal microflora. The rapid expansion of genome sequence databases and advances in biotechnology present opportunities and challenges: identification of consensus sequences from which reliable, specific phylogenetic information can be inferred for all taxonomic groups of pathogens, broad-range pathogen identification on the basis of virulence-associated gene families, and use of host gene expression response profiles as specific signatures of microbial infection.

For 100 years, efforts to detect and identify microorganisms have generally begun with the inoculation and incubation of growth media in the laboratory. Colony purification and preparation of limiting dilutions of liquid culture media have provided at least two benefits: amplification of microbial material and purification of single organisms along with their direct descendants. Because some microorganisms are not particular in their growth requirements, these efforts have yielded an array of diverse microbial cultivation types. Serial propagation of microorganisms in the presence of varied energy sources, analysis of their macromolecular composition and their metabolic by-products, and use of specific immunologic reagents have created a variety of systems for microbial classification and identification. Some isolates purified from diseased tissues of animal and human hosts produced identical disease when injected into other, previously healthy hosts. By the latter half of the 20th century, these findings had led to optimism about our ability to detect and recognize microscopic life forms, particularly forms that can cause disease.

Address for correspondence: David A. Relman, VA Palo Alto Health Care System 154T, 3801 Miranda Avenue, Palo Alto, CA 94304, USA; fax: 650-852-3291; e-mail: relman@cmgm.stanford.edu.

Microbial cultivation methods opened up an unsuspected world of microscopic life and presumed causative agents of human illness. However, much of this world remained uncharacterized. In the external environment, certain biochemical activities could best be explained by the presence of microorganisms, although they could not be cultivated in vitro. Sergei Winogradsky, a pioneering soil microbiologist of the early 20th century, spoke about the "less docile" organisms that were not satisfied with laboratory cultivation conditions. In the internal, privileged niches of animals, microorganisms were sometimes visualized in diseased tissues, and persons with typical clinical signs of infection would respond to antibiotics, despite unsuccessful efforts at microbial propagation. That conserved genomic sequences might be used to infer evolutionary ancestry and be amplified directly from natural sites of infection provided the framework for cultivation-independent approaches for microbial detection and identification. In a few years, it became clear that most extant microorganisms in the external environment had been completely overlooked because of their resistance to cultivation on artificial media.

Sequence-Based Methods for Pathogen Discovery

What features of a genetic sequence make it useful for identifying uncharacterized microorganisms? (1). First, the sequence should be conserved among a relatively large number of known organisms. Second, its rate of change should be constant over long periods and among diverse organisms and should allow inferences of evolutionary distance among a wide range of life forms; the sequence should not be subject to widely discrepant degrees of evolutionary pressure. Third, the sequence should not have been shared among different organisms by horizontal transmission. Finally, the sequence should be amenable to broad-range amplification or detection.

The sequence of the small subunit ribosomal RNA or DNA (ssu rDNA), among other genomic sequences, meets these criteria. Ssu rRNA sequences were the first to reveal a tripartite tree of cellular life, one that includes the bacteria, archaea, and eukarya (2); few genetic sequences reliably reflect the ancestry of such a wide array of cellular life as the ssu rRNA. Since this realization nearly two decades ago, a large ssu rRNA sequence database has accumulated (3), further enhancing the usefulness of this particular locus. (More than 7,000 bacterial 16S rDNA sequences are now available). Highly conserved regions of the ssu rDNA and ssu rRNA provide priming sites for broad-range polymerase chain reaction (PCR) (or RT-PCR) and obviate the need for specific information about a targeted microorganism before this procedure. Thus, a previously uncharacterized bacterium, for example, can be identified from an infected site or tissue by broad range bacterial 16S rDNA amplification, sequencing, and phylogenetic analysis (4). This approach was applied to the uncultivated bacteria of bacillary angiomatosis in 1990 and of Whipple's disease soon thereafter (5,6). Because of the usual presence of host DNA, eukaryotic pathogens (parasites, fungi) must be approached either with domainwide primers and partially purified pathogens or with range (e.g., kingdom)-restricted eukaryotic primers (7).

Broad-range PCR as a method for "pathogen discovery" is not limited to ssu rDNA as a target or to cellular life. Any phylogenetically reliable family of orthologous gene sequences found among a coherent group of microorganisms can be targeted, as long as conserved priming sites can be defined at sites that flank the informative region of sequence. For example, a newly discovered hantavirus was identified as a cause of acute pulmonary disease by using broad-range primers directed at a conserved region of a coat protein-encoding genomic segment (8). A collection of family-restricted broad-range primers is necessary to identify unrecognized viral pathogens; this collection is not yet comprehensive.

Two other independent sequence-based methods are available for pathogen discovery. One relies upon subtractive hybridization to isolate fragments of nucleic acid that are unique (different) to one member of an otherwise matched pair of specimens; these "difference" molecules are then selectively amplified by using linker sequences that had been ligated to all fragments derived from the infected specimen. Multiple rounds of subtraction and amplification are required to find rare fragments within a complex common background. Although better suited than differential display or suppressive subtractive hybridization for low copy targets and highly complex backgrounds (such as human genomic DNA), this method, known as representational difference analysis (RDA) (9), is labor-intensive and cumbersome. Nonetheless, it identified for the first time the presumed causative agent of Kaposi sarcoma, human herpesvirus 8 (9). RDA enables detection of any class of microorganism; however, it may be most useful for DNA viruses. The third sequence-based pathogen discovery method takes advantage of host immunologic recognition of an exogenous microbial agent. Immune sera are used to screen an expression genomic library created from an infected specimen. While laborious, this method has also uncovered an important previously unrecognized pathogen for humans: hepatitis C virus (10).

Sequence-based approaches take advantage of the speed and sensitivity of rapidly evolving molecular biologic methods and the specificity of genotypic characterization. Consensus PCR has the additional advantage of being able to target families of sequences preselected for their reliability in the inference of evolutionary relationships. However, all approaches have limitations. One of the most important for sequence-based methods involves the processing of clinical specimens. Difficulties include heterogeneity of sample, wide variation in the numbers of microbial targets in any given sample, resistance of some microorganisms to digestion and subsequent release of nucleic acid, and presence of PCR inhibitors in varying amounts

and types—not to mention ubiquitous microbial nucleic acid contamination of PCR reagents, specimen collection materials, and externally exposed surfaces of the host. These problems reflect the intrinsic biologic variability of a highly complex, partially characterized host. Standardized procedures that produce consistent results with large numbers of clinical specimens are rare. Despite increasing attention to these issues, particularly in the private and commercial sectors, resource commitment and technology advances have lagged behind the development of methods for sequence acquisition and analysis. In fact, it is far easier to generate a putative microbial sequence from a clinical specimen than it is to understand its clinical relevance.

As the process of pathogen discovery and detection turns to the fundamental signature macromolecules of all life forms and away from reliance on cultivation, we increasingly rely on our ability to understand a putative microorganism from its genetic sequence. Many families of virulence-associated genes and gene products are recognizable from their sequence, and their targets are predictable. To predict whether the microorganism whose presence is inferred from amplified genomic fragments is the cause of the disease under study, however, is far more problematic. A replicating organism with which to observe behavior (e.g., drug resistance) and reproduce disease is not available. In fact, the viability of the putative microorganism may not be certain. Although detection of different molecular markers (e.g., specific mRNAs, rRNA/rDNA ratio, resistance-encoding loci) might help resolve some of these questions, it is difficult to determine whether these genotypes and markers all derive from the same organism in that clinical specimen. From a practical standpoint, proof of disease causation from sequence-based investigations will require data that address strength and specificity of association, target dosage effects, temporal considerations, response to therapy, and use of in situ hybridization (11). The selection of proper experimental and control specimens is paramount.

Settings for Pathogen Discovery

Explorations of microbial diversity within the external environment have yielded surprising results. Nearly all bacteria and archaea revealed by broad-range sequence "mining" in fresh water sites, oceans, surface soils, and deep geologic niches had not been recognized or ever cultivated in the laboratory. Novel kingdoms of life have been discovered with these genotypic methods (12,13). It has been estimated that only 0.4% of all extant bacterial species have been identified. Does this remarkable lack of knowledge pertain to the subset of microorganisms both capable and accomplished in causing human disease? The molecular methods described above could be applied in several settings in which one might expect to find uncharacterized microbial pathogens.

Acute, Life-Threatening Unexplained Illness

All clinicians are aware of cases characterized by sudden onset of fever, flu-like syndrome, and hemodynamic instability, often accompanied by leukocytosis or leukopenia and rapid deterioration of one or more organ systems. In some cases, despite the strong suggestion of a microbial etiology, conventional diagnostic methods cannot determine the cause. The dramatic nature of these illnesses belies their potential importance to public health and their value in revealing "emerging" agents of disease. An Unexplained Deaths and Critical Illnesses Project has been designed to identify and characterize these illnesses (14). Laboratory investigations include the application of broad-range ssu rDNA PCR. RDA is planned for carefully selected cases with matched control samples. Appropriate specimens have been obtained in only a minority of cases, but positive results from cerebrospinal fluid samples are encouraging. Two lessons have been learned. 1) Well-recognized pathogens may be the cause of some critical illnesses that cannot be explained with traditional diagnostic methods. 2) The process of clinical specimen selection and collection may need to be rethought jointly by molecular biologists and clinicians.

Chronic Idiopathic Disease

Adaptation and cooptation, features that favor long-term survival of both participants, dominate most host-pathogen relationships. Persistent or intermittent inflammation indicates host perturbation and a subtle imbalance to the relationship and gives rise to clinical manifestations. In fact, the epidemiologic, clinical, and pathologic features of many chronic inflammatory diseases are consistent with a microbial cause, but intimate or symbiotic host-pathogen relationships are among the most

difficult to decipher and mimic in the laboratory. Thus, it is not surprising that although microbial etiologies are attractive hypotheses for many chronic diseases, culture-dependent methods have not produced much evidence. Serologic approaches have been useful in providing some leads. For example, the first clues of a possible chlamydial etiology for coronary atherosclerosis were serologic findings. Corroborating data then became available from the use of molecular and in situ methods.

The list of chronic inflammatory diseases with possible microbial etiologies is extensive (15); it includes sarcoidosis, various forms of inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, Wegener granulomatosis, diabetes mellitus, primary biliary cirrhosis, tropical sprue, and Kawasaki disease. In this discussion, the concept of pathogenic mechanism should be viewed broadly. Many chronic diseases may result from damage or disruption of local immunologic surveillance systems by microbial infection or products; the microorganism is subsequently cleared away, but autoimmune responses or responses directed against commensal flora persist. By the time typical pathologic and clinical findings are produced and the disease is recognized, the inciting agent or its nucleic acids may be gone. Under these circumstances, the optimal time for specimen collection may be well before the disease takes on its characteristic features. Clinical suspicion, astute observation, and identification of disease-predisposing factors are critical. Surprisingly few published studies describe the application of broad-range molecular pathogen discovery methods to the diseases listed above or to other enigmatic chronic disease syndromes. With the finding of microbial sequences in these disease settings, experimental criteria for identifying disease causation must be rigorously pursued (11).

Commensal Microbial Flora

The human body harbors a 10-fold greater number of microbial cells than human cells. The commensal flora includes microorganisms that occasionally cause disease, especially when host defenses are impaired (due to immunosuppressive drugs, disruption of anatomic barriers, suppression of bacterial flora with antibiotics, or insertion of artificial surfaces). However, in many hosts with impaired conditions and signs and symptoms of infectious disease, an etiologic agent is not identified. If our understanding of microbial diversity within the human-associated commensal flora is as limited as it was of external environments, these clinical observations may not be surprising. That is, the inability to cultivate some of the commensal flora may explain the failure to diagnose related disease. In addition to revolutionizing environmental microbiology, molecular methods may offer rewards for clinical microbiology and the study of internal environmental niches.

Recent research has compared culture-dependent and culture-independent methods of characterizing human commensal flora (16-19). The results suggest that members of at least some phylogenetic groups, e.g., the spirochetes, have been ignored by traditional approaches. Direct comparisons of these two methods will likely show biases and deficiencies with each; nonetheless, important aspects of microbial diversity will be revealed by one and not the other. A complete enumeration of complex microbial communities is not the primary goal. Key members play crucial roles in maintaining the health of the ecosystem (20,21), and understanding community interactions and function may be the more important goal.

Arthropod Vectors and Small Animal Reservoirs

Several prominent, recently described cultivation-resistant pathogens are transmitted to humans from small animal reservoirs through airborne or vector-borne routes. These pathogens include borreliae (22), bartonellae (23), ehrlichiae, rickettsiae, babesiae, and hantaviruses. These reservoirs and the relevant vectors are attractive targets for pathogen discovery. Searches for restricted groups of microorganisms, searches within restricted host anatomic niches, or searches that include subtractive or differential techniques may be warranted, since all these targets are also hosts for their own commensal (e.g., intestinal) flora. Microorganisms that use arthropod vectors often express different sets of genes within vector versus animal host (e.g., human). Human immune recognition of differentially expressed gene products might help distinguish vector-associated pathogens from nonpathogenic vector-associated flora.

Phylogenetic Diversity of Microbial Pathogens

Nearly all kingdoms within the domain *Bacteria* contain recognized human pathogens

(Figure). Of those bacterial pathogens identified only by molecular methods, many are clustered within some kingdoms and divisions, such as the alpha-proteobacteria, which include many organisms that form endosymbiotic relationships with their hosts.

Nearly all humans harbor in the intestinal tract Archaea—among the most diverse and numerous cellular life forms on earth (24)—most

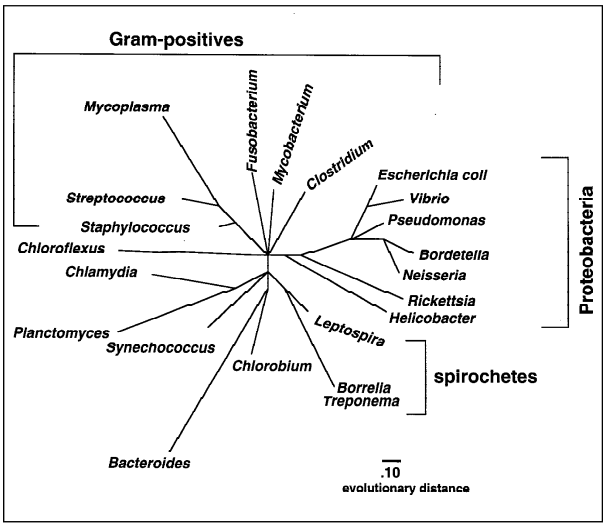


Figure. Evolutionary tree of the domain *Bacteria* based upon comparative analysis of nearly complete 16S rDNA sequences.

notably methanogens. So why are there no known archaeal pathogens? Although some of the most well-known archaea were first identified in (and were assumed to require) extreme environments, they are also found in environments similar to those found within the human body. However, in vitro cultivation methods for many archaea are unavailable, so how would we know if archaeal pathogens existed? Molecular reagents for archaeal detection and identification, i.e., rDNA-based primers and probes, have not been systematically applied to human disease-associated specimens. Without such analyses, finding these organisms in clinical samples would be unlikely.

Genomics and Newer Technologies

The ultimate genotype of a microorganism is its complete genome sequence. Approximately 15 microbial genomes have been sequenced in their entirety, and the rapid evolution of and large-scale investment in DNA sequencing technology predict full genome sequencing of approximately 50 microorganisms by the year 2000. This massive infusion of primary sequence data unleashes the potential to identify new families of broadly conserved orthologous genes that could be used to infer accurate phylogenies at every level and sector of the evolutionary tree. The number of completed genome sequences is too small to effect this goal (25). The sequence data sets for newly characterized genes are too small to assess the reliability of the phylogenies they predict. The problem imposed by horizontal gene transfer is now more apparent with the analysis of multitudes of gene families. To identify a well-characterized microorganism, an exact genotypic "hit" with a highly variable locus is sufficient. Likewise, clonality and clone identification can be determined with sequences from collections of polymorphic, but conserved loci, e.g., "housekeeping genes" (26). But for an unrecognized organism, the sequence locus or loci selected for genotyping must be highly conserved and phylogenetically informative and reliable. Over the next 5 years, with the increasing use of large-scale comparative genomic techniques, microbial sequence databases will represent the broad diversity among distant ancestral relatives, as well as the fine differences among closely related cousins. Assessment of putative universal sequences can be undertaken. All these developments and future trends apply equally well to the wide array of animal viruses and viral genomes (Table 1). As genotypes become more easily interpreted, they will continue to displace phenotypic characterization as the basis for pathogen recognition.

Often the only difference between a pathogenic and a nonpathogenic strain of the same species, e.g., enteropathogenic and nonenteropathogenic *Escherichia coli*, is a small set of virulence genes. These differences are not reflected in the ancestry inferred from more stable chromosomal markers (Table 2). Yet detection of these genes is a fundamental aspect

Table 1. Newer diagnostic technologies

1. High-density DNA microarrays
   - broad-based pathogen detection and characterization: bacteria, eukarya, viruses
   - virulence-associated gene families
   - comprehensive host gene expression profiles
2. Improved nucleic acid subtractive methods
3. Novel bioassays for toxin activity
   - neurons– or myocytes–on-a-chip

of pathogen identification. Microbial virulence is a phenotype whose genetic basis is rapidly being revealed. Families of virulence-associated genes responsible for microbial adherence, toxicity, specialized secretion, environmental sensing, and subversion of immune defenses have been defined, albeit with many sequence variations on a theme (27,28). One of the most important features of these genes is their proclivity toward horizontal transfer and over relatively rapid time scales. Genome sequencing efforts have facilitated, and will continue to facilitate, this approach to pathogen discovery. Physical clusters (or islands) of virulence genes are being identified, and their distinctive composition and boundaries are being defined (29). One might well imagine the development of a comprehensive set of consensus primers and probes for detecting these gene families, clusters, and islands (Table 1).

With increasing value placed on genotypic information and increasing numbers of potentially useful genotyping loci, the technology of sequence determination and primary genomic characterization has assumed center stage. Goals include speed, convenience, and large-scale sequencing. High density DNA microarray technology is one of the most promising in this context (Table 1). Depending on the format, microarrays can be used to detect nucleic acid polymorphisms or to sequence de novo; they can also quantitate mRNA. At least two basic applications of DNA microarray technology are available for pathogen detection and identification; neither has been fully developed or tested clinically (Table 1). The first would consist of a set of probes designed to assess ssu rDNA sequence diversity of all known monophyletic groups of bacteria, archaea, viruses, and nonanimal eukarya. Other phylogenetically reliable loci might be substituted for rDNA or included as well. In addition, consensus probes for families of virulence-associated genes, as described above, would facilitate identification of unsuspected or newly acquired pathogenic attributes in organisms not usually associated with these traits. Differential hybridizations and multiple fluorophores allow easy detection of hybridized target and normalization of quantified values to a reference sample. This sort of broad-range "pathogen detection chip" would identify mixed infections, as well as chimeric or novel microorganisms (Table 2); it could rapidly create an inventory of highly complex microbial communities and measure changes in individual members as a function of varying environmental conditions.

Table 2. Pathogens that may be difficult to detect or identify

1. Pathogens that establish intimate relationships with the host
   - endosymbionts and intracellular organisms
2. Chimeras: natural versus man-made?
3. "Nonpathogens" that acquire virulence-associated genes
4. Microorganisms without "universal" sequences?

The second theoretical use of DNA microarray technology for pathogen detection would focus on host gene responses. Arrays in current use at academic and commercial research laboratories are capable of quantitating expression responses by 10,000 to 20,000 human genes simultaneously (30-34). During most infectious diseases, directly affected tissues, secondary sites, and circulating leukocytes will likely display sets of common nonspecific expression responses; however, since each microbial pathogen interacts with and manipulates the host in a complex and unique manner, within these highly complex patterns there will also likely be critical diagnostic signatures that distinguish infection by one pathogen from infection by another. Furthermore, these stereotypic expression patterns will evolve. The time of initial host exposure to a pathogen might be determined by comparing new expression patterns with a suitable preexisting set of timed profiles. Patterns will provide clues about the pathogenesis of chronic inflammatory disease (35). Through the identification of key response genes might emerge novel diagnostic assays for their putative protein products and novel strategies for interfering with or blocking disease pathogenesis.

In many cases, infection-associated tissue damage occurs in the absence of intact microorganisms. Toxin-mediated disease is a prominent example. Often, microbial toxins act at a distance from the original site of microbial toxin production and release. In this setting, genotypic approaches for microbial detection may not be appropriate; in addition to the assessment of host responses, novel bioassays for toxin activity are attractive options (Table 1). For example, in a system designed by Greg Kovacs at Stanford University, neurons or myocytes are cultivated on the electrical contacts of a

miniaturized circuit board. The electrical output and properties of these cells can be monitored and analyzed as they are exposed to diverse membrane-active toxins. Although this technology is at an early stage of development, we know that such cells are extremely sensitive to chemical toxins, and this sensitivity can be recorded in the form of altered action potentials and changes in impedance and cell movement. Experiments are under way to test cell responses to biologic toxins in a variety of clinically relevant experimental conditions.

Relationships between Pathogen and Host

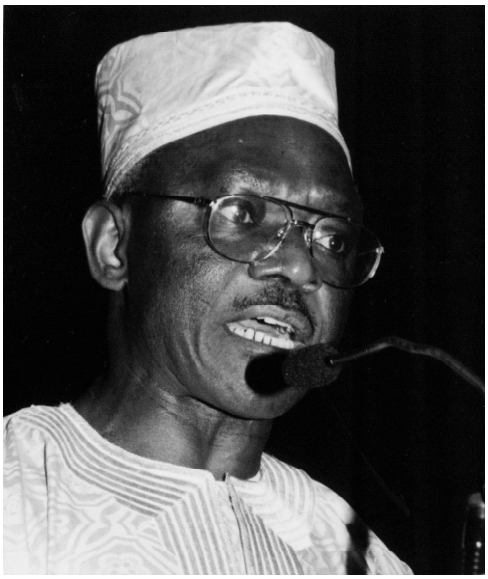
As more sensitive and comprehensive methods for uncovering human-associated pathogenic microorganisms identify previously unsuspected host-pathogen relationships, the nature of these relationships may need to be rethought (36,37). Parasitism and commensalism are probably not the complete story; mutualism may be more common in the human host than is usually taught. Evidence of coevolution between host and microbe suggests codependence. The endosymbiont theory for the origin of eukaryotic organelles is consistent with the same (38). Microbial remnants and cryptic genomic fragments may not be so uncommon within the human genome; for example, approximately 1% of the human genome is retrovirus sequence (39). Some of these viral genes may be expressed during local inflammation. The real challenges in pathogen discovery will be the problems of sequence interpretation, clinical relevance, and proof of causation. In the end, pathogen discovery will by necessity be a multidisciplinary effort (40). Only with the coordinated interaction of epidemiologists, pathologists, and clinicians will the role of microorganisms in disease be clearly defined.

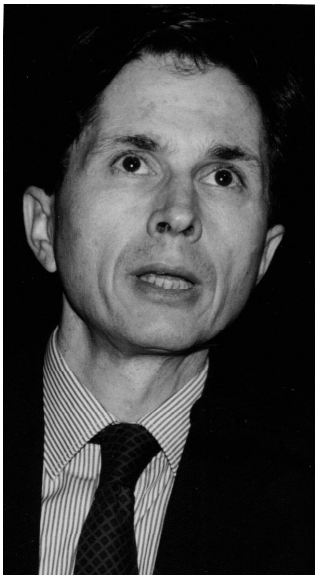
David A. Relman is assistant professor of medicine and of microbiology and immunology at Stanford University, Stanford, California; he is also staff physician at Veterans Affairs Palo Alto Health Care System, Palo Alto, California. His research interests include Bordetella pathogenesis, molecular and genomic aspects of pathogen recognition by the host, and development and application of molecular methods for the identification of unrecognized microbial pathogens.

References

1. Woese CR. Bacterial evolution. Microbiol Rev 1987;51:221-71.
2. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A 1990;87:4576-9.
3. Maidak BL, Olsen GJ, Larsen N, Overbeek R, McCaughey MJ, Woese CR. The RDP (Ribosomal Database Project). Nucleic Acids Res 1997;25:109-11.
4. Relman DA, Loutit JS, Schmidt TM, Falkow S, Tompkins LS. The agent of bacillary angiomatosis. An approach to the identification of uncultured pathogens. N Engl J Med 1990;323:1573-80.
5. Relman DA, Schmidt TM, MacDermott RP, Falkow S. Identification of the uncultured bacillus of Whipple's disease. N Engl J Med 1992;327:293-301.
6. Wilson KH, Blitchington R, Frothingham R, Wilson JA. Phylogeny of the Whipple's-disease-associated bacterium. Lancet 1991;338:474-5.
7. Santamaria-Fries M, Fajardo LF, Sogin ML, Olson PD, Relman DA. Lethal infection by a previously unrecognised metazoan parasite. Lancet 1996;347:1797-801.
8. Nichol ST, Spiropoulou CF, Morzunov S, Rollin PE, Ksiazek TG, Feldmann H, et al. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science 1993;262:914-7.
9. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 1994;266:1865-9.
10. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989;244:359-62.
11. Fredricks DN, Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. Clin Microbiol Rev 1996;9:18-33.
12. Barns SM, Delwiche CF, Palmer JD, Pace NR. Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. Proc Natl Acad Sci U S A 1996;93:9188-93.
13. Hugenholtz P, Pitulle C, Hershberger KL, Pace NR. Novel division level bacterial diversity in a Yellowstone hot spring. J Bacteriol 1998;180:366-76.
14. Perkins BA, Flood JM, Danila R, Holman RC, Reingold AL, Klug LA, et al. Unexplained deaths due to possibly infectious causes in the United States: defining the problem and designing surveillance and laboratory approaches. The Unexplained Deaths Working Group. Emerg Infect Dis 1996;2:47-53.
15. Fredricks DN, Relman DA. Infectious agents and the etiology of chronic idiopathic diseases. In: Remington JS, Swartz MN, editors. Current Clinical Topics in Infectious Diseases. Vol. 18. Boston: Blackwell Scientific Publications. In press 1998.
16. Choi BK, Paster BJ, Dewhirst FE, Gobel UB. Diversity of cultivable and uncultivable oral spirochetes from a patient with severe destructive periodontitis. Infect Immun 1994;62:1889-95.
17. Hugenholtz P, Pace NR. Identifying microbial diversity in the natural environment: a molecular phylogenetic approach. Trends in Biotechnology 1996;14:190-7.

18. Millar MR, Linton CJ, Cade A, Glancy D, Hall M, Jalal H. Application of 16S rRNA gene PCR to study bowel flora of preterm infants with and without necrotizing enterocolitis. J Clin Microbiol 1996;34:2506-10.
19. Wilson KH, Blitchington RB. Human colonic biota studied by ribosomal DNA sequence analysis. Appl Environ Microbiol 1996;62:2273-8.
20. Tilman D, Knops J, Wedin D, Reich P, Ritchie M, Siemann E. The influence of functional diversity and composition on ecosystem processes. Science 1997;277:1300-2.
21. Wardle DA, Zackrisson O, Hörnberg G, Gallet C. The influence of island area on ecosystem properties. Science 1997;277:1296-9.
22. Barbour AG, Maupin GO, Teltow GJ, Carter CJ, Piesman J. Identification of an uncultivable Borrelia species in the hard tick *Amblyomma americanum*: possible agent of a Lyme disease-like illness. J Infect Dis 1996;173:403-9.
23. Hofmeister EK, Kolbert CP, Abdulkarim AS, Magera JM, Hopkins MK, Uhl JR, et al. Cosegregation of a novel *Bartonella* species with *Borrelia burgdorferi* and *Babesia microti* in *Peromyscus leucopus*. J Infect Dis 1998;177:409-16.
24. Olsen GJ, Woese CR. Archaeal genomics: an overview. Cell 1997;89:991-4.
25. Tatusov RL, Koonin EV, Lipman DJ. A genomic perspective on protein families. Science 1997;278:631-7.
26. Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci U S A 1998;95:3140-5.
27. Finlay BB, Falkow S. Common themes in microbial pathogenicity revisited. Microbiol Mol Biol Rev 1997;61:136-69.
28. Relman DA, Falkow S. A molecular perspective of microbial pathogenicity. In: Mandell GL, Douglas RG, Bennett JE, editors. Principles and practice of infectious diseases. 4th ed. New York: Churchill Livingstone; 1994. p. 19-29.
29. Groisman EA, Ochman H. Pathogenicity islands: bacterial evolution in quantum leaps. Cell 1996;87:791-4.
30. Chee M, Yang R, Hubbell E, Berno A, Huang XC, Stern D, et al. Accessing genetic information with high-density DNA arrays. Science 1996;274:610-4.
31. DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, et al. Use of a cDNA microarray to analyse gene expression patterns in human cancer. Nat Genet 1996;14:457-60.
32. Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 1995;270:467-70.
33. Schena M, Shalon D, Heller R, Chai A, Brown PO, Davis RW. Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. Proc Natl Acad Sci U S A 1996;93:10614-9.
34. Shalon D, Smith SJ, Brown PO. A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. Genome Res 1996;6:639-45.
35. Heller RA, Schena M, Chai A, Shalon D, Bedilion T, Gilmore J, et al. Discovery and analysis of inflammatory disease-related genes using cDNA microarrays. Proc Natl Acad Sci U S A 1997;94:2150-5.
36. Krause RM. Dynamics of emergence. J Infect Dis 1994;170:265-71.
37. Lederberg J. Infectious disease as an evolutionary paradigm. Emerg Infect Dis 1997;3:417-23.
38. Gray MW. The endosymbiont hypothesis revisited. Int Rev Cytol 1992;141:233-357.
39. Lower R, Lower J, Kurth R. The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences. Proc Natl Acad Sci U S A 1996;93:5177-84.
40. Fredricks DN, Relman DA. Infectious agents and the etiology of chronic idiopathic diseases. Curr Clin Top Infect Dis 1998;18:180-200.





Thomas C. Nchinda (L)
David Heymann
World Health Organization,
Geneva, Switzerland.

The Emergence of Bovine Spongiform Encephalopathy and Related Diseases

Sir John Pattison
Medical School of University College London, London, United Kingdom

Since 1986, approximately 170,000 cases of bovine spongiform encephalopathy (BSE) have occurred among approximately one million animals infected by contaminated feed in the United Kingdom. A ruminant feed ban in 1988 resulted in the rapid decline of the epidemic. Transmissible spongiform encephalopathies due to agents indistinguishable from BSE have appeared in small numbers of exotic zoo animals; a small outbreak among domestic cats is declining. Creutzfeldt-Jakob disease (CJD) has been intensively monitored since 1990 because of the risk BSE could pose to public health. In 1995, two adolescents in the United Kingdom died of CJD, and through the early part of 1996, other relatively young people had cases of what became known as new variant CJD, whose transmissible agent (indistinguishable from that of BSE) is responsible for 26 cases in the United Kingdom and one in France. Areas of concern include how many cases will appear in the future and whether or not use of human blood and blood products may cause a second cycle of human infections.

Before the 1980s, a number of diseases of animals (scrapie, chronic wasting disease, and transmissible mink encephalopathy) and humans (Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, and kuru), in spite of distinctive individual features, could be unified by the term transmissible spongiform encephalopathies (TSEs). In 1986, bovine spongiform encephalopathy (BSE) was first identified in indigenous cattle in the United Kingdom (1). A variety of clinical signs have been observed, but the three cardinal features of the disease are nervousness, heightened reactivity to external stimuli, and difficult movement, particularly of the hind limbs (2). Spongiform change is evident in the brain (1), and neuropathologic tests remain the mainstay of a BSE diagnosis. The disease was transmitted experimentally to mice (3) and cattle (4) by use of brain homogenates from cattle with clinical BSE; thus BSE has all the features that define classical TSEs.

The BSE Epidemic

Some 1985 cases were diagnosed retrospectively; other cases occurring before 1986 probably went unnoticed. Since BSE was recognized, more than 170,000 cases were reported in the United Kingdom through the end of 1997. The epidemic curve, which peaked in 1992, is now in rapid decline (Table 1). Approximately two thirds of the dairy herds in the United Kingdom have had at least one case of BSE compared with only one sixth of the beef suckler herds. Furthermore, most of the affected suckler herds contained animals originating from dairy herds, which are fed differently.

Shortly after the recognition of BSE, epidemiologic studies indicated that the source of infection was the meat and bone meal used in concentrated cattle feed (5). Subsequently, in July 1988, ruminant protein in ruminant feed was banned. This ban immediately reduced the

Table 1. Annual incidence of bovine spongiform encephalopathy in the United Kingdom, 1985–1997

Year	Number of cases
1985	14
1986	60
1987	630
1988	2,184
1989	7,137
1990	14,181
1991	25,032
1992	36,682
1993	34,370
1994	23,945
1995	14,300
1996	8,016
1997	4,052

incidence of new infections, which began to be reflected in a diminution in the incidence of clinical cases 5 years later (the average incubation period) in 1993. Nevertheless, almost 36,000 cattle with BSE were born after the ruminant feed ban (a few as late as 1994), which indicates that the ban was not completely effective. Ruminant protein could be included in pig and poultry feed, and cross-contamination of cattle feed in the production mills and perhaps accidental exposure of cattle on the farm were possible until the feeding of mammalian protein to all farm animal species in the United Kingdom was prohibited in 1996.

The average age at which clinical BSE manifests itself is 4 to 5 years (6). Many animals in the national U.K. herd are slaughtered at significantly younger ages, and those infected with BSE would not have had a chance to develop the disease. Using methods developed for the retrospective analysis of the AIDS epidemic, Anderson and colleagues (7) calculated that approximately one million animals in the U.K. herd must have been infected to have produced 170,000 clinical cases of BSE. These same workers predicted the number of cases of BSE that would occur in 1996 and in subsequent years (Table 2). The calculations are based on a dominant feedborne source of infection; a small amount of cow-to-calf transmission was included because a long-term study, conducted by the U.K. Ministry of Agriculture, Fisheries and Food, indicated an increased incidence of BSE in calves born to mothers in the late stages of the incubation period of the disease (8). The results are compatible with a cow-to-calf transmission of approximately 10%, which in itself is not sufficient to perpetuate the BSE epidemic. The calculations predict a small number of cases and very few new infections by the beginning of the next decade. The predictions have been validated by the actual numbers in 1996 and 1997, which were 8,016 and 4,149, respectively (9).

Table 2. Predictions of new infections and cases of BSE[a] from 1996-2001[b]

	New infections		Cases	
Year	Expected value	95% Prediction interval	Expected value	95% Prediction interval
1996	189	(155-11,300)	7,386	(6,541-8,856)
1997	95	(63-236)	4,111	(3,006-7,664)
1998	38	(21-214)	1,864	(1,153-7,052)
1999	12	(5-162)	682	(388-5,909)
2000	3	(1-86)	221	(128-3,660)
2001	1	(0-33)	72	(45-1,592)

[a]Bovine spongiform encephalopathy.
[b]Information extracted from (7).

Infection in Other Animals

BSE has also been transmitted to exotic ruminants in zoos in the United Kingdom. Between 1986 and 1992, cases have occurred in bison, nyala, gemsbok, two species of oryx, greater kudu, and eland. These animals became infected by eating the same meat and bone meal-containing concentrated feed responsible for the disease in cattle. BSE infection in species other than ruminants was always considered possible. Careful watch was kept on the packs of hounds used for hunting in the United Kingdom because they are often fed carcasses unfit for human consumption. Spongiform encephalopathy has not occurred in dogs; however, in 1990, a case of spongiform encephalopathy was diagnosed in domestic cats; 81 additional cases in cats have occurred with a wide geographic spread throughout the United Kingdom. The true incidence is probably many times higher than observed because diagnosis is patchy and the disease was not statutorily notifiable until 1994. The annual incidence at the height of the outbreak was probably 10 to 15 cases per million cats (Wilesmith, pers. comm.). The most likely source of the infection was commercially produced cat food. In 1989, the pet food industry removed the dangerous bovine tissues, the specified bovine offal, before a statutory ban in 1990. The number of cases of feline spongiform encephalopathy (FSE) diagnosed in the United Kingdom has been declining since 1994 (1994, 16 cases; 1995, 6 cases; 1997, 6 cases) (Table 3). Only one cat, an adopted stray, was apparently born

Table 3. Number of cases of feline spongiform encephalopathy in the United Kingdom by year of diagnosis (MAFF, personal communication)

Year	Number of cases
1990	12
1991	12
1992	10
1993	11
1994	16
1995	8
1996	6
1997	6
1998[a]	4

[a]To May 1, 1998.

after the ban on specified bovine offal in pet food. A TSE indistinguishable from BSE has also been found in puma, cheetah, ocelot, and a tiger in zoos in the United Kingdom between 1992 and 1995. These animals became infected as a result of being fed raw meat, which would have included bovine central nervous system, a practice which has now ceased.

Human Disease and BSE

Control Measures

The risk to human health from BSE was always recognized. The principal protective measure was the November 1989 ban on the use of certain specified bovine offal in human food. As with scraple, the tissues banned were those likely to contain the highest concentrations of the transmissible agent (brain, spinal cord, tonsil, spleen, thymus, and intestine of cattle older than 6 months of age). The intestine and thymus of calves was added to the list in 1994 when a long-term pathogenesis study in cattle by the Ministry of Agriculture, Fisheries and Food indicated that the transmissible agent could be found in the terminal ileum (it is assumed that the agent was present in Peyer's patches). In 1996 the whole head, other than the tongue, was formally banned because of concern about possible contamination with brain. Since the banned tissues now contain more than offal, the tissues are referred to as specified bovine material. During 1995, it became clear that spinal cord was not being completely removed from a small number of carcasses that were subsequently certified as fit for human consumption. Consequently, in December 1995 the U.K. government banned the use of bovine vertebral column for the production of mechanically recovered meat. In March 1996, when it became clear that human disease related to BSE was "probable" rather than "theoretical," the U.K. government introduced the over-30–month scheme, which allowed only animals under the age of 30 months to be used for human food, provided that all the banned specified bovine material had been removed. This added an extra margin of safety because cattle can be reasonably accurately aged by their dentition at 30 months and because BSE is relatively rare under the age of 30 months. Only 265 cases occurred in cattle younger than 30 months, and during 1997, the youngest animal with BSE was 37 months of age (Ministry of Agriculture, Fisheries and Food, pers. comm.). In the second half of 1997, the long-term pathogenesis experiment indicated that the transmissible agent of BSE could be recovered from the dorsal root ganglia of experimentally infected animals toward the end of the incubation period. Also, in one animal the agent was transmitted by intracerebral inoculation of mice with bovine bone marrow. Accordingly, in December 1997 the U.K. government introduced legislation to ban the sale of beef on the bone, even from animals under 30 months of age. Many in the United Kingdom thought that this regulation to prevent an extremely small risk of transmitting BSE in T-bone steaks and rib of beef was unnecessary. Nevertheless, the introduction of the specified bovine offal ban in 1989 and its subsequent refinements have ensured the safety of beef and beef products that now enter the human food chain in the United Kingdom. Even so, as a consequence of the emergence of new variant CJD, a worldwide ban on the sale of U.K. beef and beef products was introduced by the European Union in March 1996 and is still in force with the exception of a recent (March 1998) relaxation for certain herds in Northern Ireland.

New Variant CJD

Clearly, the first measures to protect human health were introduced before any human disease could be related to BSE. To guard against the possible emergence of such disease (or diseases), the U.K. Department of Health set up a CJD Surveillance Unit in 1990. The purpose of the unit was to monitor the trends in incidence of CJD and any unusual features among cases. Concern was first focused on the 1995 cases of the third and fourth U.K. farmer since 1990 to be confirmed as having CJD. Statistically, the chances of four such cases occurring in 6 years in the United Kingdom were very small. However, the clinical features of the disease were typical of classical CJD, and collaboration between the CJD Surveillance Unit and other European countries indicated that farmers were overrepresented compared with CJD cases in countries with no BSE. Subsequently, classic CJD was confirmed in these farmers; no further cases have been diagnosed in U.K. farmers, and the significance of the high incidence in 1995 is diminishing.

The death in May 1995 of the first adolescent ever to be diagnosed with CJD in the United Kingdom was followed in October 1995 by the death of a second adolescent; by January 1996,

three other young (29 years of age) persons became ill. Atypical pathologic results were beginning to be defined in these patients; and on March 8, 1996, eight cases of what came to be known as new variant CJD or variant CJD (vCJD) were reported to the Spongiform Encephalopathy Advisory Committee. The cases were distinguished by the relatively young age at which the symptoms started (10,11). That age range is now 16 years to 52 years. The duration of the illness is relatively long, averaging approximately 14 months as opposed to the 4 to 5 months in classic CJD. The early symptoms are often psychiatric, and it may be 6 or 7 months before any neurologic signs appear. The characteristic electroencephalogram pattern of sporadic CJD is not seen in vCJD, and pathologic results show florid plaques and extensive cerebellar involvement with multiple PrP deposits. As with BSE and FSE, the neuropathologic appearances are the mainstay of laboratory confirmation. Magnetic resonance imaging scanning and detection of 14-3-3 protein can be helpful. Early evidence indicates that the diagnosis can often be made from tonsil biopsies (12). Otherwise, diagnosis must depend upon brain biopsy or postmortem examination.

When on March 20, 1996, the U.K. government announced the existence of 10 cases of vCJD and the opinion of the Spongiform Encephalopathy Advisory Committee that these were probably related to BSE, three questions immediately arose. The first was, "Is there really any link with BSE?" Additional evidence emerged from the work of Collinge and his colleagues (13) on the analysis of the PrP fragments after protease digestion. The position of the three fragments and the relatively high concentrations of the di-glycosylated form indicated that vCJD was distinct from the previously recognized forms of CJD and that similarities existed between the cases of vCJD and BSE and FSE. In 1997, the first results were published from the classic strain typing experiments initiated during 1996 (14). The characteristics of material from cases of vCJD, in terms of incubation period and lesion profile in RIII mice, were identical to those from cases of BSE and FSE. These observations are confirmed now in C57 black mice. Thus, vCJD can now be regarded as human BSE in the same way that FSE is regarded as feline BSE. The second question was, "What is the route of transmission from cattle to humans?" So far we have no evidence, only a working hypothesis, that transmission was likely from inclusion in the human food chain of tissues that contain the highest concentration of the transmissible agent. The major differences in human exposure to these tissues would have occurred first when sick animals were banned from the human food chain in 1988 and again in 1989 when the specified bovine offals of otherwise healthy animals were removed from the human food chain.

Studies continue in an attempt to answer the third question, "How many vCJD cases will there be in the future?" So far, 26 cases have been diagnosed in the United Kingdom (Table 4) and 1 in France. Incidence has not increased since vCJD was first diagnosed in 1995. If instead of looking at the date of death one looks at the date of onset of the symptoms in the 26 patients, two new cases occurred on average every quarter since 1994. All cases have been methionine homozygotes at codon 129 of the PrP gene. In the general population approximately 40% have such a genotype; 10% are valine homozygotes, and 50% are heterozygotes. An analysis of classic sporadic CJD indicates that 80% of those cases are methionine homozygotes, 10% valine homozygotes, and 10% heterozygotes. It is perhaps not surprising, therefore, that the first cases of vCJD to be seen are methionine homozygotes.

Only one published analysis has predicted the number of future nvCJD cases after constraining the models used to the known and surmised facts at the time (15). The total number of future cases will depend critically on the average incubation period of vCJD. At present, we have no way of determining that; therefore, it remains too early to predict with any accuracy the total number of future cases. It remains possible that the outbreak of vCJD cannot be regarded as a single curve and that the small

Table 4. Creutzfeldt-Jakob disease in the United Kingdom

	Deaths of definite and probable cases						
Year	Referrals	Sporadic	Iatrogenic	Familial	GSS[a]	nvCJD[b]	Total
1994	116	52	1	3	3	0	59
1995	86	34	4	2	3	3	46
1996	133	40	4	2	4	10	60
1997	152	42	6	3	0	10	61
1998[c]	35	3	0	1	0	2	6

[a]Gerstmann-Straussler-Scheinker syndrome.
[b]New variant Creutzfeldt-Jakob disease.
[c]To Apr 30, 1998. Figures released by U.K. Department of Health Jun 1, 1998.

number of cases have occurred in persons who are extremely susceptible for unknown reasons.

The Future

Much has happened already as a consequence of the emergence of BSE in U.K. cattle. The appropriate measures are in place to protect public health and end the BSE epidemic in cattle and other affected species. These measures are more rigorously enforced than ever before. It is difficult to see what could be done to make the BSE epidemic decline more rapidly than it already has, short of slaughtering the entire U.K. herd, which would be unnecessary and impractical. From now the question is likely to be how to withdraw some of the restrictions on U.K. beef and beef products. The exemptions are likely to be herd based (as is the case with Northern Ireland) or date based after the total ban on the use of meat and bone meal in the feed of any farm animals in the United Kingdom in 1996. In terms of the protection of public health, all the necessary measures are in place. Two further concerns remain and are actively under consideration: whether or not BSE exists in the sheep flocks and whether the cases of vCJD in the United Kingdom will be sufficient to generate concern about a second wave of transmission within the human population as a consequence of the use of blood and blood products. With respect to the former, the detection of sheep with scrapie-like diseases in the United Kingdom and the typing of strains from affected animals are being intensified. With respect to blood and blood products, some restrictions on the use of U.K. raw materials for the production of blood products are already in place, and a detailed risk assessment in relation to blood transfusion is awaited.

Sir John Pattison is a virologist and dean of the Medical School, University College London, and Chair, U.K. Spongiform Encephalopathy Advisory Committee.

References

1. Wells GAH, Scott AC, Johnson CI, Gunning RF, Hancock RD, Jeffrey M, et al. A novel progressive spongiform encephalopathy in cattle. Vet Rec 1987;121:419-20.
2. Wilesmith JW, Hoinville LJ, Ryan JBM, Sayers AR. Bovine spongiform encephalopathy: aspects of the clinical picture and analyses of possible changes. Vet Rec 1992;130:197-201.
3. Fraser H, McConnell I, Wells GAH, Dawson M. Transmission of bovine spongiform encephalopathy to mice. Vet Rec 1988;123:472.
4. Dawson M, Wells GAH, Parker BNJ. Preliminary evidence of the experimental transmissibility of bovine spongiform encephalopathy to cattle. Vet Rec 1990;126:112-3.
5. Wilesmith JW, Wells GAH, Cranwell MP, Ryan JBM. Bovine spongiform encephalopathy: epidemiological studies. Vet Rec 1988;123:638-44.
6. Stekel DJ, Nowak MA, Southwood TRE. Prediction of future BSE spread. Nature 1996;381:119.
7. Anderson RM, Donnelly CA, Ferguson NM, Woolhouse MEJ, Watt CJ, Udy HJ, et al. Transmission dynamics and epidemiology of BSE in British cattle. Nature 1996;382:779-88.
8. Donnelly CA, Ghani AC, Ferguson NM, Wilesmith JW, Anderson RM. Analysis of the bovine spongiform encephalopathy study: evidence for direct maternal transmission. Appl Statist 1997;43:321-44.
9. Lawson C, Herd L, editors. BSE Enforcement Bulletin 1998. Ministry of Agriculture, Fisheries and Food; Bull. No. 20; p. 2.
10. Will RG, Ironside JW, Zeidler SN, Cousens SN, Estibeiro K, Alperovitch A, et al. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 1996;347:921-5.
11. Zeidler M, Stewart GE, Barraclough CR, Bateman DE, Bates D, Burn DJ, et al. New variant Creutzfeldt-Jakob disease: neurological features and diagnostic tests. Lancet 1997;350:903-7.
12. Hill AF, Zeidler M, Ironside J, Collinge J. Diagnosis of new variant Creutzfeldt-Jakob diseases by tonsil biopsy. Lancet 1997;349:99-100.
13. Collinge J, Sidle KCL, Meads J, Ironside J, Hill AF. Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. Nature 1996;383:685-90.
14. Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, et al. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature 1997;389:498-501.
15. Cousens SN, Vynnycky E, Zeidler M, Will RG, Smith PG. Predicting the CJD epidemic in humans. Nature 1997;385:197-8.

The Emergence of Bovine Spongiform Encephalopathy and Related Diseases

Sir John Pattison
Medical School of University College London, London, United Kingdom

Since 1986, approximately 170,000 cases of bovine spongiform encephalopathy (BSE) have occurred among approximately one million animals infected by contaminated feed in the United Kingdom. A ruminant feed ban in 1988 resulted in the rapid decline of the epidemic. Transmissible spongiform encephalopathies due to agents indistinguishable from BSE have appeared in small numbers of exotic zoo animals; a small outbreak among domestic cats is declining. Creutzfeldt-Jakob disease (CJD) has been intensively monitored since 1990 because of the risk BSE could pose to public health. In 1995, two adolescents in the United Kingdom died of CJD, and through the early part of 1996, other relatively young people had cases of what became known as new variant CJD, whose transmissible agent (indistinguishable from that of BSE) is responsible for 26 cases in the United Kingdom and one in France. Areas of concern include how many cases will appear in the future and whether or not use of human blood and blood products may cause a second cycle of human infections.

Before the 1980s, a number of diseases of animals (scrapie, chronic wasting disease, and transmissible mink encephalopathy) and humans (Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, and kuru), in spite of distinctive individual features, could be unified by the term transmissible spongiform encephalopathies (TSEs). In 1986, bovine spongiform encephalopathy (BSE) was first identified in indigenous cattle in the United Kingdom (1). A variety of clinical signs have been observed, but the three cardinal features of the disease are nervousness, heightened reactivity to external stimuli, and difficult movement, particularly of the hind limbs (2). Spongiform change is evident in the brain (1), and neuropathologic tests remain the mainstay of a BSE diagnosis. The disease was transmitted experimentally to mice (3) and cattle (4) by use of brain homogenates from cattle with clinical BSE; thus BSE has all the features that define classical TSEs.

The BSE Epidemic

Some 1985 cases were diagnosed retrospectively; other cases occurring before 1986 probably went unnoticed. Since BSE was recognized, more than 170,000 cases were reported in the United Kingdom through the end of 1997. The epidemic curve, which peaked in 1992, is now in rapid decline (Table 1). Approximately two thirds of the dairy herds in the United Kingdom have had at least one case of BSE compared with only one sixth of the beef suckler herds. Furthermore, most of the affected suckler herds contained animals originating from dairy herds, which are fed differently.

Shortly after the recognition of BSE, epidemiologic studies indicated that the source of infection was the meat and bone meal used in concentrated cattle feed (5). Subsequently, in July 1988, ruminant protein in ruminant feed was banned. This ban immediately reduced the

Table 1. Annual incidence of bovine spongiform encephalopathy in the United Kingdom, 1985–1997

Year	Number of cases
1985	14
1986	60
1987	630
1988	2,184
1989	7,137
1990	14,181
1991	25,032
1992	36,682
1993	34,370
1994	23,945
1995	14,300
1996	8,016
1997	4,052

incidence of new infections, which began to be reflected in a diminution in the incidence of clinical cases 5 years later (the average incubation period) in 1993. Nevertheless, almost 36,000 cattle with BSE were born after the ruminant feed ban (a few as late as 1994), which indicates that the ban was not completely effective. Ruminant protein could be included in pig and poultry feed, and cross-contamination of cattle feed in the production mills and perhaps accidental exposure of cattle on the farm were possible until the feeding of mammalian protein to all farm animal species in the United Kingdom was prohibited in 1996.

The average age at which clinical BSE manifests itself is 4 to 5 years (6). Many animals in the national U.K. herd are slaughtered at significantly younger ages, and those infected with BSE would not have had a chance to develop the disease. Using methods developed for the retrospective analysis of the AIDS epidemic, Anderson and colleagues (7) calculated that approximately one million animals in the U.K. herd must have been infected to have produced 170,000 clinical cases of BSE. These same workers predicted the number of cases of BSE that would occur in 1996 and in subsequent years (Table 2). The calculations are based on a dominant feedborne source of infection; a small amount of cow-to-calf transmission was included because a long-term study, conducted by the U.K. Ministry of Agriculture, Fisheries and Food, indicated an increased incidence of BSE in calves born to mothers in the late stages of the incubation period of the disease (8). The results are compatible with a cow-to-calf transmission of approximately 10%, which in itself is not sufficient to perpetuate the BSE epidemic. The calculations predict a small number of cases and very few new infections by the beginning of the next decade. The predictions have been validated by the actual numbers in 1996 and 1997, which were 8,016 and 4,149, respectively (9).

Table 2. Predictions of new infections and cases of BSE[a] from 1996-2001[b]

	New infections		Cases	
Year	Expected value	95% Prediction interval	Expected value	95% Prediction interval
1996	189	(155-11,300)	7,386	(6,541-8,856)
1997	95	(63-236)	4,111	(3,006-7,664)
1998	38	(21-214)	1,864	(1,153-7,052)
1999	12	(5-162)	682	(388-5,909)
2000	3	(1-86)	221	(128-3,660)
2001	1	(0-33)	72	(45-1,592)

[a]Bovine spongiform encephalopathy.
[b]Information extracted from (7).

Infection in Other Animals

BSE has also been transmitted to exotic ruminants in zoos in the United Kingdom. Between 1986 and 1992, cases have occurred in bison, nyala, gemsbok, two species of oryx, greater kudu, and eland. These animals became infected by eating the same meat and bone meal-containing concentrated feed responsible for the disease in cattle. BSE infection in species other than ruminants was always considered possible. Careful watch was kept on the packs of hounds used for hunting in the United Kingdom because they are often fed carcasses unfit for human consumption. Spongiform encephalopathy has not occurred in dogs; however, in 1990, a case of spongiform encephalopathy was diagnosed in domestic cats; 81 additional cases in cats have occurred with a wide geographic spread throughout the United Kingdom. The true incidence is probably many times higher than observed because diagnosis is patchy and the disease was not statutorily notifiable until 1994. The annual incidence at the height of the outbreak was probably 10 to 15 cases per million cats (Wilesmith, pers. comm.). The most likely source of the infection was commercially produced cat food. In 1989, the pet food industry removed the dangerous bovine tissues, the specified bovine offal, before a statutory ban in 1990. The number of cases of feline spongiform encephalopathy (FSE) diagnosed in the United Kingdom has been declining since 1994 (1994, 16 cases; 1995, 6 cases; 1997, 6 cases) (Table 3). Only one cat, an adopted stray, was apparently born

Table 3. Number of cases of feline spongiform encephalopathy in the United Kingdom by year of diagnosis (MAFF, personal communication)

Year	Number of cases
1990	12
1991	12
1992	10
1993	11
1994	16
1995	8
1996	6
1997	6
1998[a]	4

[a]To May 1, 1998.

after the ban on specified bovine offal in pet food. A TSE indistinguishable from BSE has also been found in puma, cheetah, ocelot, and a tiger in zoos in the United Kingdom between 1992 and 1995. These animals became infected as a result of being fed raw meat, which would have included bovine central nervous system, a practice which has now ceased.

Human Disease and BSE

Control Measures

The risk to human health from BSE was always recognized. The principal protective measure was the November 1989 ban on the use of certain specified bovine offal in human food. As with scraple, the tissues banned were those likely to contain the highest concentrations of the transmissible agent (brain, spinal cord, tonsil, spleen, thymus, and intestine of cattle older than 6 months of age). The intestine and thymus of calves was added to the list in 1994 when a long-term pathogenesis study in cattle by the Ministry of Agriculture, Fisheries and Food indicated that the transmissible agent could be found in the terminal ileum (it is assumed that the agent was present in Peyer's patches). In 1996 the whole head, other than the tongue, was formally banned because of concern about possible contamination with brain. Since the banned tissues now contain more than offal, the tissues are referred to as specified bovine material. During 1995, it became clear that spinal cord was not being completely removed from a small number of carcasses that were subsequently certified as fit for human consumption. Consequently, in December 1995 the U.K. government banned the use of bovine vertebral column for the production of mechanically recovered meat. In March 1996, when it became clear that human disease related to BSE was "probable" rather than "theoretical," the U.K. government introduced the over-30–month scheme, which allowed only animals under the age of 30 months to be used for human food, provided that all the banned specified bovine material had been removed. This added an extra margin of safety because cattle can be reasonably accurately aged by their dentition at 30 months and because BSE is relatively rare under the age of 30 months. Only 265 cases occurred in cattle younger than 30 months, and during 1997, the youngest animal with BSE was 37 months of age (Ministry of Agriculture, Fisheries and Food, pers. comm.). In the second half of 1997, the long-term pathogenesis experiment indicated that the transmissible agent of BSE could be recovered from the dorsal root ganglia of experimentally infected animals toward the end of the incubation period. Also, in one animal the agent was transmitted by intracerebral inoculation of mice with bovine bone marrow. Accordingly, in December 1997 the U.K. government introduced legislation to ban the sale of beef on the bone, even from animals under 30 months of age. Many in the United Kingdom thought that this regulation to prevent an extremely small risk of transmitting BSE in T-bone steaks and rib of beef was unnecessary. Nevertheless, the introduction of the specified bovine offal ban in 1989 and its subsequent refinements have ensured the safety of beef and beef products that now enter the human food chain in the United Kingdom. Even so, as a consequence of the emergence of new variant CJD, a worldwide ban on the sale of U.K. beef and beef products was introduced by the European Union in March 1996 and is still in force with the exception of a recent (March 1998) relaxation for certain herds in Northern Ireland.

New Variant CJD

Clearly, the first measures to protect human health were introduced before any human disease could be related to BSE. To guard against the possible emergence of such disease (or diseases), the U.K. Department of Health set up a CJD Surveillance Unit in 1990. The purpose of the unit was to monitor the trends in incidence of CJD and any unusual features among cases. Concern was first focused on the 1995 cases of the third and fourth U.K. farmer since 1990 to be confirmed as having CJD. Statistically, the chances of four such cases occurring in 6 years in the United Kingdom were very small. However, the clinical features of the disease were typical of classical CJD, and collaboration between the CJD Surveillance Unit and other European countries indicated that farmers were overrepresented compared with CJD cases in countries with no BSE. Subsequently, classic CJD was confirmed in these farmers; no further cases have been diagnosed in U.K. farmers, and the significance of the high incidence in 1995 is diminishing.

The death in May 1995 of the first adolescent ever to be diagnosed with CJD in the United Kingdom was followed in October 1995 by the death of a second adolescent; by January 1996,

three other young (29 years of age) persons became ill. Atypical pathologic results were beginning to be defined in these patients; and on March 8, 1996, eight cases of what came to be known as new variant CJD or variant CJD (vCJD) were reported to the Spongiform Encephalopathy Advisory Committee. The cases were distinguished by the relatively young age at which the symptoms started (10,11). That age range is now 16 years to 52 years. The duration of the illness is relatively long, averaging approximately 14 months as opposed to the 4 to 5 months in classic CJD. The early symptoms are often psychiatric, and it may be 6 or 7 months before any neurologic signs appear. The characteristic electroencephalogram pattern of sporadic CJD is not seen in vCJD, and pathologic results show florid plaques and extensive cerebellar involvement with multiple PrP deposits. As with BSE and FSE, the neuropathologic appearances are the mainstay of laboratory confirmation. Magnetic resonance imaging scanning and detection of 14-3-3 protein can be helpful. Early evidence indicates that the diagnosis can often be made from tonsil biopsies (12). Otherwise, diagnosis must depend upon brain biopsy or postmortem examination.

When on March 20, 1996, the U.K. government announced the existence of 10 cases of vCJD and the opinion of the Spongiform Encephalopathy Advisory Committee that these were probably related to BSE, three questions immediately arose. The first was, "Is there really any link with BSE?" Additional evidence emerged from the work of Collinge and his colleagues (13) on the analysis of the PrP fragments after protease digestion. The position of the three fragments and the relatively high concentrations of the di-glycosylated form indicated that vCJD was distinct from the previously recognized forms of CJD and that similarities existed between the cases of vCJD and BSE and FSE. In 1997, the first results were published from the classic strain typing experiments initiated during 1996 (14). The characteristics of material from cases of vCJD, in terms of incubation period and lesion profile in RIII mice, were identical to those from cases of BSE and FSE. These observations are confirmed now in C57 black mice. Thus, vCJD can now be regarded as human BSE in the same way that FSE is regarded as feline BSE. The second question was, "What is the route of transmission from cattle to humans?" So far we have no evidence, only a working hypothesis, that transmission was likely from inclusion in the human food chain of tissues that contain the highest concentration of the transmissible agent. The major differences in human exposure to these tissues would have occurred first when sick animals were banned from the human food chain in 1988 and again in 1989 when the specified bovine offals of otherwise healthy animals were removed from the human food chain.

Studies continue in an attempt to answer the third question, "How many vCJD cases will there be in the future?" So far, 26 cases have been diagnosed in the United Kingdom (Table 4) and 1 in France. Incidence has not increased since vCJD was first diagnosed in 1995. If instead of looking at the date of death one looks at the date of onset of the symptoms in the 26 patients, two new cases occurred on average every quarter since 1994. All cases have been methionine homozygotes at codon 129 of the PrP gene. In the general population approximately 40% have such a genotype; 10% are valine homozygotes, and 50% are heterozygotes. An analysis of classic sporadic CJD indicates that 80% of those cases are methionine homozygotes, 10% valine homozygotes, and 10% heterozygotes. It is perhaps not surprising, therefore, that the first cases of vCJD to be seen are methionine homozygotes.

Only one published analysis has predicted the number of future nvCJD cases after constraining the models used to the known and surmised facts at the time (15). The total number of future cases will depend critically on the average incubation period of vCJD. At present, we have no way of determining that; therefore, it remains too early to predict with any accuracy the total number of future cases. It remains possible that the outbreak of vCJD cannot be regarded as a single curve and that the small

Table 4. Creutzfeldt-Jakob disease in the United Kingdom

	Deaths of definite and probable cases						
Year	Referrals	Sporadic	Iatrogenic	Familial	GSS[a]	nvCJD[b]	Total
1994	116	52	1	3	3	0	59
1995	86	34	4	2	3	3	46
1996	133	40	4	2	4	10	60
1997	152	42	6	3	0	10	61
1998[c]	35	3	0	1	0	2	6

[a]Gerstmann-Straussler-Scheinker syndrome.
[b]New variant Creutzfeldt-Jakob disease.
[c]To Apr 30, 1998. Figures released by U.K. Department of Health Jun 1, 1998.

number of cases have occurred in persons who are extremely susceptible for unknown reasons.

The Future

Much has happened already as a consequence of the emergence of BSE in U.K. cattle. The appropriate measures are in place to protect public health and end the BSE epidemic in cattle and other affected species. These measures are more rigorously enforced than ever before. It is difficult to see what could be done to make the BSE epidemic decline more rapidly than it already has, short of slaughtering the entire U.K. herd, which would be unnecessary and impractical. From now the question is likely to be how to withdraw some of the restrictions on U.K. beef and beef products. The exemptions are likely to be herd based (as is the case with Northern Ireland) or date based after the total ban on the use of meat and bone meal in the feed of any farm animals in the United Kingdom in 1996. In terms of the protection of public health, all the necessary measures are in place. Two further concerns remain and are actively under consideration: whether or not BSE exists in the sheep flocks and whether the cases of vCJD in the United Kingdom will be sufficient to generate concern about a second wave of transmission within the human population as a consequence of the use of blood and blood products. With respect to the former, the detection of sheep with scrapie-like diseases in the United Kingdom and the typing of strains from affected animals are being intensified. With respect to blood and blood products, some restrictions on the use of U.K. raw materials for the production of blood products are already in place, and a detailed risk assessment in relation to blood transfusion is awaited.

Sir John Pattison is a virologist and dean of the Medical School, University College London, and Chair, U.K. Spongiform Encephalopathy Advisory Committee.

References

1. Wells GAH, Scott AC, Johnson CI, Gunning RF, Hancock RD, Jeffrey M, et al. A novel progressive spongiform encephalopathy in cattle. Vet Rec 1987;121:419-20.
2. Wilesmith JW, Hoinville LJ, Ryan JBM, Sayers AR. Bovine spongiform encephalopathy: aspects of the clinical picture and analyses of possible changes. Vet Rec 1992;130:197-201.
3. Fraser H, McConnell I, Wells GAH, Dawson M. Transmission of bovine spongiform encephalopathy to mice. Vet Rec 1988;123:472.
4. Dawson M, Wells GAH, Parker BNJ. Preliminary evidence of the experimental transmissibility of bovine spongiform encephalopathy to cattle. Vet Rec 1990;126:112-3.
5. Wilesmith JW, Wells GAH, Cranwell MP, Ryan JBM. Bovine spongiform encephalopathy: epidemiological studies. Vet Rec 1988;123:638-44.
6. Stekel DJ, Nowak MA, Southwood TRE. Prediction of future BSE spread. Nature 1996;381:119.
7. Anderson RM, Donnelly CA, Ferguson NM, Woolhouse MEJ, Watt CJ, Udy HJ, et al. Transmission dynamics and epidemiology of BSE in British cattle. Nature 1996;382:779-88.
8. Donnelly CA, Ghani AC, Ferguson NM, Wilesmith JW, Anderson RM. Analysis of the bovine spongiform encephalopathy study: evidence for direct maternal transmission. Appl Statist 1997;43:321-44.
9. Lawson C, Herd L, editors. BSE Enforcement Bulletin 1998. Ministry of Agriculture, Fisheries and Food; Bull. No. 20; p. 2.
10. Will RG, Ironside JW, Zeidler SN, Cousens SN, Estibeiro K, Alperovitch A, et al. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 1996;347:921-5.
11. Zeidler M, Stewart GE, Barraclough CR, Bateman DE, Bates D, Burn DJ, et al. New variant Creutzfeldt-Jakob disease: neurological features and diagnostic tests. Lancet 1997;350:903-7.
12. Hill AF, Zeidler M, Ironside J, Collinge J. Diagnosis of new variant Creutzfeldt-Jakob diseases by tonsil biopsy. Lancet 1997;349:99-100.
13. Collinge J, Sidle KCL, Meads J, Ironside J, Hill AF. Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. Nature 1996;383:685-90.
14. Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, et al. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature 1997;389:498-501.
15. Cousens SN, Vynnycky E, Zeidler M, Will RG, Smith PG. Predicting the CJD epidemic in humans. Nature 1997;385:197-8.

Explaining the Unexplained in Clinical Infectious Diseases: Looking Forward

Bradley A. Perkins* and David Relman†
*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; and
†Palo Alto VA Medical Center, Palo Alto, California, USA

We examined the need to improve our ability to explain the unexplained in clinical infectious diseases, primarily through improvements in diagnostic technology. Part of the motivation for this effort came from an Emerging Infectious Disease Program (funded by the National Center for Infectious Diseases, Centers for Disease Control and Prevention [CDC]) to conduct surveillance for unexplained deaths and critical illnesses due to possibly infectious causes. This project has found that the number of such patients in the United States is substantial and that a probable causative agent can be identified in only a small fraction of these patients.

John Bartlett, Johns Hopkins University, Baltimore, Maryland, and Sherif Zaki, CDC, Atlanta, Georgia, addressed the current status and offered their perspectives on pneumonia, particularly acute respiratory distress syndrome (ARDS) and hemorrhagic pneumonia, syndromes frequently associated with unexplained critical illness. Greg Kovacs, Stanford University, Stanford, California, and Michael Eisen, Stanford University School of Medicine, Stanford, California, presented possible technologies and approaches to improving diagnostic capabilities—a sensitive biologic detection system (for toxins and host gene expression responses) for diagnosing infectious diseases.

Pneumonia—Evolving Diagnostic Practices

Pneumonia, the most common infectious cause of death in the United States, accounts for approximately 45,000 deaths annually. In large hospital-based studies, no causative agent can be identified in 35% of community-acquired pneumonia cases. In actual practice, this proportion is probably 50% to 75%.

Over the last three decades, the proportion of community-acquired pneumonia cases in which *Streptococcus pneumoniae* was isolated has substantially declined. In the 1970s, the proportion was 62%; in the 1980s, 40%; and in 1991, 18%. Why has the recovery of pneumococci from patients with community-acquired pneumonia changed so dramatically? Have the causes of community-acquired pneumonia changed? Standard "gumshoe" microbiology to isolate pneumococci has taken a devastating hit in the 1990s due to outsourcing of microbiology services or just decreased emphasis on standard microbiology practices (e.g., collection and handling of clinical specimens). Newly recognized agents such as *Legionella pneumophila* may also explain some of the decrease in the proportion of pneumococci isolated.

Recommendations for the evaluation and management of community-acquired pneumonia, developed by the Infectious Disease Society of America, were published in the April issue of Clinical Infectious Diseases. These recommendations detail diagnostic tests as well as inadequacies in diagnostic technologies for several of the common causes of community-acquired pneumonia, including *Chlamydia pneumoniae*, *L. pneumophila*, and *Mycoplasma pneumoniae*.

Pathologic Approach to the Diagnosis of Infectious Causes of Pulmonary Hemorrhage and Acute Respiratory Distress Syndrome

Pathologists should recognize patterns of tissue injury (especially in the lung parenchyma) that react in specific and predictable ways. This approach narrows diagnostic options and focuses testing efforts. Acute lung injury (e.g., diffuse alveolar damage or ARDS) and air space filling patterns (e.g., hemorrhage and pulmonary edema) of lung injury are two important patterns manifesting infectious disease. Examples include diffuse alveolar damage associated with adenovirus infection (smudge cells may be seen); measles (giant cells); respiratory syncytial virus (RSV) infection; influenza infections; Rocky Mountain

spotted fever; typhus; legionnella; mycoplasma; and hemorrhage associated with aspergillosis, mucormycosis, leptospirosis, dengue, yellow fever, Lassa, and Ebola virus infection. The recognition of these patterns (combined with application of special stains, immunohistochemical reagents, and in situ hybridization) is a powerful tool in the diagnosis of unexplained critical infectious diseases.

Two examples of the application of these combined methods to the identification of infectious agents are the 1993 hantavirus epidemic in the southwestern United States and the 1995 leptospirosis epidemic in Nicaragua. In the hantavirus epidemic, healthy young adults contracted fever and rapidly progressive pulmonary disease consistent with ARDS, and many died within days of the onset of illness. Testing for a wide variety of agents was negative. Lung tissue showed interstitial pneumonitis and interalveolar edema; these patterns were consistent with viral pneumonia or toxic change. After serum samples from these patients were found to cross-react with known hantaviruses, antibodies were used to demonstrate hantavirus in the lung, kidney, and muscle tissues. In the leptospirosis epidemic, after heavy rains in northern Nicaragua, a number of persons became ill with fever, headache, muscle aches, hemorrhage, and severe ARDS; no prominent renal or hepatic manifestations were observed. Initial testing focused on hantavirus, dengue, and other viral agents, but results were negative. Pathologic examination of tissue from fatal cases showed pulmonary hemorrhage and diffuse alveolar damage, as well as renal and hepatic changes. In the 1980s, reports of leptospirosis epidemics in Korea and China prompted investigators to develop an immunohistochemical test for leptospirosis; the disease was subsequently found in kidney, liver, and lung specimens of Nicaraguan patients.

Novel Bioassay for Detecting Toxin-Mediated Illness

The impetus for this project has been twofold: military detection of chemical and biological warfare agents and pharmaceutical screening. Cells are cultured on silicon chips, and their response to toxins is monitored in several ways (e.g., action potential for electrically active cells, impedance, and motility). These systems complement other approaches because they allow detection of unknown or unrecognized toxins. A cell monolayer is incredibly responsive because of its diffusion characteristics; this responsiveness can be tuned by selection of cells and through engineering. The use of cocultures can allow diversity in detection and response characteristics. In addition to detecting chemical and biological warfare agents, these systems can screen for antidotes by challenging the system with the toxin and adding a putative antidote. Pharmaceutical companies are interested in using this system for early screening of drug actions on cell physiology.

Chick myocardial cells and NG108 neuroblastoma hybrid cell lines were used to examine the shape and frequency of action potentials. Exposure of these cells to agents with known effects on cell physiology (e.g., epinephrine, verapamil, and tetrodotoxin) causes predictable changes (depending on the interaction of these toxins with transmembrane channels) on the shape of action potential curves when deviation from baseline is used as the internal control on response. Impedance measurement (alteration in electrical current after passage through a cell) can also be used to reflect changes in the cell membrane as a result of exposure to a toxin. The effect of toxins on the cytoskeleton can also be measured by cell motility through impedance. When this technology was first developed, it required approximately 1 m^3 of electronics support, but with silicon chips a laptop computer can now support the operation. A Windows application can handle the data processing, and the technology can be transferred to other laboratories.

Cellular Scouts: Genome-Wide Expression Monitoring of Peripheral Blood to Detect and Characterize Pathogens

Using an easily constructed robot, we have been building DNA microarrays in which each dot represents different open reading frames. In the fully sequenced genome of *Saccharomyces cerevisiae,* there are 6,200 dots or open reading frames. The Human Genome Project has identified approximately 50,000 distinct cDNA sequences, and we have been using microarrays with approximately 15,000 of these. By the end of 1998, we will have all 50,000 genes on a microarray. For these assays we use a control and an experimental sample. From these samples, we isolate polyadentylated RNA by using any of a variety of kits, and then make fluorescently labeled cDNA copies, with each of the two

samples labeled with a different color (e.g., one green and the other red). RNA is degraded to avoid any confounding signals; the samples are mixed and then hybridized to the microarray. The microarray is imaged by using a scanning laser confocal microscope, and through a process of quantitation, the relative representation of every gene in sample 1 versus sample 2 is calculated. These data provide a very high resolution fingerprint of what is going on in any cell(s) of interest. So when a sample from a healthy person is compared with one from an ill person, differences in gene expression should be sufficiently unique to diagnose particular infections.

First, however, we would like to know that cells respond to internal and external stimuli by at least some differences in expression of their genes, that specific stimuli result in distinct gene expression patterns, and that the response to stimuli evolves in a stereotypic temporal manner. Ideally, we would like not only to diagnose a particular infection but also to determine the stage of that infection. Preliminary data from our laboratory support these hypotheses. The patterns of human gene response to different stimuli, including T-cells stimulated with mitogens, cells exposed to DNA damaging agents, and cells infected with polio and cytomegalovirus have distinct DNA expression patterns, or "bar codes," that change over time. Although we have not processed a wide selection of infectious agents, we have evaluated approximately 60 distinct human tumor cell lines using a common control cell line. When we used these data for phylogenetic reconstruction, we found very good clustering with respect to the tissue of origin. Specific signatures are related to central nervous system tumors, kidney tumors, melanomas, and leukemias.

We would like to focus on the use of peripheral blood cells as a sort of infectious disease sensor. There are a number of reasons to believe that this may work. We have data from human lymphocytes harvested from whole blood (where one sample is exposed to interleukin-2 and the other is not) and we can demonstrate many changes in gene expression. To make this approach useful, we will need a broad range of gene array data from persons with known causes of illness.

Malaria: A Reemerging Disease in Africa

Thomas C. Nchinda
World Health Organization, Geneva, Switzerland

A recent upsurge of malaria in endemic-disease areas with explosive epidemics in many parts of Africa is probably caused by many factors, including rapidly spreading resistance to antimalarial drugs, climatic changes, and population movements. In Africa, malaria is caused by *Plasmodium falciparum* and is transmitted by *Anopheles gambiae* complex. Control efforts have been piecemeal and not coordinated. Strategies for control should have a solid research base both for developing antimalarial drugs and vaccines and for better understanding the pathogenesis, vector dynamics, epidemiology, and socioeconomic aspects of the disease. An international collaborative approach is needed to build appropriate research in a national context and to effectively translate research results into practical applications in the field. The Multilateral Initiative for Malaria in Africa can combine all of the above strategies to plan and coordinate partnerships, networking, and innovative approaches between African scientists and their Northern partners.

The global malaria eradication program of the 1950s and 1960s suffered serious setbacks in the early 1970s, and the disease was slowly increasing in areas of Asia and South America where the number of cases had been reduced to low levels. This article discusses malaria and, more specifically, malaria in Africa, where the global eradication program was never started and the disease is reemerging at an alarming and unprecedented rate.

The Disease

Malaria in humans is caused by a protozoon of the genus *Plasmodium* and the four subspecies, *falciparum, vivax, malariae*, and *ovale.* The species that causes the greatest illness and death in Africa is *P. falciparum*. The disease is transmitted by the bites of mosquitoes of the genus *Anopheles,* of which the *Anopheles gambiae* complex (the most efficient) is responsible for the transmission of disease in Africa. Fever is the main symptom of malaria. The most severe manifestations are cerebral malaria (mainly in children and persons without previous immunity), anemia (mainly in children and pregnant women), and kidney and other organ dysfunction (e.g., respiratory distress syndrome). Persons repeatedly exposed to the disease acquire a considerable degree of clinical immunity, which is unstable and disappears after a year away from the endemic-disease environment. Immunity reappears after malarial bouts if the person returns to an endemic-disease zone. Most likely to die of malaria are persons without previous immunity, primarily children or persons from parts of the same country (e.g., high altitudes) where transmission is absent, or persons from more industrialized countries where the disease does not exist.

Why Is Malaria Reemerging?

In the last decade, the prevalence of malaria has been escalating at an alarming rate, especially in Africa. An estimated 300 to 500 million cases each year cause 1.5 to 2.7 million deaths, more than 90% in children under 5 years of age in Africa (1). Malaria has been estimated to cause 2.3% of global disease and 9% of disease in Africa (1); it ranks third among major infectious disease threats in Africa after pneumococcal acute respiratory infections (3.5%) and tuberculosis (TB) (2.8%). Cases in Africa account for approximately 90% of malaria cases in the world (1). Between 1994 and 1996, malaria epidemics in 14 countries of sub-Saharan Africa caused an unacceptably high number of deaths, many in areas previously free of the disease (2).

Address for correspondence: Thomas C. Nchinda, Global Forum for Health Research, World Health Organization, 1211 Geneva 27, Switzerland; fax: 41-22-791-4394; e-mail: nchindat@who.ch.

Adolescents and young adults are now dying of severe forms of the disease. Air travel has brought the threat of the disease to the doorsteps of industrialized countries, with an increasing incidence of imported cases and deaths from malaria by visitors to endemic-disease regions. The estimated annual direct and indirect costs of malaria were US$800 million in 1987 and were expected to exceed US$1.8 billion by 1995 (3).

A number of factors appear to be contributing to the resurgence of malaria: 1) rapid spread of resistance of malaria parasites to chloroquine and the other quinolines; 2) frequent armed conflicts and civil unrest in many countries, forcing large populations to settle under difficult conditions, sometimes in areas of high malaria transmission; 3) migration (for reasons of agriculture, commerce, and trade) of nonimmune populations from nonmalarious and usually high to low parts of the same country where transmission is high; 4) changing rainfall patterns as well as water development projects such as dams and irrigation schemes, which create new mosquito breeding sites; 5) adverse socioeconomic conditions leading to a much reduced health budget and gross inadequacy of funds for drugs; 6) high birth rates leading to a rapid increase in the susceptible population under 5 years of age; and 7) changes in the behavior of the vectors, particularly in biting habits, from indoor to outdoor biters.

What Knowledge Is Needed for Effective Control?

Continental sub-Saharan Africa was never a part of the global malaria eradication program. The severity of the disease, the density and efficiency of *An. gambiae*, the problem of eradicating the disease over such a large land mass with recurrent reinvasions, high costs, and subsequent maintenance must have all contributed to the lack of will to undertake an eradication program. Also, the eradication program period coincided with the colonial and immediate postcolonial period, during which little or no indigenous capacity was available to initiate and sustain malaria eradication. After a period of laissez faire regarding malaria control, these countries have had to face the reemergence of the disease. Important questions about control include the following. Is there enough knowledge about the disease and its determinants? Are there enough tools? Are existing resources adequate? Are governments and populations of endemic-disease countries adequately prepared?

Knowledge About the Disease and Its Determinants

Falciparum malaria is a complex disease with a patchy nonuniform distribution and clinical manifestations that vary from one area to another within an endemic-disease zone, often showing space-time clustering of severe malaria in the community (4). The relationship between fevers, clinical disease, anemia, and cerebral malaria remains the subject of current research. The determinants of severe life-threatening malaria need further elucidation. Present research, focusing on the disease rather than the infection and the dynamics of its transmission, is bringing in new vision about the disease, particularly the immunologic aspects. Persons with asymptomatic parasitemia constitute an important reservoir. The epidemiology of malaria (particularly the relationship between the clinical patterns of the disease in different locations, the pattern of severe disease, and causes of deaths due to malaria) needs future research (5).

Tools for Malaria Control

The present strategy for malaria control, adopted by the Ministerial Conference on Malaria in Amsterdam in 1992, is to prevent death, reduce illness, and decrease social and economic loss due to the disease (6). Its practical implementation requires two main tools: first, drugs for early treatment of the disease, management of severe and complicated cases, and prophylactic use on the most vulnerable population (particularly pregnant women); second, insecticide-treated nets for protection against mosquito bites. Each tool has its own problems in regard to field implementation.

Chloroquine remains the first-line therapy for malaria. However, the alarming increase in resistance in eastern and southern Africa requires that sulfadoxine-pyrimethamine replace chloroquine as the first-line drug. Currently, 20% to 30% of strains are highly resistant (RIII) with in vivo levels of 40% to 60%. Resistance has been spreading westward, attaining levels of 20% to 35% in West Africa. Chloroquine remains the drug of choice in most of sub-Saharan Africa.

Resistance to mefloquine, another first-line drug, developed in the early 1980s, was noticed soon after its introduction and is now almost at

the same level as chloroquine. Sulfadoxine-pyrimethamine (Fansidar, Hoffman la Roche) is the second-line drug in many countries of West and Central Africa, but so much resistance appears to be rising in countries of East Africa that atovaquone/dapsone (Malarone, Glaxo Wellcome) is being developed as a replacement. Intravenous quinine is still the main therapy for cerebral malaria, although resistance is increasing. Development by the African strains of malaria parasites of the pattern of drug resistance now seen in Southeast Asia would be a major disaster.

More research is needed. For example, it is necessary to initiate systematic monitoring of drug resistance in Africa using standardized methods. Drug efficacy studies using in vivo methods have now been standardized by the World Health Organization (WHO)/Regional Office for Africa (AFRO) and carried out in a large number of countries in West, Central, and East Africa. Sentinel sites have also been established for monitoring resistance. No new methods are being developed. The feasibility of using polymerase chain reaction techniques should be explored. Also, management guidelines should be developed concerning when and under what conditions to change the treatment regimen for different levels of resistance at the district, regional, and central level. Development and field testing of inexpensive, effective new malaria drugs are urgently needed to replace present drugs when resistance patterns make them unusable. Drugs developed because of the more serious problem of drug resistance in Asia should be field tested in Africa. The most promising ones, artemisinin and its derivatives artemether, arteether, and artesunate, are being tested for use in cerebral malaria and cases of proven resistance to chloroquine (12); some are already used in some countries.

Research carried out in Dakar (7) demonstrated the efficacy of insecticide-treated nets for reducing infant death; subsequent large-scale multicenter studies in six countries across Africa confirmed this finding (8-10). However, costs of the nets and treatment still inhibit wide-scale use. Ongoing research seeks ways of reducing these costs, such as social marketing, possible involvement of the private sector, cost-effective methods for net treatment, the most appropriate nets, and proper procurement of insecticides and treatment of the nets. Eventually, the long-term effects on natural acquisition of partial immunity to malaria in endemic-disease areas should be evaluated. The old vector-control method of house spraying persists in some countries. The relative merits and cost-effectiveness of house spraying versus the use of treated nets should be evaluated.

The Challenge of Malaria Control to Communities and Governments

The best tools will not necessarily lead to malaria control. African populations have traditional perceptions about disease causation and management. Some diseases are considered suitable for management by western medicine, while others are considered the exclusive domain of local traditional health practitioners. Decisions to seek western medicine for any illness are often considered a last resort. Studies on health-seeking behavior, perceptions of malaria, treatments, and decision making for health care at the household level are crucial to malaria control. Such studies must be accompanied by improved public awareness of the importance of seeking appropriate treatment and complying with recommended regimens.

Management of disease in the household devolves on mothers. Fever remains the most recognized symptom of malaria. Studies are ongoing to determine the proportion of fevers actually due to malaria. Mothers should be taught to recognize the symptoms of malaria, to provide home management, and to know when to refer cases to health centers. Four countries in Africa have developed and tested teaching guides to facilitate home management of malaria (11). Also, guidelines for the management of fever at the periphery have been developed and field tested within the Sick Child Initiative and have been recommended for wide-scale application. Socioeconomic and community studies are needed to understand the extent to which the communities will participate in new malaria control measures. Finally, cost recovery of health care, including costs of drugs (the Bamako Initiative), has been the subject of many recent studies and probably holds the key to health care in rural populations.

Some study results indicate an initial fall in use of services following the introduction of cost-recovery schemes (12). However, a recent study indicates the opposite. Community health workers were trained to administer prepackaged antimalarial drugs only when paid. They also received direct remuneration for their work rather than being supported by the village on a

voluntary basis (13). This plan seems to have increased attendance. This subject needs large-scale multicenter studies.

Governments' Response—Peripheral Health Services

Health service organization, function, and governing policies are important to malaria control. Health policy and systems research have been recently identified as neglected areas of research in need of international effort (1). Many studies are researching different ways to integrate vertical malaria control programs into the general health-care system. Economic evaluation of different interventions is important, and the techniques are continually being refined and improved. They require much local capacity since they tend to be country specific. Studies in this area have now caught up with the current trend favoring decentralization of services, giving more power to the districts. Such studies include ways of improving case management where health services have been decentralized, sustaining effective interventions, and ensuring that drug supply chains function optimally. Extensive research is examining health sector reform on malaria control (12). Health sector reform holds great potential for controlling malaria and all other diseases, as it is the focal point of the central and local governments and the populations themselves. Other needed research includes different health policies, access to health services, and the issues of equity in health care.

Is There a Place for Biomedical Research?

If the emphasis appears to be on epidemiologic and socioeconomic research and studies on health policies and systems, it is because these results have immediate importance to malaria control. The argument is for better use of existing tools. However, tools alone will not provide all the knowledge needed for sustainable malaria control. Recent research by the Wellcome Trust and the National Institutes of Health on sequencing the genome of *P. falciparum* is likely to lead to development of new antimalarial drugs and vaccines. Similarly, DNA technologies are being used to search for candidate molecules for vaccines and new targets for drug development.

The development of a malaria vaccine is still in the laboratories, and no effective vaccine is in sight despite promising candidates. Subsequently, all candidate vaccine trials must be closely linked to studies on how humans acquire immunity and the correlation between protective immunity and immunologic assays. Such studies should be carried out longitudinally in multiple sites where future vaccines will be tested.

On the vector side, studies in Mali have shown that malaria transmission in this Sahel country is maintained by a relay transmission pattern, whereby the three main vectors appear at different times of the year, thus ensuring that vectors are always present (Y. Toure, pers. comm.). More research is in progress concerning the potential of using genetic engineering to make the main malaria vector, *An. gambiae*, refractory to the malaria parasite and releasing this refractory parasite into the wild population to replace the active vectors. Despite potential ethical problems, this approach probably constitutes a long-term future method for interrupting malaria transmission (14). Finally, the much-neglected issue of the pathogenesis of malaria anemia both in children and pregnant women, as well as the link of anemia in pregnancy and HIV/AIDS, needs further study and is likely to be multifactorial.

Mapping malaria transmission intensity using geographic information systems and geographic positioning systems has developed into a Pan-African research collaboration for Mapping the Malaria Risk in Africa, which has received international funding. It plays a major role in time-spatial mapping of malaria across the continent with a strong potential for predicting malaria epidemics (15) and monitoring control.

What Is the Response of the World Health Organization?

WHO developed global and regional strategies for malaria control after the Ministerial Conference on Malaria in Amsterdam in 1992. WHO/AFRO has multiplied efforts to encourage countries to embark seriously on malaria control. A WHO/AFRO Task Force for Malaria comprising a selected sample of malaria control managers, malaria experts from Africa, and technical representatives from bilateral and multilateral agencies funding malaria control in Africa was set up in 1994. This task force has met regularly to provide guidance on malaria control strategies and to recommend criteria for monitoring and evaluation as well as operational research. Some of these agencies have recently increased their malaria control funding directly to some

countries of Africa; others have preferred funding through the regional office.

In addition, the WHO Director General made a generous grant of US$10 million from the WHO regular budget for 1997 for intensified malaria control efforts. Momentum is building, strongly supported by the World Bank, for more concerted efforts at malaria control.

The Way Forward

The Multilateral Initiative on Malaria in Africa (MIM) was created in Dakar in January 1997 from the realization that success in controlling malaria in the future would be greatly enhanced by cooperation and collaborative efforts in research to support strategies for control (5). MIM capitalized on the important 1992 Ministerial Conference, which led to the adoption of a Global Plan of Action for Malaria Control and the World Health Assembly Resolution on this subject (WHA 49.11), urging increased efforts on malaria control. Composed of scientists from Africa and their colleagues from industrialized countries as well as representatives from major funding agencies, MIM plans to facilitate collaboration between governments, research scientists, research funding agencies, and the private (pharmaceutical industry) sector for concerted action through research to combat malaria.

Like other diseases of low-income countries, malaria has been grossly underfunded. From 1990 to 1992, $58 million a year was spent on malaria research, while $56 billion was spent on health research worldwide. Expressed as research investment per death, malaria research receives about US$42 per fatal case, much less than for other diseases such as HIV/AIDS (US$3,270) and asthma (US$789) (3). Rather than the duplicative efforts of the past, MIM encourages a common goal with common research priorities, which should create a greater spirit of cooperation.

Strengthening Research Capability

MIM took a firm stand on indigenous capacity building for malaria research in Africa, an important prerequisite for sustainable research and control of malaria in that continent. Training would be carried out in Africa as far as possible but not exclusively so. Training would be carried out for all health-care workers within the malaria research and control pyramid, including Ministry of Health personnel and those in research institutes and universities, with no exclusion. Flexible training programs would be developed to meet the needs of individual research centers and countries. A good start has been made. Using funds provided late in 1997 to WHO's Tropical Diseases Research Programme, a task force was set up for Malaria Research Capability Strengthening in Africa. The money funded North/South and South/South collaborative research in malaria. All the principal investigators were to be from Africa. Training was central to the projects so that more hands-on and practical research training would be given to trainees, and practical refresher training and technology transfer would be given to experienced scientists.

Research centers also need to be strengthened. Laboratories need refurbishing and equipment and supplies (including computer equipment and software), and vehicles are needed for field studies. Suitable research careers should be created to encourage the best scientists to remain in research.

Because scientific isolation constitutes a major constraint to African scientists, communication facilities need urgent attention. One of MIM's highest priorities is to enhance the capacity of African scientists to communicate electronically with each other and with colleagues around the world and to access needed scientific information from local and remote libraries and the Internet. NIH's National Library of Medicine is playing a lead role in this critical area.

The Future

Malaria is an important social, economic, and developmental problem affecting individuals, families, communities, and countries. The best chance for successfully combating the disease requires a collaboration particularly of those responsible for control and research. Such collaboration, particularly between South and North, is being actively developed, and MIM presents itself as a worthwhile initiative (16). Important factors are 1) placing the control strategy on a strong research base, 2) strong international collaboration, and 3) sustained government support.

Smallpox was eradicated because of the development of freeze-dried vaccine, the development of the multiple-use nozzle jet injector and bifurcated needle, and the replacement of mass vaccination by selective vaccination, coupled with a strong international effort. Onchocercia-

sis is being controlled because research results were immediately applied to control. Translating research findings into control methods has also been pursued for Chagas disease and leprosy. Concerted action between the research and control communities is needed to ensure that malaria follows the same path. MIM strongly advocates this approach. Research must be a constant feature throughout the entire process of malaria control.

Dr. Nchinda is senior health specialist at the Global Forum for Health Research based at the World Health Organization headquarters in Geneva. His research interests are in tropical diseases research and control, particularly malaria, training and utilization of health personnel, research capacity strengthening, health services research, and organization of community health services.

References

1. World Health Organization. Investing in health research for development. Report of the Ad Hoc Committee on Health Research Relating to Future Intervention Options. Geneva:The Organization; 1996. Report No.: TDR/Gen/96.1
2. Harare declaration on malaria prevention and control in the context of African economic recovery and development. In: Proceeding of the 33rd Ordinary Session of the Assembly of Heads of State and Government, Organization of African Unity; 1997 2-4 June; Harare, Zimbabwe.
3. Anderson J, MacLean M, Davies C. Malaria research: an audit of international activity. Prism Report No. 7, The Wellcome Trust; 1996.
4. Snow RW, Schellenberg JR, Peshu N, Foster D, Newton CR, Witstanley PA, et al. Periodicity and space-time clustering of severe childhood malaria on the coast of Kenya. Trans R Soc Trop Med Hyg 1993;87:386-90.
5. Final report: International Conference on Malaria in Africa, 6-9 January 1997, Dakar, Senegal. [document online] Available from: url: http://www.niaid.nih/dmid/malafr/.
6. World Health Organization. Control of Tropical Diseases: 1. Progress Report. Geneva: The Organization, Division of Control of Tropical Diseases; 1994. Report No.: CTD/MIP/94.4
7. Alonso PL, Lindsay SW, Armstrong JRM, Conteh M, Hill AG, David PH, et al. The effect of insecticide-treated bed nets on mortality of Gambian children. Lancet 1991;337:1499-502.
8. Nevill CG, Some ES, Mung'ala VO, Mutemi W, New L, Marsh K, et al. Insecticide treated bednets reduce mortality and severe morbidity among children in the Kenyan Coast. Trop Med Int Health 1996;1:139-46.
9. Binka FN, Kubaje A, Adjuik M, Williams LA, Lengeler C, Maude CH, et al. Impact of Permethrine impregnated bednets on child mortality in Kassena-Nankana district of Ghana: a randomized controlled trial. Trop Med Int Health 1996;1:147-54.
10. Lengeler C, Cattani J, de Savigny D, editors. Net gain: a new method for preventing malaria deaths. Ottawa, Canada: International Development Centre/World Health Organization; 1996.
11. World Health Organization. Toward healthy women counseling guide: ideas from the gender and health research group. Geneva: The Organization, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). Report No.: TDR/GEN/95.1
12. World Health Organization. Tropical diseases research: progress 1995-96. 13th Programme Report. Geneva: The Organization, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases(TDR), Geneva.
13. Pagnoni F, Convelbo N, Tiendrebeago H, Cousens S, Esposito F. A community-based programme to provide prompt and adequate treatment of presumptive malaria in children. Trans R Soc Trop Med Hyg 1997;91:512-7.
14. Carlson J, Olson K, Higgs S, Beaty B. Molecular genetic manipulation of mosquito vectors. Annu Rev Entomol 1995;40:359-88.
15. Omumbo J, Ouma J, Rapouda B, Craig M, le Sueur D, Snow RW. Mapping malaria transmission intensity using geographic information systems: an example from Kenya. Ann Trop Med Parasitol. In press 1998.
16. Mons B, Klasen E, van Kessel R, Nchinda T. Partnerships between South and North crystallizes around malaria. Science 1998;279:498-9.

Vaccine-Preventable Diseases

Alison C. Mawle
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

The panel addressed four main areas of vaccine development and use: diseases of public health importance for which no vaccine is available; diseases for which an existing licensed vaccine is not optimal and alternative vaccines are under development; diseases for which a vaccine exists but is not being used optimally; and diseases requiring vaccines of specialized or limited use, such as those needed for controlling outbreaks or for military use. The specific diseases chosen to illustrate each area were malaria, influenza, meningitis, and filovirus infections (Ebola and Marburg), respectively.

Lee Hall, National Institute of Allergy and Infectious Diseases, addressed the basic challenges in developing a malaria vaccine. The historical norm of vaccine development has been an empirical process; in contrast, modern vaccines take advantage of basic knowledge of the organism and of the immune response to it. In vaccine development, certain elements are prerequisite: demonstrable protective immunity and intimate knowledge of the organism's life cycle, including the DNA sequence. Vaccine development has three potential goals: prevent infection, prevent disease, and prevent transmission. A successful vaccine may address any or all these areas; for malaria, a vaccine able to perform any of these would have a significant impact. Vaccine development, often thought of as a flow, is in fact an iterative process; it addresses scientific, technical, manufacturing, and clinical issues and is affected by economic, political, and social issues usually outside the scientific sphere of influence. Several downstream gaps in vaccine development include resource limitation, lack of standardization, and problems in clinical and industrial interest.

Claude Hannoun, Institut Pasteur, addressed problems in influenza vaccine development. Influenza, the quintessential emerging infectious disease, needs a new vaccine each year to protect against the predominant strains. The disease poses additional challenges; one is the need for vaccine against a potential pandemic strain, particularly a pandemic strain whose epidemiologic characteristics are different from those of usual strains (e.g., the 1918 strain killed young adults). Vaccine production problems pose another challenge. Identifying an appropriate seed strain can delay initiation of production for 4 to 6 months after the need for a vaccine has been identified, and growing sufficient quantities of vaccine is difficult. The issue of an appropriate vaccine regimen (one dose or two) was also addressed. The many vaccination issues involved in a pandemic situation make the adoption of a credible pandemic plan imperative. The emergence of the H5N1 strain in Hong Kong has underlined this imperative.

Brad Perkins, Centers for Disease Control and Prevention, presented the challenge of the African meningitis belt, where periodic large epidemics affect approximately 1% of the population with a 10% death rate. Since the current vaccine does not confer lasting protection, prediction of these epidemics can trigger vaccination campaigns to prevent deaths. A model using an epidemic threshold of 15 cases per 100,000 demonstrated potential lives saved. Obstacles to the use of the vaccine include inadequate surveillance, high cost of the vaccine, inadequate delivery systems, and inadequate vaccine supply.

Vaccines for agents such as Marburg and Ebola were discussed by Alan Schmaljohn, U.S. Army Medical Research Institute of Infectious Diseases. Limited-use vaccines do not have a global market but are potentially important against the threat of biological weapons or epidemics. These vaccines have unique problems, such as inadequate efficacy testing (since there is no disease-endemic area) and high production costs (since there is no target population).

All the presentations addressed public policy issues: who will use the vaccine and under what circumstances, what is the time frame for vaccine development (short-term versus long-term), what is the cost of a vaccine (who bears the brunt of development costs), how are these costs recouped, and what is the role of partnerships in determining vaccine need and use.

Travelers' Health

Martin Cetron,* Jay Keystone,† David Shlim,‡ and Robert Steffen§
*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; †The Toronto Hospital, Toronto, Ontario, Canada; ‡The Canadian International Water and Energy Consultants Clinic Travel Medicine Center, Kathmandu, Nepal; §University of Zurich and World Health Organization Collaborating Centre for Travelers' Health, Zurich, Switzerland

Over the last century and a half, the world's population has grown from one billion to almost six billion, and the time required to circumnavigate the globe has decreased from 365 days to fewer than 3. Currently, 1.4 million persons travel internationally by air every day. The speed and volume of international travel are cited by the Institute of Medicine as principal factors contributing to the global emergence of infectious diseases. This panel assessed the effect of travel on emerging infections from the perspectives of both industrialized (Jay Keystone) and developing countries (David Shlim), discussed the role of vaccination in preventing travel-related infections (Robert Steffen), and explored unique health issues associated with travel into space (David Shlim and Thomas Marshburn, National Aeronautics and Space Administration [NASA]).

Travel and the Spread of Emerging Infectious Diseases

In the past decade, international travel has grown dramatically to the point where more than 500 million travelers cross international borders annually by commercial aircraft alone. Mass migrations of refugees, workers, and displaced persons have led to a steady growth of urban centers at the expense of rural areas. These population movements have been ideal conduits for the global spread of new and reemerging infectious diseases. Virtually any place in the world can be reached within 36 hours, less than the incubation period for most infectious diseases. Infectious agents can spread from person to person directly or to vectors at the traveler's destination. Vehicles of human transport, such as aircraft and ships, also transport the infectious agent and its vector. In addition, mass migrations have facilitated the rapid and immediate spread of communicable disease among refugees and displaced persons.

If travelers are responsible for the spread of infectious agents, they are also ideal sentinels for the arrival of an infectious agent in a new community. In 1969, the first documented outbreak of Lassa fever was noted among American missionaries in Lagos, Nigeria. In 1992, two Peace Corps volunteers contracted neuroschistosomiasis; a subsequent investigation confirmed that Lake Malawi was an important source for the transmission of *Schistosoma haematobium*. More recently, chloroquine-resistant *Plasmodium vivax* was documented for the first time in North Africa in U.S. Army troops returning from Somalia.

These and many other examples show that travel is instrumental in the spread of new and reemerging infectious diseases. As international travel grows by more than 10% per year and major population shifts continue because of political, economic, and social instability, public health agencies will have to focus their infectious disease surveillance programs on travelers, migrants, and the vehicles of transport. Recognition that travelers are important sentinels for emerging infectious diseases has stimulated the International Society of Travel Medicine and the Centers for Disease Control and Prevention to carry out a joint surveillance project, Geosentinel, in 22 travel clinics around the world.

Vaccinating Travelers

Vaccination is only one of several strategies of prophylaxis in travel medicine. Selection of immunizations should be based on requirements and on risk for infection. According to the International Health Regulations, many countries require proof of yellow fever vaccination on the International Certificate of Vaccination. The revised International Health Regulations, to be submitted to the World Health Assembly next year, will likely maintain this requirement. Addition-

ally, a few countries still require proof of vaccination against cholera, diphtheria, and meningococcal disease under specific circumstances.

Recommended immunizations often are more important for travelers' health than the required or routine ones. The most frequent vaccine-preventable infection in travelers to developing countries is hepatitis A, with an average incidence rate of 0.3% per month in susceptible persons; in high-risk backpackers or foreign-aid volunteers, this rate is 2.0%. The World Health Organization and many national expert groups recommend that all travelers visiting developing countries be immunized against hepatitis A. Several other immunizations are recommended for special risk groups. Hepatitis B is a problem among expatriates, particularly if they live close to the native population or engage in high-risk behavior (e.g., unprotected casual sex or intravenous drug use); rabies is also a problem, with approximately 0.2% to 0.4% of long-term residents bitten by animals each month—travelers to selected geographic regions with high endemicity (Southeast Asia) and exposure risk profiles warrant rabies vaccination; typhoid fever (often diagnosed among travelers to the Indian subcontinent, North and West Africa—except Tunisia—and Peru) poses a higher risk in long-term residents and in those who consume food and beverages prepared under substandard hygienic conditions (estimated incidence 0.03% to 0.003% per month); anecdotally, meningococcal disease, Japanese encephalitis, and tick-borne encephalitis have been reported in travelers (monthly incidence less than one per million travelers); immunizations against cholera and tuberculosis are only rarely recommended.

In the not-too-distant future, travelers will be offered a variety of oral vaccines against pathogens causing travelers' diarrhea, including all enterotoxigenic *Escherichia coli* as well as *Campylobacter* and *Shigella*. Additionally, immunization against Lyme disease and dengue fever will be available within the next years; vaccines against malaria and AIDS, the infections causing most death in travelers, are much further in the future.

Emerging Diseases in a Developing Country: Observations from a Travel Medicine Clinic

The CIWEC Clinic Travel Medicine Center was the world's first self-supporting destination travel medicine clinic. Started in 1982 to provide western medical care for expatriates and tourists in Nepal, the clinic has set a standard for investigating disease risks for tourists in a developing country.

Nepal (population 22 million, resident foreign population 3,000) has an annual influx of approximately 250,000 non-Indian tourists a year. The Travel Medicine Center is situated in the capital, Kathmandu, through which 90% of tourists enter and exit the country; a high percentage of expatriates and tourists use the clinic for medical care, creating a favorable situation for health-risk studies.

Emerging diseases can be divided into three categories: 1) truly new diseases, such as diarrhea caused by *Cyclospora*; 2) newly noted or increasing diseases, such as meningococcal meningitis and Japanese encephalitis; and 3) unexplained illnesses, such as a cluster of cases of sudden death among young trekkers in teahouses. The Travel Medicine Center has made it possible to observe all three categories. Most of the above conditions would likely have remained unnoticed and unreported were it not for the presence of a clinic focused on the health risks of foreigners in Nepal.

The value of a research-oriented travel medicine center in Kathmandu extends beyond the borders of Nepal. Research into diarrheal disease, hepatitis, typhoid fever, rabies immunoprophylaxis, and altitude illness has been relevant to other locations. Specific disease risks for travelers to Nepal have been carefully defined. Clinics in other developing countries should be identified, and similar data gathering and observations should be encouraged.

Travel Medicine in the 21st Century: NASA Perspectives

Space travel entails unique circumstances that influence the health of astronauts, most notably, the limitations imposed by zero-gravity environments. The impact of weightlessness on human physiology is substantial and affects various body systems: neurologic, psychologic, vestibular, cardiovascular, musculoskeletal, endocrine, hematologic, and immunologic. Prominent sequelae of prolonged weightlessness may include profound motor weakness and 1% to 2% loss of mineralized bone for each month in orbit; these effects can be countered by maintaining a vigorous daily exercise program while in orbit. In addition, recent studies of the immune system have

documented a decrease in cell-mediated immunity among astronauts spending extended time in space.

Environmental stressors (prolonged exposure to increased temperatures, high-velocity travel [17,000 miles per hour], and prolonged confinement to limited physical space) may also affect the health of space travelers. The space environment also challenges the growth of microorganisms. Increased antimicrobial resistance has been noted among *E. coli* and *Staphylococcus aureus* bacteria in space, and fungal overgrowth can be a problem because of changes in humidity and pockets of increased condensation aboard spacecraft and space stations.

The primary strategies used by NASA to prevent health-related mishaps associated with space travel include selecting exceptionally fit, healthy crew members and imposing preflight protective quarantine. Access to the astronauts is restricted to family members beginning 10 days before departure, and a strict 7-day prelaunch quarantine is imposed.

Thus far, infectious diseases have rarely been an important problem at NASA; launch delays due to infectious diseases have occurred in only two Apollo flights and one shuttle mission. One instance of infectious prostatitis in an astronaut on the MIR space station resulted in the premature termination of his stay. Recent forays into long-term habitation in space such as those aboard MIR (3 to 6 months) will provide new insights into the feasibility of extended space travel and human colonization beyond our planet.

Global Tuberculosis Challenges

Kenneth G. Castro
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Mario Raviglione, World Health Organization (WHO), described the epidemiology of global tuberculosis (TB) using surveillance data available to WHO from 212 countries and data from a recent survey of antituberculosis drug resistance in 32 countries. Countries were categorized according to the degree of TB directly observed treatment strategy (DOTS) implementation. Performance of national TB programs was assessed by using treatment outcome indicators. In 1996, 3.8 million TB cases (887,731 from areas with DOTS) were reported to WHO. In developing countries, the bulk of TB cases are found in all age groups of native-born populations, while in many industrialized countries a large proportion of TB cases are in foreign-born residents. In countries of the former Soviet Union, TB cases and deaths have doubled in just a few years. Drug resistance and HIV infection related to TB are found only in limited foci. Acquired multidrug-resistant TB (MDRTB) was present in 27% to 54% of culture-positive TB cases from the Baltic countries and Russia. The effect of the HIV epidemic on TB has been major in Africa, where HIV seroprevalence among TB cases is 50% to 70% and TB case notifications have at times tripled. Countries with inadequate TB control are particularly exposed to the consequences of both epidemics. In Southeast Asia, cases are increasing, and MDRTB is common in Thailand, China, and Vietnam.

One hundred eighty-one countries and territories (97% of the global population) have reported on the status of DOTS to WHO. Of these, 96 implemented DOTS (63 countrywide). Approximately 32% of the global population lives in areas where DOTS is available. Twenty countries have adopted DOTS since the 1996 survey, and an additional 9% of the global population were benefitting from it. However, most of these new countries had small populations; DOTS was only slowly implemented in countries with high TB prevalence. In areas that used DOTS, treatment outcome evaluation remains high (94%), and treatment success rose from 76% in 1994 to 78% in 1995. In areas that did not use DOTS, 45% of reported TB cases were not evaluated, and treatment success remained low (45%). Among the 22 countries with the highest TB prevalence, six showed progress in DOTS implementation, seven showed little progress, and nine did not implement DOTS. In summary, TB remains an important public health problem in many areas of the world where DOTS has not been implemented. Because treatment outcomes were better in countries where DOTS has been used, the strategy needs to be expanded rapidly and new tools to facilitate its implementation need to be developed.

Barry Bloom, Howard Hughes Medical Institute, described advances in TB vaccine development. The available bacillus Calmette-Guerin (BCG) vaccine has a demonstrated efficacy ranging from no protection to 80% protection. Most recently, a meta analysis estimated that the overall efficacy of BCG is 50%. Because of case reports of disseminated BCG infection, this vaccine is contraindicated in immunocompromised persons, and safer and more efficacious vaccines are clearly needed. Identifying such new vaccines for use in humans will take several years. However, recent advances in this area provide optimism. Recent research activities have improved our understanding of the immunologic response to *Mycobacterium tuberculosis* and identified major protein antigens of *M. tuberculosis* and recombinant BCG forms that overexpress protective antigens. Additionally, avirulent auxotrophic mutants of both BCG and *M. tuberculosis* have been used in animal models. The recent sequencing of the *M. tuberculosis* genome has presented additional opportunities to identify virulence factors that could be deleted and other target sites that could be genetically engineered. DNA constituents can also be used to develop candidate vaccines. In animal studies, subunit vaccines consisting of pooled mycobacterial culture-filtrate proteins have been protective. Auxotrophic mutants may also prove useful in immuno-

compromised patients, as may recombinant BCG vaccines that secrete host-specific cytokines. Clearly, a major national effort is required for TB vaccine development, recommendations on policies and priorities, and cooperation between the government and private sector in these efforts.

Christopher Murray, Harvard School of Public Health, described a mathematical model developed to forecast the future impact of improvements in TB prevention and control. Specifically, this model projected the number of TB cases and deaths averted through the year 2050. Different scenarios were simulated to project the effect of adding TB vaccines to existing interventions. Six specific scenarios assessed the effect of vaccines (with efficacy levels of 20%, 50%, and 80%) to protect from *M. tuberculosis* infection, as well as the effect of vaccines of the same levels of efficacy to protect latently infected persons from "breakdown" to active TB. Although a TB infection vaccine with 20% efficacy would prevent more than 30 million TB cases, the best protection is obtained from a TB breakdown vaccine with 80% efficacy, which would prevent almost 130 million TB cases. The breakdown vaccine could be used in the large number of persons with latent *M. tuberculosis* infection, now estimated at almost one third of the world's population. Such anticipated gains justify the effort to develop better TB vaccines.

Denise Garrett, Centers for Disease Control and Prevention, presented the findings of recent tuberculin skin test studies regarding the risk for TB among health-care workers in Thailand and Brazil. In Thailand, 35% of 911 health-care workers had a positive test at the 15-mm cutoff, while 69% were positive at the 10-mm cutoff. BCG scar was associated with positive skin test reaction at the lower cutoff value, but not at the 15-mm cutoff. Additionally, tuberculin skin test reactivity correlated with more than 1 year's employment as a health-care worker, and with occasional or frequent patient contact. In Brazil, 48% of 524 health-care workers had a reaction of 10 mm, while 26% had a reaction of 15 mm. As in Thailand, BCG scar correlated only with 10-mm skin test reactivity but not with 15-mm. Workers with occasional or frequent patient contact were also more likely to have a positive tuberculin test. Factors that appear to contribute to the risk for TB in these workers include delays in the diagnosis of TB, inadequate isolation practices, and lack of personal protection during high-risk procedures. Important measures to reduce the risk for TB in these settings include increasing the awareness and training of health-care workers about the risk for TB, improving the ability to establish the diagnosis of TB by smear microscopy, reducing the need for hospitalization of TB patients, considering the establishment of chest clinics at separate times or in separate areas, and improving ventilation by keeping windows open. Laboratories should contain all needed safety features.

Blood Safety

Mary E. Chamberland,* Jay Epstein,† Roger Y. Dodd,‡ David Persing,§ Robert G. Will,¶ Alfred DeMaria, Jr.,# Jean C. Emmanuel, Beatrice Pierce,†† and Rima Khabbaz***

*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; †Food and Drug Administration, Washington, D.C., USA; ‡American Red Cross, Washington, D.C., USA; §Western General Hospital, Edinburgh, Scotland; ¶The Mayo Clinic, Rochester, Minnesota, USA; #Massachusetts Department of Public Health, Boston, Masschusetts, USA; **World Health Organization, Geneva, Switzerland; and ††National Hemophilia Foundation, New York, New York, USA

The blood supply in industrialized countries is safer than ever. However, blood (a human tissue) is a natural vehicle for transmission of infectious agents. In recent years, numerous pathogens have emerged in the United States and worldwide with the potential to affect the safety of the blood supply.

International Movement of Infectious Agents

Movement of transfusable blood and blood components between countries is relatively uncommon. However, infectious agents can cross international borders through immigration or travel. For example, malaria is an important problem in much of the world, with an estimated 300 to 500 million cases per year. On average, 1,000 cases are reported each year in the United States, most in persons who travel to malaria-endemic areas. Only a small number of cases (approximately three per year) are transmitted by exposure to infected blood products. Current measures (which temporarily defer donors with a history of origin in a malarious country, clinical malaria, or travel to malaria-endemic areas) appear to be effective. Similarly, Chagas disease, a vector-borne disease caused by the parasite *Trypanosoma cruzi,* is endemic in parts of Central and South America and Mexico, where infected persons can transmit the disease through transfusion. The immigration of millions of persons from *T. cruzi*–endemic areas and increased international travel have raised concerns about the potential for transfusion-transmitted Chagas disease. Five cases of *T. cruzi* transmission from transfusions have been reported in North America. Recent seroprevalence studies showed that approximately 0.1% of blood donors likely to have been born in or have traveled to disease-endemic countries were seropositive for *T. cruzi*. Moreover, American Red Cross studies of recipients of *T. cruzi*–seropositive blood and blood products showed no evidence of transmission. Finally, variant forms of recognized pathogens can potentially affect the safety of the blood supply. Current serologic tests do not consistently detect HIV-1 group O infections, which are common in some West and Central African countries but very rare (two cases) in the United States. Efforts are under way to modify existing serologic tests to improve detection of group O strains without compromising sensitivity for the predominant group M viruses. As an interim measure, the Food and Drug Administration has recommended that donors at increased risk for HIV-1 group O on the basis of residence and risk exposure be deferred from donating blood or plasma.

Creutzfeldt-Jakob Disease and Blood Safety

Risk for transmission by transfusion is poorly characterized for a number of emerging agents. One of these is Creutzfeldt-Jakob disease (CJD), a rare, fatal neurodegenerative disease believed to be caused by an abnormal form of prion protein. CJD has been transmitted iatrogenically through human pituitary-derived growth hormones, human dura mater grafts, corneal transplants, and contaminated surface electroencephalogram electrodes and neurosurgical instruments. Incubation was as long as 30 years in some cases. Concerns regarding bloodborne transmission of the CJD agent derive primarily from laboratory studies, including animal models, which suggest such a potential. However, no proven cases of blood

transmission are reported in humans, and accumulating epidemiologic information (surveillance, follow-up of recipients of blood from donors who subsequently developed CJD, and case-control data) indicates that the risk (if any) for transmission of CJD by blood products is extremely small. At present, CJD is considered a remote, theoretical risk.

In March 1996, health officials in the United Kingdom announced that the agent responsible for the decade-old bovine spongiform encephalopathy epizootic might have spread to humans. As of March 1998, 24 persons have been reported with this apparently new variant form of CJD (nvCJD). The possibility of nvCJD transmission through the blood supply has been debated. Currently, this risk is theoretical. However, because the infectious agent of nvCJD is new in humans, it may present risks that differ from those of classic CJD. In addition, important differences have been noted in the two diseases. For example, human spleen and tonsil tissues contain abnormal prion protein in nvCJD but not in classic CJD. In view of this uncertainty, U.K. health officials have undertaken a conservative approach, including 1) withdrawal of blood products donated by persons subsequently confirmed or strongly suspected to have nvCJD; 2) discontinuation of the use of British plasma in plasma-derived products; and 3) consideration of leukodepletion of all blood donations, in view of experimental studies suggesting that B lymphocytes may play a role in the development of scrapie.

Tick-Borne Agents and Transfusion Risk

In the United States, the most commonly reported transfusion-associated tickborne infection is babesiosis. At least 21 reported cases of babesiosis, mostly caused by *Babesia microti* but also by the more recently recognized WA1-type *Babesia* parasite, have been transmitted by transfusion of blood from asymptomatic infected blood donors. With the expansion of deer populations (natural host of *B. microti*) in the northeastern United States, the incidence of transfusion-transmitted babesiosis may increase. The tick vector and animal reservoir of the *Babesia* more recently found in the northwestern and western United States remain to be defined. The parasite survives blood-banking conditions and is transmissible by transfusion of red blood cells and platelet concentrates. Although babesiosis classically causes a febrile illness with hemolytic anemia, infection can also cause chronic asymptomatic or mildly symptomatic parasitemia. Recent studies suggest that untreated persons have evidence of *B. microti* DNA for longer periods, despite mild or absent symptoms, and may transmit infection for months or possibly longer. The potential for transmission of other tick-borne agents is unclear. Like babesiosis, Lyme disease or ehrlichiosis (caused by an obligate intracellular gram-negative rickettsia) may be asymptomatic or mildly symptomatic; spirochetemia or rickettsemia can precede prodromal symptoms by 24 to 72 hours, making transmission by transfusion a possibility. One case of transfusion-transmitted Rocky Mountain spotted fever has been reported.

Summary

Since blood is a biologic product, it is unlikely that the risk for transfusion-transmitted infection will ever be reduced to zero. The approach to emerging infections associated with transfusion of blood and blood products includes assessing the transmissibility of the agent by this route; developing effective prevention strategies, including screening tests and donor deferral policies; improving viral and bacterial inactivation procedures; and surveillance for known, as well as emerging and poorly characterized, transfusion-transmitted agents. Vigilance is needed to help ensure proper balance between safety and the availability of blood. Finally, vigilance needs to extend to the developing world, where the basic elements to reduce transfusion-transmitted infections and systems of disease surveillance are often not available.

Confronting Emerging Infections: Lessons from the Smallpox Eradication Campaign

William H. Foege
Emory University, Atlanta, Georgia, USA

Ralph Waldo Emerson in 1860 said, "We learn geology the day after the earthquake." Traditionally, the world learns prevention the day after the epidemic. Today, we have the responsibility of preparing for the prevention and control not only of known but also unknown conditions. Eradication is a focused field exercise in which approaches have been tested and from which public health lessons can be learned.

Lessons from Eradication

Calculated Risks

It is clear, in retrospect, that we didn't know how to eradicate smallpox when the eradication effort began. Thirty years ago, in the middle of the smallpox campaign in West and Central Africa (charged with ending transmission in 20 countries in 5 years), we tried a new strategy, converting from mass vaccination to surveillance and containment. Although we were 1 1/2 years into the campaign when the strategy shift occurred, we still reached the goal of zero cases on time and under budget. The lesson is that we do not have the luxury of waiting until we know everything before doing something. We are always called upon to make decisions with insufficient information and make corrections midcourse.

Interdependence

Disease eradication campaigns illustrate the value of working as global citizens rather than as a collection of national programs. First promoted by the Soviet Union in 1958, smallpox eradication did not get the approval of the World Health Assembly until 8 years later in 1966, when it became a joint proposal of the Soviet Union and the United States. If we could form this alliance during the cold war, how many alliances can we form now? No country alone can prevent or control emerging infections.

Knowledge

We did not understand the limitations of smallpox transmission; we knew nothing about fetishes or the role of nomads. As organisms, the environment, people, and tools change, programs must change. Appropriate response requires good epidemiologic analysis. The epidemiology, in turn, can be no better than the facts assembled. Knowledge is dependent on the information system; in public health, the surveillance system forms the foundation of knowledge.

Vision

With eradication, the vision is no more cases. With emerging infections, the vision is rapid, appropriate, effective response, being prepared to protect the world because you are ready to act.

Performance

With eradication, to get global support, we must demonstrate that a disease can be eliminated from a geographic area. With emerging infections, the value of surveillance (for making decisions, for deciding on interventions) must be demonstrated.

Humility

With all our experience, we have not gone far on the road to eradicating disease. This knowledge keeps us humble. We have trouble outthinking a virus. Even smallpox humbled us until the very end. That virus seemed to have a better understanding of nature, human behavior, and ways to achieve immortality than the entire smallpox eradication team. The emergence and reemergence of infections must be approached with humility.

Enemies

Some anthropologists think conflict is not only inevitable but needed. Will Durant once

doubted the world could ever combine forces without fear of an alien invasion. Perhaps disease could be used as a surrogate enemy? Emerging infections are a powerful common enemy well suited as a global challenge.

Focused Energy

Energy focused on a specific end can also build infrastructure. Energy focused on eradication improved infrastructure. Surveillance, logistic systems, evaluation, field teams, and cluster sampling are concepts used during eradication that are now part of primary health care.

Optimism

The pessimists and cynics were not just wrong with smallpox; they were harmful. They diverted attention, generated doubts in those who could provide resources, invented problems far beyond the vast array of existing ones. Even though negative news can be of value, their usefulness is limited. Large problems should be approached with optimism.

Conclusions

Nine hundred years ago, building inventions converged and reached a peak, leading builders and architects of the time to try ever bolder structures. Cathedrals were built that in turn led to new innovations. For several hundred years Europe was rewarded not only with cathedrals but also with better building techniques for all structures. The infrastructure changed. Historians, in a thousand years, will look on the public health cathedrals that resulted from better building materials in a period of 75 years, from the mid-20th century until the early 21st century. The control and eradication of infectious diseases that once caused great trepidation produced better diagnostic systems, treatment, and vaccines, the elements with which to strengthen and improve the public health system and confront new disease challenges.

The Guinea Worm Eradication Effort: Lessons for the Future

Donald R. Hopkins
The Carter Center, Atlanta, Georgia, USA

The dracunculiasis (Guinea worm) eradication campaign has specific implications for efforts to control other emerging infectious diseases. Guinea worm, a painful disfiguring disease, affects primarily adults, who often become ill in very large numbers (usually 30% or more of a village's population) during the planting or harvest season. The disease used to be transmitted in parts of Asia and in Africa in open standing stagnant water. The intermediate host of the parasite, the copepod, contains the larva of the worm in such open drinking water; these organisms are barely visible in a glass of drinking water held up to the light. Thirteen years ago, the disease was still endemic in parts of the Indian subcontinent, a small part of Pakistan and India, Yemen, and the band of countries across Africa from east to west.

The Guinea Worm Eradication Campaign

Several interventions have been used to end transmission of Guinea worm disease: health education (teaching people to filter their water through a finely woven cloth and not to enter water when they or their neighbors are infectious), safe drinking water from such sources as underground borehole wells, and vector control (using Abate).

The Guinea worm campaign, like other campaigns in the past, has illustrated the importance of political mobilization, including the mobilization of national leaders. For example, General Amadou Toumani Touré (a charismatic former head of state of the Republic of Mali), with the encouragement of President Carter in 1992, made the eradication of Guinea worm disease in Mali and in the nine other French-speaking countries in West Africa his personal mission.

The campaign faces a problem common to many other efforts to control infectious diseases in the industrialized and the developing world: underreporting. For example, in Ghana, as in Nigeria 10 years ago, and in many other countries, only three or four thousand cases of Guinea worm disease were officially reported; but the actual numbers were much higher. In 1989 when Ghana conducted a nationwide village-by-village search, almost 180,000 cases were found. Sudan began its eradication program late because of civil war. In 1996 and 1997, an apparent decline of cases in Sudan was due to less complete reporting because of increased fighting in 1997.

The Campaign's Implications for Other Diseases

The Guinea worm campaign has demonstrated very graphically the possibility of village-based monthly reporting in Africa. In Ghana and Nigeria at the beginning of this program 10 years ago, such reporting did not exist. Now in those countries, more than 6,000 disease-endemic villages have volunteers who report to the national capital monthly.

The Guinea worm campaign has also demonstrated very clearly the efficacy of health education. In the beginning, many were skeptical because Guinea worm could not be combated with a vaccine, and eradication efforts had to rely on behavior change. However, behavior has changed. While we have been successful in helping to bring safe drinking water to many disease-endemic villages, the fastest and most effective intervention has been health education, which helped people understand where the parasite was coming from, how they were being infected, and the importance of using cloth filters to protect themselves and their families.

The campaign has underscored the potential of local volunteers. Many years ago in the Americas, village volunteers were used as part of malaria control efforts. The onchocerciasis control program in Africa is also using village volunteers successfully. The Guinea worm campaign has been another illustration of how volunteers can be used to diagnose, report, and provide, in this instance, on-the-spot treatment

to neighbors for a specific infection. Those responsible for the campaign's success are often not members of the general health services.

With the help of the World Bank, the Guinea worm campaign demonstrates the importance of disease eradication to the national economy. The World Bank has estimated that the economic rate of return on the investment in Guinea worm eradication will be on the order of 29% per year once the disease is eradicated. That figure is based on a very conservative estimate of the average amount of time infected workers are unable to perform agricultural tasks.

The campaign has also created a group of trained health-care workers of a different generation from those involved in the smallpox eradication program. These workers have gone from beginning to end, from hearing the doubters and seeing the difficulty of initiating the campaign to tasting victory in their own countries. These workers can contribute to subsequent campaigns. Moreover, the concept of eradication, which was in disrepute only 5, 10 years ago, has been revived. Soon we will confirm that a nonviral disease for which vaccine is not available can be eradicated.

Like the smallpox eradication campaign, the Guinea worm campaign has illustrated very vividly in many different ways and at many different levels (from international to village level) the power of data. In the Guinea worm campaign, we have used surveillance data to promote health policy. One key lesson from the smallpox campaign we are deliberately applying in the Guinea worm campaign is to distill what needs to be done in terms of interventions to a handful, or almost a handful, of indexes (seven on an international level) to know what is most important and (as rapidly as possible) how well we are doing. That unleashes inordinate amounts of energy.

Finally, the Guinea worm eradication campaign will have illustrated again the power of demonstration. Eradication can happen because it has happened.

Nosocomial Infection Update

Robert A. Weinstein
Cook County Hospital & Rush Medical College, Chicago, Illinois, USA

Historically, staphylococci, pseudomonads, and *Escherichia coli* have been the nosocomial infection troika; nosocomial pneumonia, surgical wound infections, and vascular access–related bacteremia have caused the most illness and death in hospitalized patients; and intensive care units have been the epicenters of antibiotic resistance. Acquired antimicrobial resistance is the major problem, and vancomycin-resistant *Staphylococcus aureus* is the pathogen of greatest concern. The shift to outpatient care is leaving the most vulnerable patients in hospitals. Aging of our population and increasingly aggressive medical and surgical interventions, including implanted foreign bodies, organ transplantations, and xenotransplantation, create a cohort of particularly susceptible persons. Renovation of aging hospitals increases risk of airborne fungal and other infections. To prevent and control these emerging nosocomial infections, we need to increase national surveillance, "risk adjust" infection rates so that interhospital comparisons are valid, develop more noninvasive infection-resistant devices, and work with health-care workers on better implementation of existing control measures such as hand washing.

As we enter the next millennium of infection control, we stand on the shoulders of giants—Jenner, Semmelweis, Nightingale, Oliver Wendell Holmes, and my own personal favorite, Thomas Crapper, the father of indoor plumbing. Modern infection control is grounded in the work of Ignaz Semmelweis, who in the 1840s demonstrated the importance of hand hygiene for controlling transmission of infection in hospitals. However, infection control efforts were spotty for almost a century. In 1976, the Joint Commission on Accreditation of Healthcare Organizations published accreditation standards for infection control, creating the impetus and need for hospitals to provide administrative and financial support for infection control programs. In 1985, the Centers for Disease Control and Prevention's (CDC's) Study on the Efficacy of Nosocomial Infection Control reported that hospitals with four key infection control components—an effective hospital epidemiologist, one infection control practitioner for every 250 beds, active surveillance mechanisms, and ongoing control efforts—reduced nosocomial infection rates by approximately one third (1).

Address for correspondence: Robert A. Weinstein, Cook County Hospital, Division of Infectious Diseases, 129 Durand, 1835 W. Harrison St., Chicago, IL 60612, USA; fax: 312-572-3523; e-mail: rweinste@rush.edu.

Over the past 25 years, CDC's National Nosocomial Infections Surveillance (NNIS) system has received monthly reports of nosocomial infections from a nonrandom sample of United States hospitals; more than 270 institutions report. The nosocomial infection rate has remained remarkably stable (approximately five to six hospital-acquired infections per 100 admissions); however, because of progressively shorter inpatient stays over the last 20 years, the rate of nosocomial infections per 1,000 patient days has actually increased 36%, from 7.2 in 1975 to 9.8 in 1995 (Table 1). It is estimated that in 1995, nosocomial infections cost $4.5 billion and contributed to more than 88,000 deaths—one death every 6 minutes.

Table 1. Nosocomial infections, United States (2,3)

Year	Admissions ($x10^6$)	Patient days[a] ($x10^6$)	Length of stay (days)	Nosocomial infection ($x10^6$)	Nosocomial infections (/1000 patient days)
1975	38	299	7.9	2.1	7.2
1995	36	190	5.3	1.9	9.8

[a]Patient days = total inpatient days.

Which Nosocomial Infections Are Emerging?

We have witnessed a cyclical parade of pathogens in hospitals. In Semmelweis's era, group A streptococci created most nosocomial problems. For the next 50 to 60 years, gram-positive cocci, particularly streptococci and *Staphylococcus aureus*, were the hospital pathogens of major concern. These problems culminated in the pandemic of 1940 to 1950, when *S. aureus* phage type 94/96 caused major nosocomial problems. In the 1970s, gram-negative bacilli, particularly *Pseudomonas aeruginosa* and *Enterobacteriaceae*, became synonymous with nosocomial infection. By the late 1980s and early 1990s, several different classes of antimicrobial drugs effective against gram-negative bacilli provided a brief respite. During this time, methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE) emerged, signaling the return of the "blue bugs." In 1990 to 1996, the three most common gram-positive pathogens—*S. aureus*, coagulase-negative staphylococci, and enterococci—accounted for 34% of nosocomial infections, and the four most common gram-negative pathogens—*Escherichia coli*, *P. aeruginosa*, *Enterobacter* spp., and *Klebsiella pneumoniae*—accounted for 32% (3).

Bloodstream infections and pneumonias have increased in frequency from 1975 to 1996 (Table 2). However, tracking nosocomial infections by site has become difficult in the last few years because of shorter inpatient stays. For example, the average postoperative stay, now approximately 5 days, is usually shorter than the 5- to 7-day incubation period for *S. aureus* surgical wound infections.

Acquired antimicrobial resistance is the major anticipated problem in hospitals. VRE and MRSA are the major gram-positive pathogens of concern (5,6). *P. aeruginosa*, *Klebsiella*, and *Enterobacter* that harbor chromosomal or plasmid-mediated beta-lactamase enzymes are the major resistant gram-negative pathogens.

Table 2. Sites of nosocomial infections (2,4)

Year	Urinary tract (%)	Surgical wound (%)	Lower respiratory tract (%)	Bloodstream (%)	Other (%)
1975	42	24	10	5	19
1990–96	34	17	13	14	21

The contribution of antibiotic resistance to excessive death rates in hospitals is difficult to evaluate, often depending on whether studies are population-based or case-control, but evidence is mounting that antimicrobial resistance contributes to nosocomial deaths.

While bacterial resistance is clearly the major threat, viral and fungal resistance could become important because of the small number of therapeutic options for these pathogens. Herpes viruses with acquired resistance to acyclovir and ganciclovir have emerged as problems, particularly in HIV-infected patients. Pathogens with intrinsic resistance often have lower pathogenicity and have disproportionately affected immunocompromised patients. For example, *Candida* spp. with intrinsic resistance to azole antifungal agents (e.g., *C. krusei*) and to amphotericin B (e.g., *C. lusitaniae*) have emerged as problem pathogens in oncology units.

While we are facing the era of opportunists, including fungi, viruses, and parasites in immunocompromised patients, the one we fear most is the postantibiotic era. The first nosocomial inkling is MRSA with reduced susceptibility to vancomycin (7). Beyond the postantibiotic era lies the era of xenogenic infections as organs, transplanted from nonhuman primates, bring with them a variety of potential zoonotic pathogens. Nevertheless, traditional respiratory pathogens may yet prove to be our greatest challenge; for example, a major shift in strain type (8) could result in devastating pandemic community and nosocomial influenza A outbreaks.

Who Is Affected by Emerging Nosocomial Pathogens?

Nosocomial infections typically affect patients who are immunocompromised because of age, underlying diseases, or medical or surgical treatments. Aging of our population and increasingly aggressive medical and therapeutic interventions, including implanted foreign bodies, organ transplantations, and xenotransplantations, have created a cohort of particularly vulnerable persons. As a result, the highest infection rates are in intensive care unit (ICU) patients. Nosocomial infection rates in adult and pediatric ICUs are approximately three times higher than elsewhere in hospitals. The sites of infection and the pathogens involved are directly related to treatment in ICUs. In these areas, patients with invasive vascular catheters and monitoring

devices have more bloodstream infections due to coagulase-negative staphylococci. In fact, most cases of occult bacteremia in ICU patients are probably due to vascular access-related infections. Fungal urinary tract infections have also increased in ICU patients, presumably because of extensive exposure to broad-spectrum antibiotics. In the National Nosocomial Infections Surveillance system, *Candida* spp. are the main cause of nosocomial urinary infections in ICUs (9).

Why Are Nosocomial Infections Emerging Now?

Three major forces are involved in nosocomial infections. The first is antimicrobial use in hospitals and long-term care facilities. The increased concern about gram-negative bacilli infections in the 1970s to 1980s led to increased use of cephalosporin antibiotics. As gram-negative bacilli became resistant to earlier generations of cephalosporin antibiotics, newer generations were developed. Widespread use of cephalosporin antibiotics is often cited as a cause of the emergence of enterococci as nosocomial pathogens. About the same time, MRSA, perhaps also in response to extensive use of cephalosporin antibiotics, became a major nosocomial threat. Widespread empiric use of vancomycin, as a response to concerns about MRSA and for treatment of vascular catheter–associated infection by resistant coagulase-negative staphylococci, is the major initial selective pressure for VRE. Use of antimicrobial drugs in long-term care facilities and transfer of patients between these facilities and hospitals have created a large reservoir of resistant strains in nursing homes.

Second, many hospital personnel fail to follow basic infection control, such as hand washing between patient contacts. In ICUs, asepsis is often overlooked in the rush of crisis care (10).

Third, patients in hospitals are increasingly immunocompromised. The shift of surgical care to outpatient centers leaves the sickest patients in hospitals, which are becoming more like large ICUs (11). This shift has led to the greater prevalence of vascular access–associated bloodstream infections and ventilator-associated pneumonias.

Other precipitating factors also can be anticipated in hospitals. Transplantation is a double-edged sword because of the combined effects of immunosuppression of transplant patients and of infectious diseases that come with some transplanted organs. The blood supply will continue to be a source of emerging infectious diseases. Moreover, as hospitals age, infrastructure repairs and renovations will create risks of airborne fungal diseases caused by dust and spores released during demolition and construction. Infections due to other pathogens, such as Legionella, may also result from such disruptions.

How Can We Prevent and Control Emerging Nosocomial Infections?

Infection control can be very cost-effective. Approximately one third of nosocomial infections are preventable. To meet and exceed this level of prevention, we need to pursue several strategies simultaneously (12). First, we need to continue to improve national surveillance of nosocomial infections so that we have more representative data. We must assess the sensitivity and specificity of our surveillance and of our case definitions, particularly for difficult-to-diagnose infections like ventilator-associated pneumonia. We also need to develop systems for surveillance of "nosocomial" infections that occur out of the hospital, where much health care is now given.

Second, we need to ensure that surveillance uses are valid. The Joint Commission on Accreditation of Healthcare Organization's ORYX initiative for monitoring health-care processes and outcomes will lead to core indicators and sentinel event monitoring. This initiative will be followed by increased outpatient surveillance, which ultimately may lead to systemwide real-time surveillance and reporting. Because we want to use nosocomial infection rates as a core indicator of quality of care, we need to improve our ability to "risk adjust" infection rates so we know that our interprovider and interhospital comparisons are valid. Risk stratification will ultimately depend on organic-based computer systems that will mimic biologic events.

Third, many of our successes in controlling nosocomial infections have come from improving the design of invasive devices. This is particularly important given the marked increase in frequency of vascular access–associated bloodstream infections, particularly in ICU patients. Given the choice of changing human behavior (e.g., improving aseptic technique) or designing a better device, the device will always be more successful. Of particular importance is the development of noninvasive monitoring devices and minimally invasive surgical techniques that avoid the high risk associated with bypassing

normal host defense barriers (e.g., the skin and mucous membranes).

Fourth, forestalling the postantibiotic era will require aggressive antibiotic control programs (13); these may become mandated for hospitals that receive federal reimbursements, as happened in the past with infection control programs. Risks for antibiotic-resistant strains also may be reduced in the future by controlling colonization through use of immunization or competing flora.

Fifth, antimicrobial resistance problems and the advent of xenotransplantation emphasize the importance of newer microbiologic methods. For investigation of outbreaks of multidrug-resistant pathogens, pulsed-field gel electrophoresis has become a routine epidemiologic tool (14). Molecular epidemiologic analysis also may help us better understand the factors that lead to the emergence of resistant strains. For diagnosis of syndromes caused by unusual pathogens, representational difference analysis and speciation by use of the pathogen's phylogenetic r-RNA "clock" may become routine.

Sixth, control of tuberculosis (TB) in hospitals is an excellent example of the successful collaboration of the infection control community, CDC, and regulatory agencies. But we can anticipate that the Occupational Safety and Health Administration may have many new employee health issues—beyond TB and bloodborne pathogens—to evaluate in hospitals, such as health problems related to exposure to magnetic fields, to new polymers, and to medications that contaminate the environment. Problems of mental stress due to unrelenting exposure to pagers, faxes, e-mail, holograms, and telephonic implanted communicators will require special attention.

Conclusion

Several enduring truths characterize the field of infection control. Hospitals will become more like ICUs, and more routine care will be delivered on an outpatient basis. Given the choice of improving technology or improving human behavior, technology is the better choice. All infection control measures will need to continue to pass the test of the "four Ps" (15): Are the recommendations Plausible biologically (e.g., is it likely to work)? Are they Practical (e.g., are they affordable)? Are they Politically acceptable (e.g., will the administration agree)? And, will Personnel follow them (e.g., can they and will they)?

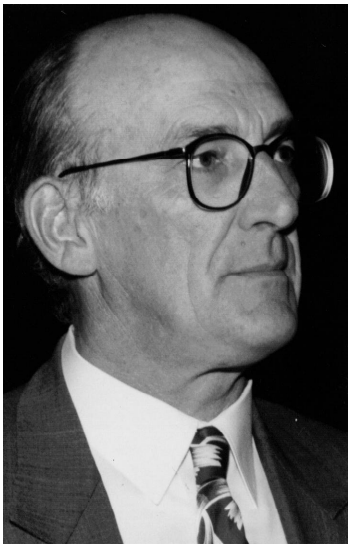
The major advances in overall control of infectious diseases have resulted from immunization and improved hygiene, particularly hand washing. We must work with hospital personnel on better implementation of existing infection control technologies so that we will not need to rely solely on technologic advances.

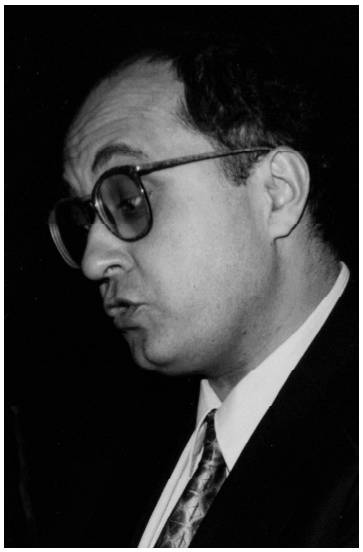
Dr. Weinstein is chair, Division of Infectious Diseases, Cook County Hospital; director of Infectious Diseases Services for the Cook County Bureau of Health Services; and professor of Medicine, Rush Medical College. He also oversees the CORE Center for the Prevention, Care and Research of Infectious Disease and directs the Cook County Hospital component of the Rush/Cook County Infectious Disease Fellowship Program. His areas of research include nosocomial infections (particularly the epidemiology and control of antimicrobial resistance and infections in intensive care units) and health-care outcomes for patients with HIV/AIDS.

References

1. Haley RW, Culver DH, White J, Morgan WM, Amber TG, Mann VP, et al. The efficacy of infection surveillance and control programs in preventing nosocomial infections in US hospitals. Am J Epidemiol 1985;121:182-205.
2. Haley RW, Culver DH, White JW, Morgan WM, Emori TG. The nationwide nosocomial infection rate: a new need for vital statistics. Am J Epidemiol 1985;121:159-67.
3. New York Times 1998 Mar 12; Sect. A12.
4. Centers for Disease Control and Prevention, Hospital Infections Program. National Nosocomial Infections Surveillance (NNIS) report, data summary from October 1986-April 1996, issued May 1996: A report from the NNIS System. Am J Infect Control 1996;24:380-8.
5. Slaughter S, Hayden MK, Nathan C, Hu TC, Rice T, Van Voorhis J, et al. A comparison of the effect of universal use of gloves and gowns with that of glove use alone on acquisition of vancomycin-resistant enterococci in a medical intensive care unit. Ann Intern Med 1996;125:448-56.
6. Bonten MJM, Hayden MK, Nathan C, Van Voorhis J, Matushek M, Slaughter S, et al. Epidemiology of colonisation of patients and environment with vancomycin-resistant enterococci. Lancet 1996;348:1615-9.
7. Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, et al. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. Lancet 1997;350:1670-3.
8. Webster RG. Influenza: an emerging disease. Emerg Infect Dis 1998;4(3).
9. Fridkin SK, Welbel SF, Weinstein RA. Magnitude and prevention of nosocomial infections in the intensive care unit. Infect Dis Clin North Am 1997;11:479-96.
10. Weinstein RA. Epidemiology and control of nosocomial infections in adult intensive care units. Am J Med 1991;91:179-84.

11. Archibald L, Phillips L, Monnet D, McGowan JE, Tenover F, Gaynes R. Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit. Clin Infect Dis 1997;24:211-5.
12. Scheckler WE, Brimhall D, Buck AS, Farr BM, Friedman C, Garibaldi RA, et al. Requirements for infrastructure and essential activities of infection control and epidemiology in hospitals: a consensus panel report. Infect Control Hosp Epidemiol 1998;19:114-24.
13. Goldmann DA, Weinstein RA, Wenzel RP, Tablan OC, Duma RJ, Gaynes RP, et al. Strategies to prevent and control the emergence and spread of antimicrobial-resistant microorganisms in hospitals. A challenge to hospital leadership. JAMA 1996;275:234-40.
14. Tenover FC, Arbeit RD, Goering RV. How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Infect Control Hosp Epidemiol 1997;18:426-39.
15. Weinstein RA. SHEA consensus panel report: a smooth takeoff. Infect Control Hosp Epidemiol 1998;19:91-3.





Sir John Pattison (L)
Medical School of University College, London
Sherif Zaki
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Opportunistic Infections in Immunodeficient Populations

Jonathan E. Kaplan,* Gary Roselle,† and Kent Sepkowitz‡
*Centers for Disease Control and Prevention, Atlanta, Georgia, USA;
†Veterans Administration Medical Center, Cincinnati, Ohio, USA; and
‡Memorial Sloan-Kettering Cancer Center, New York, New York, USA

Opportunistic infections occur with greater frequency or severity in patients with impaired host defenses. Growing numbers of HIV-infected persons, transplant recipients, and elderly persons are at increased risk.

Alison Grant, London School of Hygiene and Tropical Medicine, discussed opportunistic infections due to HIV. In 1997, more than 30 million HIV-infected persons lived in the world, with more than two thirds of them in sub-Saharan Africa and an additional 20% in Asia and Latin America. Assessments of the prevalence and incidence of opportunistic infections in these areas and comparability of the available data are hampered by limited access to care, diagnostic capabilities, and surveillance data. Despite these limitations, we know that tuberculosis (TB) is the most frequent serious opportunistic infection in the developing world. Other such infections common in sub-Saharan Africa include septicemia (of which nontyphoid salmonella is the most common cause), toxoplasmosis, and bacterial pneumonia. *Pneumocystis carinii* infection, for unknown reasons, is uncommon among adults in East and West Africa but appears to be more common in South Africa. *Penicillium marneffei* infection, common in Thailand, is an example of an opportunistic infection of importance in a specific region; risk factors in these regions are largely unknown. Additional challenges are posed by the different HIV subtypes in the developing world and the possibility that some may be associated with a differential risk for opportunistic infections. Prevention efforts in developing countries have been limited. More work is needed to evaluate prophylactic regimens appropriate to different regions. Prevention of TB with isoniazid; of pneumocystosis, toxoplasmosis, and some bacterial infections with cotrimoxazole; and of pneumococcal infections with 23-valent pneumococcal vaccine have potential.

Robert Hogg, University of British Columbia, discussed the remarkable changes in the natural history of HIV in North America, specifically in British Columbia, as a result of highly active antiretroviral therapy (HAART). Of more than 5,000 HIV-infected persons receiving care in British Columbia, more than 2,000 are receiving HAART. HIV viral loads have been reduced to undetectable levels in approximately half of these patients, with corresponding decreases in the incidence of opportunistic infections, hospitalizations, and deaths. However, even for persons who have access to the therapy, these successes may be short-lived as resistance to HAART becomes more widespread. HAART use has resulted in new syndromes that may occur soon after therapy, probably representing preexisting, subclinical infections that are unmasked by the immunologic improvement that accompanies HAART; these syndromes include lymphadenitis associated with *Mycobacterium avium* complex, cytomegalovirus retinitis, and miliary TB on chest X-ray. Hepatitis C infection, common in HIV-infected injection drug users, may pose increasing problems as coinfected persons live longer. Therefore, surveillance for old and new syndromes remains critical even with the reduced incidence of opportunistic infections that has been associated with HAART.

Robert Rubin, Harvard University and Massachusetts Institute of Technology, discussed opportunistic infections in hematopoietic stem cell (bone marrow) and solid-organ transplant recipients; the number of these transplant recipients has increased dramatically in the United States in the past decade. The opportunistic infections in these patients originate from endogenous flora (e.g, invasive candidiasis), from the general (nonhospital) environment (e.g,

histoplasmosis, TB, disseminated strongyloidiasis), or from the hospital environment (e.g, aspergillosis, legionellosis, and infections with vancomycin-resistant enterococci or multiply resistant gram-negative bacteria). These infections characteristically occur in a time-dependent pattern posttransplant, corresponding with the nature of the immunodeficiency. For example, in bone marrow transplant recipients, infections within 1 month of transplantation (pre-engraftment) occur as a result of neutropenia and disruption of mucosal surfaces; infections that occur in the second or third months are due to deficiencies in cell-mediated immunity and are more frequent in the setting of graft versus host disease. In solid-organ transplant recipients, infections within the first month are generally associated with technical problems related to surgery; infections that occur later are due to immunodeficiency associated with immunosuppressive therapy. These timetables are useful in that infections that are unusual or occur outside the expected time frame may serve as sentinels for emerging opportunistic infections. Research priorities in this area include development of therapies that will enhance successful transplantation without increasing the risk for opportunistic infections, strategies to reduce the risk of drug-resistant opportunistic infections, and greater understanding of the role of cytokines in the relationship between graft versus host disease and opportunistic infections.

Carol Kauffman, University of Michigan and the Ann Arbor Veterans Administration Medical Center, discussed infections in the elderly, a population that is increasing in the United States and worldwide. Persons ≥65 years of age already constitute approximately one eighth of the U.S. population; this proportion is expected to double in the next 50 years. Elderly persons have defects in T-cell immunity that result in increased incidence and death from TB. B-cell defects result in increased susceptibility to *Streptococcus pneumoniae* and respiratory syncytial virus and a decreased response to 23-valent pneumococcal vaccine. Elderly persons are at increased risk for cancer, so various treatments associated with immunosuppression (such as organ transplantation and aggressive cancer chemotherapy) are increasingly being used in this population. Chronic corticosteroid therapy is frequently used for treatment of temporal arteritis. Although HIV infection is relatively uncommon in the elderly, when it does occur, it is likely to go undiagnosed. Because of higher rates of hospitalization, elderly persons are more susceptible to nosocomial infections (including those caused by antibiotic-resistant organisms). Moreover, the elderly are more likely to reside in long-term care facilities, which may serve as sources or amplifiers of infections such as influenza. Susceptibility to infection may be further increased by malnutrition, diabetes, and chronic renal failure. Finally, healthy, more affluent older persons are at risk for infections associated with travel.

In summary, opportunistic infections are a threat in the increasing populations of immunocompromised persons. In these populations, opportunistic infections pose challenges for surveillance and determination of risk factors, including those for infection with antibiotic-resistant organisms.

Host Genes and Infectious Diseases

Janet McNicholl
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

This panel presented data on host genes that influence susceptibility to or manifestations of four infectious diseases: Puumala hantavirus infection, tuberculosis (TB), Lyme disease, and AIDS. Gus Birkhead, Council of State and Territorial Epidemiologists, introduced the session, highlighting its timeliness in relation to the rapidly emerging body of data on our 100,000 human genes that stems from the Human Genome and related projects.

The presentations introduced several approaches to identifying a host gene–infectious disease interaction. Panelists presented case-control studies of hantavirus infection, AIDS, TB, and Lyme disease that used the candidate gene approach; the new approach, genome scanning by microsatellites to identify genes associated with TB susceptibility, was also described. Candidate genes were chosen on the basis of pathology of the infectious disease (human leukocyte antigen [HLA], tumor necrosis factor [TNF], the antigen processing [TAP]), mouse genetic studies of the pathogen (*NRAMP1* in TB), or epidemiologic findings of disease severity (Vitamin D and TB).

Susceptibility-Associated Major Histocompatibility Complex (MHC) Haplotype in Severe Puumala Hantavirus Infection

Annti Vaheri, Haartman Institute, University of Helsinki, described the epidemiology of hantavirus infections in Northern Europe. The pathogens, enveloped RNA viruses primarily of the Puumala and Dobrava genotypes, are carried by rodents such as mice and voles and cause a range of disease in humans. While the epidemics in the United States are of hantaviruses that cause primarily pulmonary disease, in northern Europe, renal disease is the primary pathologic manifestation, as evidenced by increased capillary permeability, infiltrates of CD8+ T cells, high levels of ICAM-1, and expression of TNF-α and transforming growth factor (TGF)-ß. Although most infections with these viruses are probably subclinical or cause mild disease, in 10% of patients disease may progress to shock, 5% may require dialysis, and some may die. Of those who recover, renal damage may later result in chronic hypertension. Because hantaviruses are variable and are usually transmitted as swarms of viruses, it was proposed that host factors, such as HLA genes, might influence the spectrum of disease. Indeed, this has been shown to be the case. Persons who express the HLA-B8 genes had more severe disease with lower blood pressures, higher creatinine (1), and more virus in the urine and blood by polymerase chain reaction (PCR) (2). Persons with HLA-B27 had milder disease (3). The finding of TNF-α expression in the kidney of infected patients prompted an analysis of the TNF 1 and 2 alleles (at positions -308 and -238) by restriction fragment length polymorphism (RFLP), and as might have been predicted from their linkage to the HLA-A1-B8-DR3 haplotype, nearly all who progressed to shock expressed the TNF 2 allele (M. Kanerva, unpub. data). This allele has been linked to high TNF production (4).

Because the HLA-A1-B8-DR3 MHC haplotype is associated with insulin-dependent diabetes mellitus and other autoimmune diseases that may have a viral etiology, it was asked if molecular mimicry could explain the association of this haplotype with the renal disease of Puumala virus infection. Dr. Vaheri stated that no such evidence exists and that the association probably reflects a propensity to a particular type of immune response that results in disease. Whether the genetic associations observed with Puumala hantavirus disease are due to a primary association with the TNF 2 allele or the linked HLA alleles is not known and deserves future research. Another important field is the mapping of HLA-restricted epitopes in hantaviruses.

Host Susceptibility to TB in Africa

Although TB has been present in human populations for millennia, its reemergence as a public health problem and the new tools of molecular genetics have provided an impetus to study host genetic susceptibility to TB disease.

Richard Bellamy, Wellcome Trust Center for Human Genetics, presented studies that used both candidate- and genome-screening approaches to define these factors in African populations. As stated during the question-and-answer session, many studies of TB should be considered studies of TB disease rather than TB susceptibility, since most persons, particularly in Africa, are TB infected, but (at least in HIV-negative populations) fewer than 10% become ill. Dr. Bellamy's studies were carried out in populations with low HIV prevalence, HIV-infected persons were excluded, and disease was defined as smear-positive TB. Historically, in most populations, particularly in The Gambia and South Africa, the sources of patients and controls for these studies, TB is predominantly a disease of males. Previous studies of mono- and dizygotic twins have also suggested a genetic component (reviewed in 5).

One of the studies described by Dr. Bellamy used new tools from the human genome and other projects called microsatellite markers (intronic sections of cytosine, adenine repeats) and automated robotic DNA typing using four-color fluorescent labels with 20 markers per lane of large gels that are scanned and analyzed by software such as GeneScan 672 and Genotyper 1.2. He analyzed 92 sibling-pairs from The Gambia and South Africa. Cosegregation with TB was identified for markers on chromosomes 3, 5, 6, 8, 9, 15, and the X chromosome. A second study of 83 sibling-pairs from the same countries again linked the same sites on Xq and 15p with lod scores of >2. While these studies do not identify the genes in question, further studies of these regions may reveal the relevant genes (e.g., the microsatellite region identified on Xq is close to genes encoding the CD40 ligand and human LAMP).

Bellamy's group identified two additional genes associated with TB in candidate gene association studies of African TB cases and ethnically matched controls (6). The human homologue *NRAMP1* of the mouse *Bcg* gene that confers resistance to bacillus Calmette-Guérin has been located on chromosome 2q35. Four polymorphisms in *NRAMP1* were studied with microsatellite markers and probes that distinguished single-base substitutions and a 4-bp deletion in the gene. While all four polymorphisms were associated with TB, two, one intronic and another in the 3' untranslated region, were particularly overrepresented in TB patients; persons heterozygous for INT4 GC and 3'UTR deletion had a fourfold increased risk of having TB (6). The 3'UTR allele is of unusually high prevalence in the West African population studies but is uncommon in Europeans. This may partly explain the higher susceptibility to TB in African Americans compared with other ethnic groups. While the physiologic function of *NRAMP1* has not been defined, it may affect phagolysosome function. Dr. Bellamy's data suggest that the polymorphisms they have defined or linked polymorphisms may alter *NRAMP1* function and therefore the host's ability to clear intracellular pathogens. In vitro studies to address the effect of these polymorphisms on macrophage function are in progress.

Dr. Bellamy also presented unpublished data on vitamin D receptor genotypes and susceptibility to TB disease. This gene was chosen because of clinical and laboratory data suggesting vitamin D may be important in host defenses against TB (7,8). He observed a low prevalence of the homozygous t vitamin D receptor genotype in TB cases but not in controls. This genotype is also associated with increased risk for osteoporosis (9,10). These findings raise the question whether administering vitamin D to populations at risk for TB disease might be a simple public health measure to reduce the disease. However, the effect of such therapy might be hard to estimate because of the low prevalence of the tt homozygous genotype.

The genes (e.g., HLA) identified in these and other studies are certainly not the only genes involved in host susceptibility to TB. Dr. Bellamy estimated that together they account for less than 2% of the total familial clustering effect in this disease.

HLA and the Pathogenesis of Lyme Arthritis

Host responses to another bacterium, the spirochete *Borrelia burgdorferi*, and the clinical spectrum of Lyme arthritis were discussed by Allen Steere, Department of Rheumatology and Immunology, New England Medical Center, Boston, Massachusetts. Another vector-borne human pathogen, *B. burgdorferi* causes a multisystem disease that may affect the skin, nervous system, heart, or joints. Arthritis is a major late manifestation of the illness. Although all manifestations are usually treatable with antibiotic therapy, approximately 10% of patients

with Lyme arthritis have persistent joint inflammation for months or even years after antibiotic therapy. In these patients, PCR tests for *B. burgdorferi* DNA in joint fluid have been negative after antibiotic treatment, which suggests that joint inflammation may sometimes continue after the spirochete has been eradicated from the joint.

Dr. Steere's group is studying host factors that may be important in the pathogenesis of chronic, treatment-resistant Lyme arthritis. Studies of HLA class II alleles have shown that HLA-DRB1*0401 alleles are associated with chronic Lyme arthritis and lack of response to antibiotic therapy (11). This allele is also associated with an increased risk of developing severe rheumatoid arthritis (12). In a study of antibody responses in patients throughout the course of Lyme disease, immunoglobulin G (IgG) responses to outer-surface protein A (OspA) and OspB of the spirochete often developed near the beginning of prolonged episodes of arthritis (13). Arthritis lasted considerably longer after treatment in patients with HLA-DR4 and OspA and OspB antibody reactivity than in those who lacked responses to these proteins (13). The cellular arm of the immune response has also been examined by Dr. Steere's group, and persons with treatment-resistant Lyme arthritis usually have T cells that react with many OspA epitopes, whereas treatment-responsive patients usually do not. A possible explanation for these findings is that the T-cell response to OspA in patients with treatment-resistant Lyme arthritis may cross-react with a self antigen in the joint, and the response to this self antigen may continue to cause joint inflammation for months or even years after the eradication of the spirochete from the joint.

How does one treat patients with Lyme arthritis who do not appear to respond to therapy? Dr. Steere recommended that if they have not responded to antibiotics after 2 months and the PCR test on joint fluid is negative for *B. burgdorferi* DNA, patients should be treated with antiinflammatory agents. When asked whether HLA genes might influence Osp-based vaccines for Lyme disease, Dr. Steere noted that studies to address this question have not yet been carried out.

Host Genes, HIV Susceptibility, and Disease Course

The rapidly growing and complex body of knowledge on the host genes that influence susceptibility to HIV infection and progression to AIDS was reviewed by Richard Kaslow, Department of Epidemiology, University of Alabama, Birmingham, Alabama. The studies reported by Kaslow and others in the last 2 years have greatly benefited from several longitudinal cohort studies, some focusing on HIV-infected seroconverters or HIV-exposed persons in the United States and Europe. More than 10 years after these cohorts have been established, adequate power to address the role of candidate genes in transmitting HIV horizontally and vertically and in affecting the rate of disease progression has been obtained, while increased knowledge of HIV's mode of cellular entry has provided new candidate genes to study. HIV enters cells through an interaction with both CD4 and a chemokine receptor of the 7 Tm family (14). Dr. Kaslow first reviewed the role of genes in encoding chemokine receptors (CCR5 and CCR2) and chemokines (SDF-1) in HIV disease. While CCR5 has multiple allelic variants in its coding region (15), the deletion of a 32-bp segment results in a nonfunctional receptor (reviewed in 16), thus preventing HIV entry; two copies of this gene provide strong protection against HIV infection in epidemiologic studies, although the protection is not absolute. This gene is found in up to 20% of Europeans but is rare in Africans and Asians. Multiple studies of HIV-infected persons have shown that presence of one copy of this gene delays progression to AIDS by about 2 years. A mutation in another chemokine receptor gene, that coding for CCR2, has also been reported by several groups to be associated with a delayed progression to AIDS (reviewed in 17). This polymorphism (a position 64 Val→Ile substitution) does not appear likely to affect receptor function, and the mutation may be linked to another polymorphism in the promotor of CCR5 (18). Nevertheless, studies of persons with both CCR2 64I polymorphism and CCR5 delta 32 deletion suggest the effect of both genes on HIV disease progression is additive (19). A polymorphism in the chemokine SDF-1, which binds to another HIV entry receptor, CXCR4, also delays HIV progression and similarly appears additive to the effects of the CCR2 and CCR5 polymorphisms (20).

Dr. Kaslow also reviewed studies of the HLA system (at the Class I HLA A, B, C and Class II DR and DQ and the antigen processing [TAP] loci) and how complex combinations of different HLA alleles alter the risk of developing AIDS in several cohorts of HIV-infected persons (21). The effects of different

combinations of HLA alleles appear to delay HIV progression by a variable number of years and to be additive to the effects of the chemokine gene polymorphisms described above.

These new findings about HIV and host genes have led to new approaches to AIDS treatments, such as those directed at chemokine receptors, and hold great promise for advancing our ability to combat this disease.

References

1. Mustonen J, Partanen J, Kanerva M, Pietilä K, Vapalahti O, Pasternack A, et al. Genetic susceptibility to severe course of nephropathia epidemica caused by Puumala hantavirus. Kidney Int 1996;49:217-21.
2. Plyusnin A, Hörling J, Kanerva M, Mustonen J, Cheng Y, Partanen J, et al. Puumala hantavirus genome in patients with nephropathia epidemica: correlation of PCR positivity with HLA haplotype and link to viral sequences in local rodents. J Clin Microbiol 1997;35:1090-6.
3. Mustonen J, Partanen J, Kanerva M, Pietilä K, Vapalahti O, Pasternack A, et al. Asssociation of HLA B27 with benign clinical course of nephropathia epidemica caused by Puumala hantavirus. Scand J Immunol. In press 1998**.**
4. Wilson AG, Symons JA, McDowell TL, McDevit HO, Duff GW. Effects of polymorphism in the tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A 1997;94:3195-9.
5. Bloom BR, Small PM. Editorial. The evolving relation between humans and *Mycobacterium tuberculosis*. N Engl J Med 1998;338:677-8.
6. Bellamy R, Ruwende C, Tumani C, McAdam PWJ, Whittle HC, Hill AVS. Variations in the *NRAMP1* gene and susceptibility to tuberculosis in West Africans. N Engl J Med 1998;338:640-4.
7. Davies PD. A possible link between vitamin D deficiency and impaired host defence to *Mycobacterium tuberculosis*. Tubercle 1985;66:301-6.
8. Rook GA, Steele J, Fraher L, Barker S, Karmali R, O'Riordan J, et al. Vitamin D3, gamma interferon, and control of proliferation of *Mycobacterium tuberculosis* by human monocytes. Immunology. 1986;57:159-63.
9. Sainz J, Van Tornout JM, Loro ML, Sayre J, Roe TF, Gilsanz V. Vitamin D-receptor gene polymorphisms and bone density in prepubertal American girls of Mexican descent. N Engl J Med. 1997;337(2):77-82.
10. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, et al. Prediction of bone density from vitamin D receptor alleles. Nature 1994;367(6460):284-287.
11. Steere AC, Dwyer E, Winchester R. Association of chronic Lyme arthritis with HLA-DR4 and HLA-DR2 alleles. N Engl J Med 1990;323:219-23.
12. Gregerson PK, Silber J, Winchester RJ. The shared epitope hypothesis: an approach to understanding the molecular genetics of rheumatoid arthritis. Arthritis Rheum 1987;30:1205-13.
13. Kalish RA, Leong JM, Steere AC. Association of treatment-resistant chronic Lyme arthritis with HLA-DR4 and antibody reactivity with OspA and OspB of *Borrelia burgdorferi*. Infect Immun 1993;61:2774-9.
14. Murphy PM. Chemokine receptors: structure, function and role in microbial pathogenesis. Cytokine & Growth Factor Reviews 1996;7:47-64.
15. Carrington M, Kissner T, Gerrard B, Ivanov S, O'Brien SJ, Dean M. Novel alleles of the chemokine-receptor gene CCR5. Am J Hum Genet 1997;61:1261-7.
16. McNicholl JM, Smith DK, Qari SH, Hodge T. Host genes and HIV: the role of the chemokine receptor gene CCR5 and its allele (Δ32 CCR5). Emerg Infect Dis 1997:3:261-71.
17. Garred P. Chemokine-receptor polymorphisms: clarity or confusion for HIV prognosis [editorial]. Lancet 1998;351:2-3.
18. Kostrikis LG, Huang Y, Moore JP, Wolinsky SM, Zhang L, Guo Y, et al. A chemokine receptor CCR2 allele delays HIV progression and is associated with a CCR5 promotor mutation. Nature Med 1998;4;350-3.
19. Smith MW, Dean M, Carrington M, Winkler C, Huttley GA, Lomb DA, et al. Contrasting genetic influence of CCR 2 and CCR5 variants on HIV infection and disease progression. Science 1997;277;959-65.
20. Winkler C, Modi W, Smith MW, Nelson GW, Wu X, Carrington M, et al. Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. Science 1998;279;380-93.
21. Kaslow RA, Carrington M, Apple R, Park L, Munoz A, Saah AJ, et al. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. Nature Medicine 1996;2:405-11.

Immigrant and Refugee Health

Susan Cookson,* Ronald Waldman,* Brian Gushulak,† Douglas MacPherson,‡ Frederick Burkle, Jr.,§ Christophe Paquet,¶ Erich Kliewer,# and Patricia Walker**

*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; †The International Organization for Migration, Geneva, Switzerland; ‡St. Joseph's Hospital, Hamilton, Ontario, Canada; §University of Hawaii, Honolulu, Hawaii, USA; ¶Epicentre, Paris, France; #Manitoba Cancer Treatment and Research Foundation, Winnipeg, Manitoba, Canada; and **Regions Hospital, St. Paul, Minnesota, USA

Each year, more than 15 million people seek political asylum or become refugees in various parts of the world. Most of these displaced persons are from developing countries where infectious diseases (e.g., tuberculosis, hepatitis, malaria, various parasitic and emerging diseases) are prevalent. These persons migrate mainly to the United States, Australia, and Canada, nations that receive inflows of migrants proportional to their mainstream population.

Because of the speed and efficiency of modern transportation systems, health interventions applicable to all persons who cross international borders are difficult to introduce and monitor. Identifying and addressing individual and public health risks necessitate international and quarantine health legislation, health policy and social economic evaluation, risk-benefit and utility analysis, and risk-predictive modeling. Ultimately, improving the health of migrants is at the heart of reducing the public health risk to the international community from infectious disease spread by travel.

Medical intelligence systems that can survey, detect, and confirm the emergence of new infectious diseases are still in their infancy. The global ability to generate numerators (cases of existing and emerging infectious disease) has been limited to the relatively few diseases listed in the old World Health Organization (WHO) International Health Regulations (yellow fever, plague, cholera, smallpox); further limitations stem from poor detection systems and incomplete reports (with the exception of smallpox). The dynamic problem of defining numerators and denominators (displaced persons at risk) is compounded by the need for improved diagnostics, heightened recognition, and effective medical interventions for the causative agents of diseases that affect these vulnerable populations.

Migrant populations have been displaced by disasters (natural, technologic, and human), which test the public health resources of a nation and expose weaknesses. Public health workers increasingly appreciate the fragile interaction between individual host, environment, and infectious and noninfectious agents capable of producing disease. The consequences of these relationships, including the real and potential vulnerability of populations, are becoming increasingly important indicators of national security.

Cholera, a disease that affects migrant populations, was examined. In Malawi, 11 outbreaks were documented in Mozambican refugees between 1987 and 1991, with attack rates of 0.6% to 9.3%. In 1994, an estimated 60,000 cases of cholera and 10,000 deaths occurred during a 1-month massive epidemic among Rwandan refugees (population 800,000) in Goma, Democratic Republic of Congo. Epidemic preparedness during the 1996 return of the epidemic proved the cornerstone of cholera control in these refugees. Properly implemented, active case-finding and rehydration therapy in specialized treatment centers can keep the case-fatality ratio below 1%.

In Australia, use of hospital and medical services by immigrants and refugees was examined. Foreign-born persons had lower hospitalization rates than native Australians, although some immigrant groups had higher rates for some diagnoses. Hospital data may help define trends in immigrant disease profiles; the data, however, do not indicate whether the generally lower hospitalization rates among immigrants were due to better health status or to barriers in accessing the medical system. In the

United States, Minnesota has been a leader in refugee resettlement since 1979; one center for international health has established a unique multidisciplinary primary and specialty care program for refugees and immigrants. Hmong, Cambodian, Vietnamese, Russian, Ukrainian, African, and Latin American refugees and immigrants have been seen; diseases such as hepatitis B, tuberculosis, and parasitic diseases, as well as mental health problems, have been diagnosed; and prevention strategies or therapies have been implemented.

Successful integration of migrant populations into their new communities' health-care systems is critical to the prevention and control of new and reemerging infectious diseases.

Emerging Zoonoses

Frederick A. Murphy
University of California, Davis, California, USA

In the past few years, emergent disease episodes have increased; nearly all have involved zoonotic or species-jumping infectious agents. Because there is no way to predict when or where the next important new zoonotic pathogen will emerge or what its ultimate importance might be, investigation at the first sign of emergence of a new zoonotic disease is particularly important. Such investigation may be described in terms of a discovery-to-control continuum: from recognition of a new disease in a new setting to complex phases involving the hard science disciplines pertaining to discovery, the epidemiologic sciences pertaining to risk assessment, and activities pertaining to risk management. Today, many activities involving zoonotic disease control are at risk because of a failed investigative infrastructure or financial base. Because zoonotic diseases are distinct, their prevention and control will require unique strategies, based more on fundamental research than on traditional approaches. Such strategies require that we rebuild a cadre of career-committed professionals with a holistic appreciation of several medical and biologic sciences.

In the past few years, emergent disease episodes have increased in the United States and globally. The list of important emergent diseases is impressive indeed and, given what we know about disease ecology, it will only continue to grow. Nearly all of these emergent disease episodes have involved zoonotic infectious agents; that is, they have involved the transmission of the etiologic agent to humans from an ongoing reservoir life cycle in animals or arthropods, without the permanent establishment of a new life cycle in humans. Fewer episodes have involved species-jumping by the etiologic agent; that is, they derive from an ancient reservoir life cycle in animals but have subsequently established a new life cycle in humans that no longer involves an animal reservoir.

Distinct Prevention and Control Strategies

Nearly all of the major topics for discussion at this conference involve either zoonotic or species-jumping infectious agents. Prevention and control strategies for diseases caused by these agents are different from those required for diseases whose etiologic agent has long relied on human-to-human transmission for its survival. The Centers for Disease Control and Prevention's (CDC) acute infectious disease prevention and control strategies were largely developed from experiences with vaccine-preventable childhood diseases, sexually transmitted diseases, hepatitis, and other diseases for which traditional clinically based or laboratory-based surveillance can provide the base for intervention activities such as vaccination or antimicrobial chemotherapy. For the zoonoses and for diseases caused by species-jumping agents, prevention and control strategies have come from diverse bases. At the heart of this research have been individual scientists who have spent whole careers accumulating highly specialized knowledge and experience. In fact, the work of these scientists might best be described as fundamental research—research seeking the means for disease control and prevention.

Predicting the Emergence of Zoonotic and Species-Jumping Pathogens

In general, there is no way to predict when or where the next important new zoonotic pathogen will emerge or what its ultimate importance might be. A pathogen might emerge as the cause of a geographically limited curiosity, intermittent disease outbreaks, or a new epidemic. No one could have predicted the emergence or zoonotic

Address for correspondence: Frederick A. Murphy, School of Veterinary Medicine, University of California, Davis, CA 95616-8734, USA; fax: 530-752-2801; e-mail: famurphy@ucdavis.edu.

nature of the bovine spongiform encephalopathy prion in cattle in the United Kingdom in 1986, the emergence or zoonotic potential of Sin Nombre virus as the cause of hantavirus pulmonary syndrome in the Southwest in 1993, and certainly not the species-jumping emergence of HIV as the cause of AIDS in 1981. Consequently, investigation at the first sign of emergence of a new zoonotic disease is particularly important, although the investigation usually resembles a field- and laboratory-based research project rather than a typical case-control–based outbreak investigation. This reality must drive strategic planning for dealing with new zoonotic diseases.

Factors Contributing to the Emergence of Zoonotic Diseases

Many elements can contribute to the emergence of a new zoonotic disease: microbial/virologic determinants, such as mutation, natural selection, and evolutionary progression; individual host determinants, such as acquired immunity and physiologic factors; host population determinants, such as host behavioral characteristics and societal, transport, commercial, and iatrogenic factors; and environmental determinants, such as ecologic and climatologic influences.

Emergence of new zoonotic pathogens seems to be accelerating for several reasons: global human and livestock animal populations have continued to grow, bringing increasingly larger numbers of people and animals into close contact; transportation has advanced, making it possible to circumnavigate the globe in less than the incubation period of most infectious agents; ecologic and environmental changes brought about by human activity are massive; and bioterroristic activities, supported by rogue governments as well as organized amateurs, are increasing, and in most instances the infectious agents of choice seem to be zoonotic.

Ecologic Factors Contributing to the Emergence of Zoonotic Diseases, as Exemplified by Arbovirus Diseases

Contributing to the emergence of zoonotic diseases is the capacity of microorganisms and viruses to adapt to extremely diverse and changing econiches. One of the most complex sets of adaptations concerns the arboviruses and their transmission by specific arthropods. When ecosystems are altered, disease problems of humans and animals follow. Population movements and the intrusion of humans and domestic animals into arthropod habitats have resulted in emergent disease episodes, some of which are the stuff of fiction. The classic example is the emergence of yellow fever when humans entered the Central American jungle to build the Panama Canal—many contemporary examples suggest that similar events will continue to occur. Deforestation and settlement of new tropical forest and farm margins have exposed farmers and domestic animals to new arthropods and the viruses they carry. Mayaro and Oropouche virus infections in Brazilian woodcutters who cleared the Amazonian forest in recent years is a case in point. The opening up of isolated ecosystems has contributed to emergent disease episodes. Remote econiches, such as islands, with immunologically naive potential reservoir hosts and vectors are often particularly vulnerable to an introduced virus. For example, the initial Pacific island-hopping of Ross River virus in the 1980s from its original econiche in Australia caused "virgin soil" epidemics of arthritis-myalgia syndrome in Fiji and Samoa—this virus will surely reemerge. Increased long-distance air travel facilitates the movement of infected persons and exotic arthropod vectors around the world. The introduction of the Asian mosquito *Aedes albopictus* to the United States in water contained in used tires represents an unsolved problem of this kind. Increased long-distance livestock transportation facilitates the movement of viruses and arthropods (especially ticks) around the world. The introduction and emergence of African swine fever virus from Africa into the Americas in the 1960s and 1970s seem prophetic; although this virus is not zoonotic (it does not infect humans), this experience should raise the question concerning possible transport of Crimean-Congo hemorrhagic fever virus or other tick-borne pathogens to new locales. Ecologic factors pertaining to uncontrolled urbanization and environmental pollution are contributing to many emergent disease episodes. Arthropod vectors breeding in accumulations of water (e.g., tin cans, old tires) and sewage-laden water are a problem worldwide. Environmental chemical toxicants (herbicides, pesticides, residues) can also affect vector-virus relationships directly or indirectly. Ecologic factors related to expanding primitive irrigation systems are becoming important in virus

disease emergence, as exemplified by the emergence of Japanese encephalitis in newly developed rice-growing areas of southern Asia. New routings of long-distance bird migrations, brought about by new man-made water impoundments, represent an important yet still untested risk of introduction of arboviruses into new areas. Global warming, which affects sea level, estuarine wetlands, fresh water swamps, and human habitation patterns, may also be affecting vector-virus relationships throughout the tropics; however, data are scarce and long-term programs to study the effect of global warming have too often not included the participation of tropical medicine experts.

Of all the ecologic factors contributing to arthropod-borne zoonotic viral disease emergence, uncontrolled urbanization is the most important. The mega cities of the tropics, with their lack of sanitary systems, serve as incubators for emerging zoonoses—they represent the most difficult zoonotic disease risks of the next century. Who will pay to control disease in these cities? How will the World Health Organization (WHO) and the Pan American Health Organization (PAHO) serve the needs of the people in these cities? How will CDC serve the interests of the people of the United States in preventing emergent zoonotic diseases from emigrating from these cities? Lessons from the past suggest that we need a larger national and international enterprise to deal with emergent zoonoses in such settings, but even more we need an adaptable enterprise, one that can adjust quickly to diverse episodes.

Lessons from Venezuelan Equine Encephalitis Epidemics

Past Venezuelan equine encephalitis epidemics provide lessons regarding today's zoonotic disease prevention and control systems. In 1971, as the virus crossed from Mexico into Texas, agricultural disease control authorities were prepared to start shooting and burying horses in a massive slaughter campaign. Scientists from CDC and the Middle America Research Unit (at the time a unit of the National Institutes of Health) provided the virologic and epidemiologic base to override the sanitary rifle strategy of agricultural authorities, and the U.S. Army provided its then new TC83 vaccine. Conflict between agricultural and public health agencies was rampant; if this kind of emergency happened again, the response might not be much different. If the epidemic in Venezuela and Columbia in 1995 had progressed and jumped north, which agency would have stepped forward to direct control activities? What would have been done? Do we have an interagency plan? The same question might be asked in regard to the possible introduction of Rift Valley fever virus into the United States. In my view, our government institutional culture fails in long-term, interdisciplinary, interagency strategy development—we need strategies that are proof-tested to ensure success.

There is another lesson from the 1971 and 1995 Venezuelan equine encephalitis epidemics. Thirty years ago the arbovirus community was large, very experienced in field work and disease control actions, and holistic in perspective and expertise. Arbovirologists were able to bring together all necessary expertise—entomology and vector biology, ecology, mammology, ornithology, epidemiology, and virology. However, today this community, like so many others supporting zoonotic public health programs, is very small, rather poorly experienced in field work, and scientifically fragmented. Experts on mosquito biology, genetics, ecology, and vector competence are becoming more and more separated from the people in local mosquito control agencies who are expected to terminate epidemics. We had better fix this, organizationally and culturally, if we are to deal with mosquito-borne diseases in the 21st century.

Lessons from the Equine Morbillivirus Outbreak in Australia

Recent experiences in Australia with a new morbillivirus disease add still more lessons in zoonotic disease prevention and control. In 1994, horses on a property in Queensland developed acute respiratory distress with hemorrhagic manifestations—14 of 21 infected horses died. A horse trainer and a stable-hand became ill after nursing a sick horse—the trainer died. The disease was found to be caused by a previously unknown morbillivirus. Remarkably, in 1996 fruit bats (flying foxes) were found to be the natural host of the virus. Studies are under way to unravel these findings.

One lesson is similar to that taught by experiences with Venezuelan equine encephalitis. In Australia, where animal disease research is organized on a national basis but human disease research (and prevention and control

activities) on a state basis, this disease was given over to the Australian Animal Health Laboratory. One can imagine the public outcry if it had turned out that humans were at greater risk than horses. Again, cooperation across a wide range of institutions is required to deal with zoonoses, but when human health is at risk, I cannot imagine our public health institutions deferring to animal disease and agricultural institutions. Similar turf issues have been raised in the United States and in the United Kingdom in regard to the recent episode of H5N1 influenza in chickens and humans in Hong Kong.

Lessons from Ebola Hemorrhagic Fever Epidemics

Should we be concerned about Ebola virus? Is there a risk to Africa that compares with the everyday problems of other zoonoses such as malaria or yellow fever? Is there a risk to people in North America or Europe? If the worst that might happen is an occasional importation resulting in a small cluster of cases, should we be concerned? If the time and place of such episodes are unpredictable, should we not just wait and react after the fact? The risk reflected in these questions is difficult to evaluate because we know so little. However, we can say that as western-style hospitals become more affordable for Africans, nosocomial Ebola amplification will increase, and epidemics will get larger.

These viruses and the diseases they cause need to be understood because the risk they represent is unknown and the risk for future episodes is so unpredictable—the same should be said in regard to all similarly lethal zoonotic pathogens. For example, we need to find the natural reservoir of Ebola virus and learn how its prevalence in its natural environment and how transmission to humans are regulated. In Africa, the emergence of Ebola virus could dramatically increase if its still unknown reservoir host(s) increased, the virus changed its behavior, or ecologic factors brought additional reservoir hosts into play. We need to know enough to detect such changes quickly. The concerned public would not be satisfied if public health leaders decided on a wait-and-see approach for dealing with Ebola hemorrhagic fever or other diseases with similar pathogenic potential.

Dealing with Ebola virus and similar very dangerous infectious agents need not be thought of as so expansive or expensive as to be unrealistic. Field-based epidemiologic studies are needed; diagnostic systems require better placement in laboratories in Africa. Training is a major need—not through short courses, but rather through advanced career training and experience; transcending these is the need for an expanded research base, which in turn requires more national laboratory facilities and resources for work at biosafety level (BSL) 4. These needs must be met in all industrialized countries on behalf of developing countries.

Lessons from Rabies Epidemics

Rabies provides many lessons in how viral adaptation contributes to emergence in new econiches. Often, the necessary ecologic elements are in place and the recipe for emergence simply involves the introduction of virus; a dramatic illustration was the appearance of epidemic raccoon rabies in the eastern United States. The epidemic was traced to raccoons imported from Florida to West Virginia in 1977—as usual, human perturbation of an ecosystem, in this instance involving the transport of wild raccoons from an endemic site, caused trouble. One key to our understanding of this episode was the discovery that rabies virus is not one virus; rather, it is a set of different genotypes, each transmitted within a separate reservoir host econiche. In North America, there are six terrestrial animal genotypes, including the raccoon virus genotype. Raccoons bite raccoons that bite raccoons, and after some time, their virus becomes a distinct genotype, highly adapted to the host cycle. When the full significance of this discovery was realized, many mysteries of rabies ecology were clarified. The lesson here is that modern virologic research is the key for prevention and control programs such as those carried out by the CDC Rabies Laboratory and the Texas State Health Department, which is achieving much success with its coyote vaccination program.

Lessons from the Hantavirus Pulmonary Syndrome Epidemic

In 1993, hantavirus pulmonary syndrome was first recognized in the southwestern United States. Cases have been found in 28 states; as of 1997, more than 164 cases had been confirmed in

the United States and more than 400 throughout the Americas—the death rate has been approximately 45%. At the beginning of the investigation, serologic tests provided the first clue about the nature of the causative virus. Viral RNA was amplified from patient specimens, and a previously unknown hantavirus, now named Sin Nombre virus, was uncovered. Later, scientists from CDC, the University of New Mexico, and elsewhere found that several variant viruses were distributed over large areas of the United States, all previously unknown, all entrenched in specific rodent reservoirs, all capable of zoonotic transmission to humans.

The laboratory and field work resembled fundamental field- and laboratory-based research, not a traditional outbreak investigation. Sin Nombre virus and its relatives could only be dealt with in laboratories with the most sophisticated molecular biologic and immunologic technologies, the most expert staff scientists, and the kind of global perspective seen in WHO international reference centers. If scientists in these laboratories compete rather than collaborate, how will public health be given priority? How will technology transfer occur as rapidly as needed? How will the full capacity of more specialized biomedical research laboratories be brought to bear?

The tradition of public service holds the answer. When the rabies immunofluorescence test was developed at CDC, it was made available immediately to state and other laboratories. When *Legionella pneumophila* was discovered, cultures and reagents were made available immediately to everyone concerned. This tradition, in turn, has led over the years to the immediate transfer to CDC of new infectious agents isolated in other laboratories—Marburg virus from Germany, Lassa virus from Yale, HIV from France, poliovirus isolates from everywhere. Research competition has never been the point—public health has been the purpose at hand. The perpetuation of this tradition seems extremely important.

Lessons from the Bovine Spongiform Encephalopathy Epidemic in Cattle and New-Variant Creutzfeldt-Jakob Disease in Humans

Bovine spongiform encephalopathy (BSE) in the United Kingdom may provide more lessons than any other recent emergent zoonotic disease episode. The disease was first diagnosed in the United Kingdom in 1986; as of 1997, more than 170,000 cattle had been reported as infected, but modern statistical methods have indicated that about one million cattle had been infected, roughly half of which entered the human food chain in the United Kingdom.

Today, with the wisdom of hindsight, it might be said that the ministry of agriculture in the United Kingdom failed to react in time to what was clearly a great risk to the livestock and related food industries of the country—every element of its disease prevention and control responsibilities might be called into question. By 1990, the front pages of British newspapers were filled with BSE articles, forcing the question "…does BSE pose a risk to human health?" British government officials responded, "…there is nothing to worry about…" This of course led the public to become more skeptical. The editors of the journal Nature reacted as follows:

> …Never say there is no danger [risk]. Instead, say that there is always a danger [risk], and that the problem is to calculate what it is… Never say that the risk is negligible unless you are sure that your listeners share your own philosophy of life…

In my view, this response sums up one of the central precepts of public health practice.

In 1995, the BSE agent was reported to be the cause of a new human zoonotic disease, new-variant Creutzfeldt-Jakob disease. By 1997, 26 cases had been reported in the United Kingdom and one in France. A recent report from The Royal Society states that there is now a compelling case regarding new-variant Creutzfeldt-Jakob disease as the human manifestation of BSE. With such a small number of cases, it is impossible to predict future numbers of cases of the human disease, but clearly the damage to the livestock and related food industries of the United Kingdom will continue. BSE may be instructive in other ways, especially in its extension into the worlds of macroeconomics, international trade, political science, and even global governance.

In all these lessons, one of the most important points is the need for greater epidemiologic resources and better trained professionals for dealing with human and animal diseases or with

the zoonotic interface between the two. This training component requires consideration of all steps along the discovery-to-control continuum.

The Discovery-to-Control Continuum as Applied to Zoonotic Diseases

Initial investigation at the first sign of emergence of a new zoonotic disease must focus on practical characteristics such as death rate, severity of disease, transmissibility, and remote spread, all of which are important predictors of epidemic potential and societal risk. Various elements of a discovery-to-control continuum are usually called for: discovery, the recognition of a new zoonotic disease in a new setting; epidemiologic field investigation; etiologic investigation; diagnostics development; focused research; technology transfer; training and outreach; and ultimately control, elimination, and eradication. Of course, not all of these elements are appropriate in every emerging zoonotic disease episode—decisions must be made and priorities must be set.

In the initial phases in the discovery-to-control continuum, people outside the "citadel" (the traditional federal community of investigators and officials) must be recognized—local clinicians, pathologists (including medical examiners and forensic pathologists), veterinarians and animal scientists, ecologists, wildlife scientists, as well as local public health officials, many of whom have not been enamored of their experiences in dealing with those inside the citadel. The important early role of primary diagnostic laboratories and the reference laboratory networks that support them must also be recognized. In this era of the primacy of molecular microbiology and virology, it bears reminding that many of the early investigative activities surrounding the identification of a possibly emergent zoonotic disease must be carried out in the field, not in the laboratory. This is the world of shoe-leather epidemiology (the logo of CDC's Epidemic Intelligence Service program is the outline of the sole of a shoe with a prominent hole worn in it), as well as of molecular microbiology and virology.

In the intermediate phases in the discovery-to-control continuum, the continuum progresses to the general area of risk management, the area represented not by the question what's going on here? but by the question what are we going to do about it? This phase may include expansion of many elements: technology transfer involving diagnostics development and proof testing, vaccine and drug development and proof testing, sanitation and vector control, and medical and veterinary care activities and their adaptation to the circumstances of the disease locale; commercialization, where appropriate, of diagnostics, vaccines, and therapeutic agents in quantities needed and provision of these materials through nongovernment organizations or government sources; training, outreach, continuing education, and public education, each requiring professional expertise and adaptation to the special circumstances of the disease locale; and communications, employing the technologies of the day such as the Internet and professional expertise.

Further along the discovery-to-control continuum, activities become more complex. Frustration often occurs at intermediate points as administrators and politicians drag their feet in regard to resource allocation. This frustration, in turn, drives scientists back to their laboratories, to the world of research, to the front end of the continuum. Younger scientists, particularly, become cynical of the harsh political world of risk management, even though this is the arena in which their discoveries must prove themselves.

More expensive and specialized expertise and resources come into play in the final phases of the discovery-to-control continuum: public health systems, including rapid case-reporting systems, surveillance systems, vital records and disease registers, staffing and staff support, logistic support, legislation and regulation, and expanded administration; special clinical systems, including isolation of cases, quarantine, and patient care; and public infrastructure systems, including sanitation and sewerage, safe food and water supplies, and reservoir host and vector control.

The question of facilities needs in the United States is an element of our capacity to fulfill the discovery-to-control continuum. What about BSL-3+ and BSL-4 laboratory facilities west of the Appalachians? Recent debate makes it clear that having two BSL-4 facilities in the United States (CDC in Atlanta, and the U.S. Army Medical Research Institute of Infectious Diseases in Frederick, Maryland) and one in Canada (at the new center in Winnipeg) is not enough. Plans for a few small BSL-4 labs in U.S. academic centers may help in expanding basic research

supported by competitive grants, but they will not support expanded field-based research. Which government agency will step forward to solve this problem? And in a related way, which government agency will step forward to solve the unique problem of career-committed professional personnel needs for dealing with emerging zoonotic diseases?

Conclusions

Who will be the world's doctor? Who will be the world's expert on zoonotic diseases? These questions are taken from an editorial in the New York Times, May 12, 1995. It seems that many authorities, including those at CDC, are saying that they have the answer to these questions in regard to all emerging diseases. Their answers have been in the form of proposals and funding requests to expand global disease surveillance, diagnostics, communications, and emergency response systems, a global training program, and a global stable funding base. However, somewhat distinct strategies are needed to deal specifically with emerging zoonotic diseases, and these strategies have not been fully developed. Examples have been given in this paper to suggest that these strategies must involve more of a field and laboratory research enterprise than a traditional surveillance and reference diagnostics enterprise. In some cases, it is not even clear who might do the focused applied research that must underpin advances in zoonotic disease prevention and control. In present circumstances, where the survival of institutions is at stake, turf battles are exacerbated, and competition rather than cooperation between academic institutions and government agencies ensues. CDC may be getting new funds, but there is no parallel sense of "good times ahead" out in the country. This is happening in contradiction to public expectations. Data clearly show that the concerned public wants more disease control and intervention actions, more of the medical research needed to drive such actions, and more participation across the country. Numerous surveys of public opinion done by Research!America show that the concerned public is willing to pay. In my view, public expectations can only be met by the integration of the nascent global public health emerging infectious disease network, with networks focused on threats posed by livestock animal diseases, crop plant diseases, and bioterrorism. The public would see such an overall system as having a high benefit:cost ratio, which would solve several high priority problems most efficiently.

Frederick Murphy is professor of virology at the School of Veterinary Medicine, University of California, Davis. He has served as director of the Division of Viral and Rickettsial Diseases as well as director of the National Center for Infectious Diseases, Centers for Disease Control, and dean of the School of Veterinary Medicine, UC Davis. His professional interests include the pathogenesis and ultrastructural pathology of viral diseases, viral characterization and taxonomy, rabies, arboviruses, viral hemorrhagic fevers, viral encephalitides, public health policy, vaccine development, and new and reemerging infectious diseases.

George Hill (L)
Meharry Medical College,
Nashville, Tennessee, USA
Frederick A. Murphy
University of California,
Davis, California, USA

Influenza: An Emerging Disease

Robert G. Webster
St. Jude Children's Research Hospital, Memphis, Tennessee, USA

Because all known influenza A subtypes exist in the aquatic bird reservoir, influenza is not an eradicable disease; prevention and control are the only realistic goals. If people, pigs, and aquatic birds are the principal variables associated with interspecies transfer of influenza virus and the emergence of new human pandemic strains, influenza surveillance in these species is indicated. Live-bird markets housing a wide variety of avian species together (chickens, ducks, geese, pigeon, turkeys, pheasants, guinea fowl), occasionally with pigs, for sale directly to the public provide outstanding conditions for genetic mixing and spreading of influenza viruses; therefore, these birds should be monitored for influenza viruses. Moreover, if pigs are the mixing vessel for influenza viruses, surveillance in this population may also provide an early warning system for humans.

The influenza virus continues to evolve, and new antigenic variants (drift strains) emerge constantly, giving rise to yearly epidemics. In addition, strains to which most humans have no immunity appear suddenly, and the resulting pandemics vary from serious to catastrophic.

Influenza viruses are unique among respiratory tract viruses in that they undergo considerable antigenic variation. Both surface antigens of the influenza A viruses undergo two types of variation: drift and shift (1). Antigenic drift involves minor changes in the hemagglutinin (HA) and neuraminidase (NA), whereas antigenic shift involves major changes in these molecules resulting from replacement of the gene segment.

The Reservoirs of Influenza A Viruses

Aquatic birds are the reservoirs of all 15 subtypes of influenza A viruses. In wild ducks, influenza viruses replicate preferentially in the cells lining the intestinal tract, cause no disease signs, and are excreted in high concentrations in the feces (up to $10^{8.7}$ 50% egg infectious doses/g) (2). Avian influenza viruses have been isolated from freshly deposited fecal material and from unconcentrated lake water, which indicates that waterfowl have a very efficient way to transmit viruses, i.e., by fecal material in the water supply. Since a large number of susceptible young ducks are hatched each year throughout the world, many birds are infected by virus shed into water. This would explain the high incidence of virus infection in Canadian ducks, particularly juveniles, when up to 30% can shed virus before fall migration. Transmission by feces also provides a way for wild ducks as they migrate through an area to spread their viruses to other domestic and feral birds (3).

The avirulent nature of avian influenza infection in ducks and wading birds may result from virus adaptation to this host over many centuries, which created a reservoir that ensures perpetuation of the virus; therefore, ducks and wading birds may be occupying an important position in the natural history of influenza viruses. Influenza viruses of avian origin have been implicated in outbreaks of influenza in mammals, such as seals (4), whales (5), and pigs (6), as well as in domestic poultry (7).

Evolutionary Pathways for Influenza Viruses

Studies on the ecology of influenza viruses have led to the hypothesis that all mammalian influenza viruses derive from the avian influenza reservoir. Support for this theory comes from phylogenetic analyses of nucleic acid sequences of influenza A viruses from a variety of hosts, geographic regions, and virus subtypes. Analyses of the nucleoprotein (NP) gene show that avian influenza viruses have evolved into five host-

Address for correspondence: Robert G. Webster, St. Jude Children's Research Hospital, 332 North Lauderdale St., Memphis TN 38105-2794, USA; fax: 901-523-2622; e-mail: robert.webster@stjude.org.

specific lineages: ancient equine, which has not been isolated in over 15 years; recent equine; gull; swine; and human. The human and classic swine viruses have a genetic "sister group" relationship, which shows that they evolved from a common origin. The ancestor of the human and classic swine virus appears to have been an intact avian virus that, like the influenza virus currently circulating in pigs in Europe, derived all its genes from avian sources (8,9).

Studies on the NP and other gene lineages in avian species show separate sublineages of influenza in Eurasia and the Americas, indicating that migratory birds moving between these continents (latitudinal migration) have little or no role in the transmission of influenza, while birds that migrate longitudinally appear to play a key role in the continuing process of virus evolution.

Phylogenetic analyses of amino acid changes show that avian influenza viruses, unlike mammalian strains, have low evolutionary rates (8). In fact, influenza viruses in aquatic birds appear to be in evolutionary stasis, with no evidence of net evolution over the past 60 years. Nucleotide changes have continued at a similar rate in avian and mammalian influenza viruses; however, these changes no longer result in amino acid changes in the avian viruses, whereas all eight mammalian influenza gene segments continue to accumulate changes in amino acids. The high level of genetic conservation suggests that avian viruses are approaching or have reached optimum, wherein nucleotide changes provide no selective advantage. It also means that the source of genes for pandemic influenza viruses exists phenotypically unchanged in the aquatic bird reservoir. The most important implication of phylogenetic studies is that the ancestral viruses that caused the Spanish flu in 1918, as well as the viruses that provided gene segments for the Asian/1957 and Hong Kong/1968 pandemics, are still circulating in wild birds, with few or no mutational changes.

Emergence and Reemergence of "New" Influenza A Virus in Humans

Over the past two and a half centuries, 10 to 20 human influenza pandemics have swept the globe; the most devastating, the so-called Spanish flu of 1918 to 1919, caused more than 20 million deaths and affected more than 200 million people. Both pandemics probably originated from aquatic birds.

Since the first human influenza virus was isolated in 1933, new subtypes of human type A influenza viruses have occurred: H2N2 (Asian influenza) replaced H1N1 in 1957, Hong Kong (H3N2) virus appeared in 1968, and H1N1 virus reappeared in 1977. Each of these new subtypes first appeared in China, and anecdotal records suggest that previous epidemics also had their origin in China. Serologic and virologic evidence suggests that since 1889 there have been six instances of the introduction of a virus bearing an HA subtype that had been absent from the human population for some time. Three human subtypes of HA have appeared cyclically—H2 viruses in 1889, H3 in 1900, H1 in 1918, H2 again in 1957, H3 again in 1968, and H1 again in 1977. Phylogenetic evidence indicates that a totally new H1N1 virus of avian origin (not a reassortant) could have appeared in humans or swine before the 1918 influenza and replaced the previous human virus strains. Whether the virus was first introduced into humans and then transmitted to pigs, or vice versa, remains unknown. The reappearance of the H1N1 Russian 1977 influenza virus remains a mystery.

How Are Influenza Viruses Spread?

Avian influenza viruses in wild aquatic birds are spread by fecal-oral transmission through the water supply (10); initial transmission of avian influenza viruses to mammals, including pigs and horses, probably also occurs by fecal contamination of water. Scholtissek has postulated that the use of fecal material from ducks for fish farming in Asia may contribute to transmission of avian influenza viruses to pigs (11). Another direct method of transfer is by feeding pigs untreated garbage or the carcasses of dead birds. Raising pigs under chicken houses and feeding them dead avian carcasses has been observed on rare occasions in the United States; H5N2 influenza virus was isolated from pigs living under chicken houses in Pennsylvania during the outbreak in 1982. Both pigs and poultry are commonly raised on the same commercial farms. From the perspective of the control of interspecies transmission of influenza, this is undesirable, for it may facilitate interspecies transmission of influenza viruses. After transmission to pigs, horses, or humans, the method of spread of influenza is mainly respiratory.

Emergence of H5N2 Influenza Viruses in North America

In 1983 an H5N2 influenza virus infected chickens and turkeys in Pennsylvania and became highly pathogenic for poultry. Virologic and serologic studies provided no evidence of transmission to humans (12). The virus was eventually eradicated by quarantine and extermination of more than 17 million birds at a direct cost of more than US$60 million and an indirect cost to the industry of more than US$250 million.

More recently, a highly pathogenic H5N2 influenza virus emerged in domestic chickens in Mexico (7). In October 1993, egg production decreased and deaths increased among Mexican chickens in association with serologic evidence of an H5N2 influenza virus. H5N2 virus was isolated in May 1994. By the end of 1994, the virus had mutated to contain a highly cleavable HA, but remained only mildly pathogenic in chickens. Within months, however, it had become lethal in poultry. Phylogenetic analysis of the HA of H5 avian influenza viruses, including the Mexican isolates, indicated that the epidemic virus had originated from the introduction of a single virus of the North American lineage into Mexican chickens (Figure 1). This virus was eradicated from chickens by quarantine and use of inactivated vaccine.

Live Bird Markets and the Epidemiology of Influenza

The chicken/Pennsylvania (H5N2) influenza outbreak in 1983 to 1984 demonstrated that live bird markets play an important part in the spread of influenza viruses in avian species. In 1992, Senne et al. (13) described live bird markets as the "missing link in the epidemiology of avian influenza," for H5N2 viruses had been isolated from live birds until 1986. These H5N2 viruses caused subclinical infection in chickens in the markets, as did H5N1 viruses in live bird markets in Hong Kong in 1997 (Figure 2). Moreover, ducks in the markets in the United States were infected with many different subtypes of influenza A viruses, including H2N2 viruses related antigenically to the Asian/57 (H2N2) viruses that have disappeared from humans.

The live bird markets in the United States continue to harbor many influenza viruses. The ancestor of the H5N2 influenza virus that caused the epidemic in Mexico in 1993 to 1995 was isolated from market birds, and H7NX subtypes are still found in live bird markets. These viruses are potentially pathogenic for chickens and are of

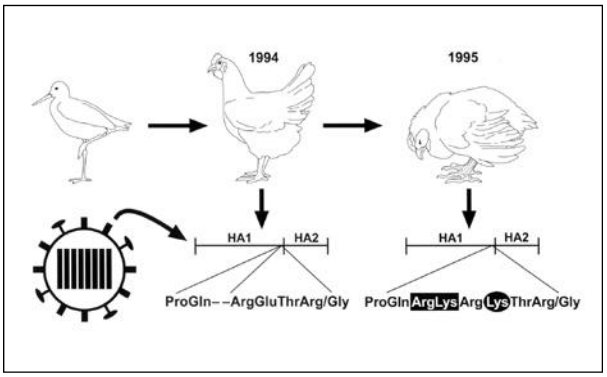


Figure 1. Molecular changes associated with emergence of a highly pathogenic H5N2 influenza virus in chickens in Mexico. In 1994, a nonpathogenic H5N2 influenza virus in Mexican chickens was related to an H5N2 virus isolated from shorebirds (ruddy turnstones) in Delaware Bay, United States, in 1991. The 1994 H5N2 isolates from chickens replicated mainly in the respiratory tract, spread rapidly among chickens, and were not highly pathogenic. Over the next year the virus became highly pathogenic, and the hemagglutinin acquired an insert of two basic amino acids (Arg-Lys), possibly by classic recombination and a mutation of Glu to Lys at position – 3 from the cleavage site of HA1/HA2.

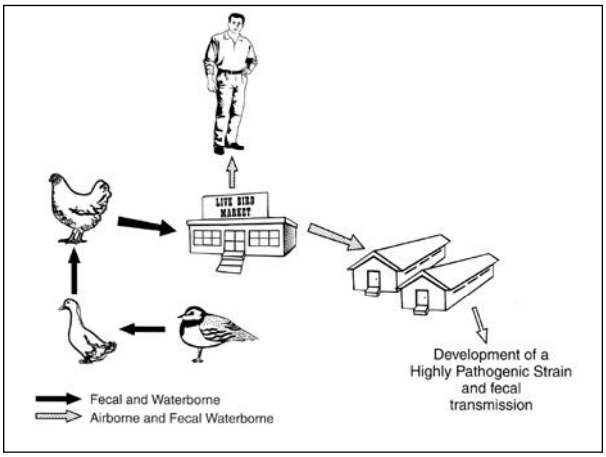


Figure 2. The emergence of H5N1 influenza in Hong Kong. It is postulated that a nonpathogenic H5N1 influenza spread from migrating shorebirds to ducks by fecal contamination of water. The virus was transmitted to chickens and became established in live bird markets in Hong Kong. During transmission between different species, the virus became highly pathogenic for chickens and occasionally was transmitted to humans from chickens in the markets. Despite high pathogenicity for chickens (and humans), H5N1 were nonpathogenic for ducks and geese.

great concern to chicken farmers in the northeastern United States.

The depopulation of live bird markets and farms in the New Territories of Hong Kong (December 29, 1997) stopped the spread of H5N1 influenza viruses. An important lesson can be learned from this action in Hong Kong. Live bird markets are potential breeding grounds for both avian and mammalian influenza viruses. Serologic monitoring of the chickens in Hong Kong markets for H5N1 influenza virus was an important first step in stopping the spread of the viruses. An even more important step would be to reduce the opportunity for interspecies transmission by marketing chickens separately from other avian species.

The Index Case of H5N1 in Humans in Hong Kong

On May 21, 1997, a 3-year-old boy from Hong Kong died in an intensive care unit in Hong Kong on the fifth day of his hospitalization, with a final diagnosis of Reye syndrome, acute influenza pneumonia, and respiratory distress syndrome (14). He had no indications of other underlying disease, including immunodeficiency or cardiopulmonary disease. From a tracheal aspirate, we isolated an influenza virus in MDCK cells but were unable to grow any pathogenic bacteria from respiratory specimens. In hemagglutination inhibition assays, the virus did not react with ferret antisera to recent isolates of human and swine subtypes.

Hemagglutination inhibition assays using antisera to 14 H subtypes showed that the isolate was an H5 influenza A virus. Neuraminidase inhibition tests, using antisera to nine N subtypes, indicated that the neuraminidase was of the N1 subtype. Nucleotide sequence analyses of parts of the HA and NA genes of the virus allowed a phylogenetic comparison with other influenza viruses. Our analyses confirmed that the virus was of the H5N1 subtypes. Each of the eight RNA segments wase of avian origin, and the virus was highly pathogenic for chickens. The contribution of the influenza A H5N1 virus infection to the child's disease, eventually leading to death, was complicated by the child's treatment with aspirin. The virus identification is important because it is the first documented isolation of an influenza A virus of this subtype from humans (15).

Characterization of the Human and Chicken H5N1 Viruses from Hong Kong

Avian influenza outbreaks occurred in Hong Kong from late March to early May of 1997. Three chicken farms were separately affected; the death rate for the total of 6,800 chickens exceeded 70%. A comparison between the nucleotide sequences of the H5 genes from both the human virus A/Hong Kong/156/97 (H5N1) (HK97) and a representative of the chicken viruses from the March outbreak, A/chicken/Hong Kong/258/97 (CkHK97), showed a high degree of homology in their respective H5 HA1 sequences. Only three amino-acid differences were observed in the HA1 of the HA, confirming the close phylogenetic relationship between these viruses, belonging to the Eurasian lineage of the subtype H5 viruses.

Sequence analyses of the HA of multiple human and chicken H5N1 isolates show that they form two subgroups with close linkage between chicken and human isolates. An analysis of the amino acids expected to be involved in the assembly of the receptor binding site showed no differences could be observed between the human isolate and avian H5 viruses. Therefore, the H5 HA of HK97 had probably not acquired mutations that favor binding to sialic acids with 2,6 linkage to the galactoside over the 2,3 linked sialic acid receptor preferred by human and avian viruses respectively. However, the loss of a potential N-linked glycosylation site at amino acid 156 Asn, close to the receptor binding site, could affect binding to the cellular receptor.

The amino acid sequence motif at the cleavage site of the HA molecule has been associated with high virulence of avian influenza viruses. Experimental infection of chickens with HK97 showed that even after passaging in mammalian cells (once in the child and twice in MDCK cells), the virus remained highly pathogenic for chickens: all eight chickens inoculated intratracheally with MDCK-grown HK97 died within 3 days after infection. A comparison of the reactivity of a panel of 17 monoclonal antibodies (MAb) directed against A/chicken/Pennsylvania/83 (H5N2) with HK97 and CkHK97 in a hemagglutination inhibition assay showed similar antigenic reactivities with all but one MAb, indicating antigenic cross-reactivity between these viruses and the usefulness of these antibodies for diagnosis.

The fetuin-cleaving activity of the NA proved to be inhibited by anti-NA antiserum. Reverse

transcriptase polymerase chain reaction using primer sets that amplified the 5' end of the NA gene segments showed that this gene was of the N1 genotype. Nucleotide sequence analysis and comparison to published NA sequences confirmed this finding genetically. The NA sequences unequivocally showed a close molecular relationship between HK97 and CkHK97, as a unique 57-nucleotide deletion was observed in the stalk region of the N1 gene of both viruses. Each of the eight gene segments showed close genetic homology between the HK97 and Ck/HK97 viruses, the lowest being 98.2% for the nucleoprotein; the remaining genes varied from 98.8% to 100% homology (16).

Can the Emergence of Pandemic Strains Be Prevented?

Because all known influenza A virus subtypes are found in aquatic wild birds in nature, agricultural authorities have recommended avoiding direct or indirect contact between domestic poultry and wild birds. A classic mistake made by chicken and turkey farmers is to raise a few domestic ducks on a pond near poultry barns; these birds attract wild ducks. The highly pathogenic outbreaks of H5N2 avian influenza in chickens and turkeys in Pennsylvania and surrounding states in 1983 to 1984 (12) and the H5N2 in Mexico in 1993 (7) could probably have been prevented if domestic poultry had been raised in ecologically controlled houses with a high standard of security and limited access.

If we assume that people, pigs, and aquatic birds are the principal variables associated with the emergence of new human pandemic stains, human pandemics of influenza may be prevented. The principles applied to preventing outbreaks of influenza in domestic animals should apply equally here. Pandemic strains of human influenza emerge only rarely; however, interspecies transmission of influenza viruses may not be so rare, for up to 10% of persons with occupational exposure to pigs develop antibodies to swine influenza virus (17). Most transfers of influenza viruses from pigs to humans are dead-end transfers (they do not spread efficiently from human to human). As indicated above, we do not know the frequency of virus transfer between the suspect species in southern China. If there is an epicenter for pandemic influenza and a detectable frequency of transfer between people, pigs, and ducks and if we understand the ecologic and agricultural features involved in the transfer, pandemics may be preventable. If pigs are the major mixing vessel for influenza viruses, changes in the agricultural practices that separate pigs from people and ducks could prevent future pandemics. Most importantly, we may influence the appearance of pandemics by changing the methods of live bird marketing by separating chickens from other species, especially from aquatic birds.

This work was supported by Public Health Service grants AI-29680 and AI-08831 from the National Institute of Allergy and Infectious Diseases, by Cancer Center Support (CORE) grant CA-21765, and by the American Lebanese Syrian Associated Charities.

Dr. Webster holds the Rose Marie Thomas Chair in the Department of Virology and Molecular Biology at St. Jude Children's Research Hospital, Memphis, Tennessee. In addition, he is director of the World Health Organization Collaborating Center for Ecology of Influenza Viruses in Lower Animals and Birds. He has devoted his life to the understanding of the emergence of pandemic influenza viruses, the structure and function of the viral proteins, and methods for developing new and improved antiviral drugs and vaccines.

References

1. Murphy BR, Webster RG. Orthomyxoviruses. In: Fields BN, Knipe DM, Howley PM, Chanock RM, Melnick JL, Monath TP, Roizman R, Straus SE, editors. Fields virology. New York: Raven Press; 1996. p. 1397-445.
2. Webster RG, Yakhno MA, Hinshaw VS, Bean WJ, Murti KG. Intestinal influenza: replication and characterization of influenza viruses in ducks. Virology 1978;84:268-78.
3. Halvorson D, Karunakaran D, Senne D, Kelleher C, Bailey C, Abraham A, et al. Epizootiology of avian influenza-simultaneous monitoring of sentinel ducks and turkeys in Minnesota. Avian Dis 1983;27:77-85.
4. Geraci JR, St. Aubin DJ, Barker IK, Webster RG, Hinshaw VS, Bean WJ, et al. Science 1982;215:1129-31.
5. Hinshaw VS, Bean WJ, Geraci JR, Fiorelli P, Early G, Webster RG. Characterization of two influenza A viruses from a pilot whale. J Virol 1986;58:655-6.
6. Scholtissek C, Burger H, Bachmann PA, Hannoun C. Genetic relatedness of hemagglutinins of the H1 subtype of influenza A viruses isolated from swine and birds. Virology 1983;129:521-3.
7. Horimoto T, Rivera E, Pearson J, Senne D, Krauss S, Kawaoka Y, et al. Origin and molecular changes associated with emergence of a highly pathogenic H5N2 influenza virus in Mexico. Virology 1995;213:223-30.

8. Gorman OT, Bean WJ, Kawaoka Y, Webster RG. Evolution of the nucleoprotein gene of influenza A virus. J Virol 1990;64:1487-97.
9. Gammelin M, Altmuller A, Reinhardt U, Mandler J, Harley VR, Hudson PJ, et al. Phylogenetic analysis of nucleoproteins suggests that human influenza A viruses emerged from a 19th-century avian ancestor. Mol Biol Evol 1990;7:194-200.
10. Hinshaw VS, Webster RG. The natural history of influenza A viruses. In: Beare AS, editor. Basic and applied influenza research. Boca Raton (FL): CRC Press; 1982. p. 79-104.
11. Scholtissek C, Naylor E. Fish farming and influenza pandemics. Nature 1988;331:215.
12. Bean WJ, Kawaoka Y, Wood JM, Pearson JE, Webster RG. Characterization of virulent and avirulent A/Chicken/Pennsylvania/83 influenza A viruses: potential role of defective interfering RNAs in nature. J Virol 1985;54:151-60.
13. Senne DA, Pearson JE, Panigrahy B. Live poultry markets: a missing link in the epidemiology of avian influenza. In: Proceedings of the 3rd International Symposium on Avian Influenza; 1997 27-29 May; The Wisconsin Center, The University of Wisconsin-Madison. p. 50-8.
14. De Jong JC, Claas ECJ, Osterhaus ADME, Webster RG, Lim WL. A pandemic warning. Nature 1997;389:554.
15. Subbarao K, Klimov A, Katz J, Regnery H, Lim W, Hall H, et al. Characterization of an Avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. Science 1998;279:393-6.
16. Claas ECJ, Osterhaus ADME, van Beek R, De Jong JC, Rimmelzwaan GF, Senne DA, et al. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet 1998;351:472-7.
17. Schnurrenberger PR, Woods GT, Martin RJ. Serologic evidence of human infection with swine influenza virus. Am Rev Respir Dis 1970;102:356-61.

Resurgent Vector-Borne Diseases as a Global Health Problem

Duane J. Gubler
Centers for Disease Control and Prevention, Fort Collins, Colorado, USA

Vector-borne infectious diseases are emerging or resurging as a result of changes in public health policy, insecticide and drug resistance, shift in emphasis from prevention to emergency response, demographic and societal changes, and genetic changes in pathogens. Effective prevention strategies can reverse this trend. Research on vaccines, environmentally safe insecticides, alternative approaches to vector control, and training programs for health-care workers are needed.

In the 120 years since arthropods were shown to transmit human disease, hundreds of viruses, bacteria, protozoa, and helminths have been found to require a hematophagous (blood-sucking) arthropod for transmission between vertebrate hosts (1). Historically, malaria, dengue, yellow fever, plague, filariasis, louse-borne typhus, trypanosomiasis, leishmaniasis, and other vector-borne diseases were responsible for more human disease and death in the 17th through the early 20th centuries than all other causes combined (1). During the 19th and 20th centuries, vector-borne diseases prevented the development of large areas of the tropics, especially in Africa; it was not until these diseases were controlled that engineering feats such as the Panama Canal could be completed (1,2).

Not long after the 1877 discovery that mosquitoes transmitted filariasis from human to human, malaria (1898), yellow fever (1900), and dengue (1903) were shown to have similar transmission cycles (2). By 1910, other major vector-borne diseases such as African sleeping sickness, plague, Rocky Mountain spotted fever, relapsing fever, Chagas disease, sandfly fever, and louse-borne typhus had all been shown to require a blood-sucking arthropod vector for transmission to humans (2).

Prevention and control programs were soon based on controlling the arthropod vector. Yellow fever in Cuba was the first vector-borne disease to be effectively controlled in this manner, followed quickly by yellow fever and malaria in Panama. Over the next 50 years, most of the important vector-borne public health problems were effectively controlled (Table 1). Most of these programs established vertically structured vector control organizations that emphasized elimination of arthropod breeding sites (source reduction) through environmental hygiene along with limited use of chemical insecticides. By the 1960s, vector-borne diseases were no longer considered major public health problems outside Africa. Urban yellow fever and dengue, both

Table 1. Successful vector-borne disease control/elimination programs

Disease	Location	Year(s)
Yellow fever	Cuba	1900-1901
Yellow fever	Panama	1904
Yellow fever	Brazil	1932
Anopheles gambiae infestation	Brazil	1938
An. gambiae infestation	Egypt	1942
Louse-borne typhus	Italy	1942
Malaria	Sardinia	1946
Yellow fever (*Aedes aegypti*)	Americas	1947-1970
Malaria	Americas	1954-1975
Malaria	Global	1955-1975
Yellow fever	West Africa	1950-1970
Onchocerciasis	West Africa	1974-present
Bancroftian filariasis	South Pacific	1970s
Chagas disease	South America	1991-present

Address for correspondence: Duane J. Gubler, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, P.O. Box 2087, Fort Collins, CO 80522, USA; fax: 970-221-6476; e-mail: djg2@cdc.gov.

transmitted by *Aedes aegypti*, were effectively controlled in Central and South America and eliminated from North America; malaria was nearly eradicated in the Americas, the Pacific Islands, and Asia. The discovery and effective use of residual insecticides in the 1940s, 1950s, and 1960s contributed greatly to these successes.

However, the benefits of vector-borne disease control programs were short-lived. A number of vector-borne diseases began to reemerge in the 1970s, a resurgence that has greatly intensified in the past 20 years (3-7). Although the reasons for the failure of these programs are complex and not well understood, two factors played important roles: 1) the diversion of financial support and subsequent loss of public health infrastructure and 2) reliance on quick-fix solutions such as insecticides and drugs.

The Global Emergence/Resurgence of Vector-Borne Diseases

Evidence of the reemergence of vector-borne diseases such as malaria and dengue was first observed in the 1970s in Asia and the Americas (5-9). Warnings, however, were largely ignored until recently (10), and now it may be difficult to reverse the trend.

Figure 1 shows some vector-borne parasitic, bacterial, and viral diseases that have caused epidemics in the 1990s. While malaria is the most important vector-borne disease because of its global distribution, the numbers of people affected, and the large number of deaths, the vector-borne viruses (arboviruses) are clearly the most numerous.

Malaria

The resurgence of malaria in Asia in the late 1960s and early 1970s provides a dramatic example of how quickly vector-borne disease trends can change. Malaria, transmitted to humans by anopheline mosquitoes, had been nearly eliminated in Sri Lanka in the 1960s, with only 31 and 17 cases reported in 1962 and 1963, respectively. By 1967, 3,468 cases were reported. In 1968, however, a major epidemic caused 440,644 cases. In 1969, 537,705 cases were reported (Figure 2a); the disease has never been effectively controlled since then. In India, a similar resurgence of malaria occurred (Figure 2b), with sporadic outbreaks of disease beginning in the early 1970s and nearly seven million cases by 1976. Sri Lanka and India are classic examples of the lack of sustainability of vertically structured prevention/control/elimination programs. Complacency, dwindling financial and political support, and a change in strategy from vector control to case finding and drug treatment were mainly responsible for the resurgence of malaria in these countries.

More recently, vivax malaria has reemerged in Korea (Figure 2c). Urban malaria in the Indian subcontinent and in parts of South America (Figure 2d) is also a major concern. In 1998, malaria is the most important tropical disease with more than half of the world's population living in areas of risk and with an estimated 200 million cases and two million deaths each year (11). Widespread drug resistance of the parasites and insecticide resistance among anopheline mosquito vectors have complicated malaria control (4).

Malaria is the most common imported disease in the United States, where anopheline mosquito vectors still exist (12). Approximately 1,000 suspected malaria cases are imported into the United States each year, associated with increased frequency of autochthonous cases; since 1987, 16 incidents of autochthonous malaria have occurred in nearly all parts of the United States. In each incident, however, transmission was limited to only a few cases (12).

African Trypanosomiasis

Historically, African sleeping sickness, transmitted by the tsetse fly, has been a major impediment to the social and economic development of Central and East Africa. With the use of modern drugs, insecticides, and other control methods, this disease was effectively controlled in most countries by the mid-1960s. In the past 20 years, however, major epidemics have occurred in East and Central Africa, mainly because control programs were disrupted by war (13). In the Sudan, the Republic of Congo, and Angola, which have high prevalence, poor surveillance, no drugs, and no vector control, the disease poses a major public health threat. Although available, some new drugs, vector control approaches, and diagnostic tests are not being used because of lack of funding support.

African sleeping sickness is a low-priority rural disease. Effective, sustainable control is unlikely until traditional uses of land change and socioeconomic conditions improve in rural Africa (13). The primary approach to control is treatment with drugs that are expensive and not

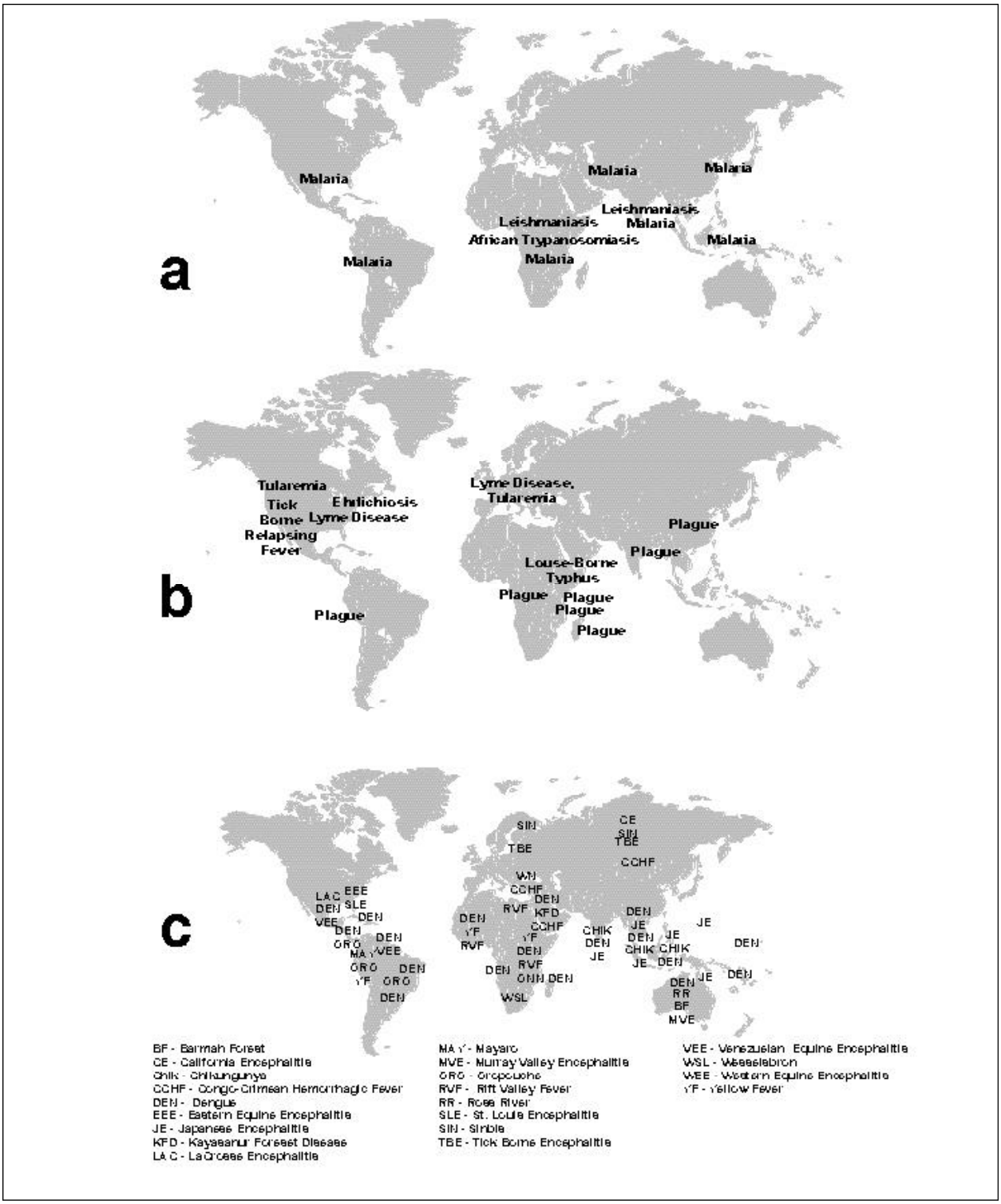


Figure 1. Epidemic vector-borne diseases, 1990–1997. A. parasitic diseases, B. bacterial diseases, C. arboviral diseases.

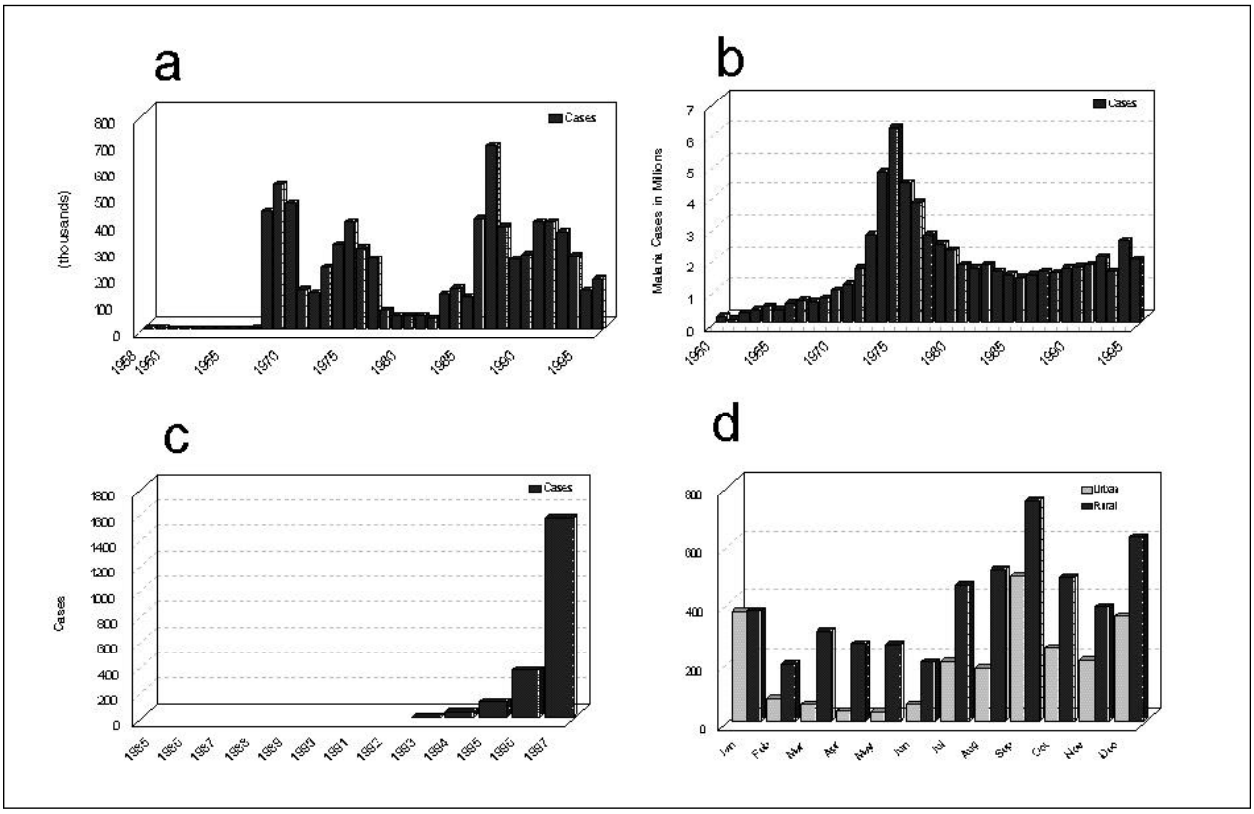


Figure 2. The resurgence of malaria. A. Sri Lanka (data from Tissa Vitarana, Office of Science and Technology, Sri Lanka); B. India (data from Shiv Lal, Director, National Malaria Eradication Program, India); C. Korea (data from Dan Strickman, Walter Reed Army Institute of Research; D. Manaus, Brazil (data from Bedsy Dutary, National Institute of Research of the Amazon).

readily available (11). To reverse this trend, an integrated sustainable control program must be implemented, including effective surveillance for case finding, a network of treatment centers with a supply of drugs, and vector control using trapping techniques (13).

Lyme Disease

Lyme disease, a bacterial tick-borne infection, is caused by *Borrelia burgdorferi*. Discovered in the United States in 1975, the disease has continued to increase in incidence and geographic distribution since national surveillance was initiated in 1982. At that time, 497 cases were reported compared with 11,700 to 16,455 cases each year between 1994 and 1997 (Figure 3) (cumulative total cases reported more than 109,000) (14). Lyme disease has a global distribution in the temperate regions. Because of the multistage disease and chronic complications associated with *B. burgdorferi* infection, Lyme disease has major public health and economic effects.

Lyme disease is transmitted by *Ixodes ricinus* complex hard ticks. In the United States, *I. scapularis*, the deer tick, is the vector in the eastern and midwestern states, and *I. pacificus* is the vector in the far western states. While human cases of apparent Lyme disease are reported from most states and many enzootic cycles of *B. burgdorferi* occur throughout the country, the public health importance of these cases is uncertain. Approximately 90% of reported Lyme disease cases occur each year in the Northeast (Connecticut, Maryland, Massachusetts, New Jersey, New York, Pennsylvania, and Rhode Island), upper Midwest (Minnesota and Wisconsin), and Northwest (California) (14).

Plague

Plague is the original emerging disease, having caused major pandemics; the most recent (late 19th century) is believed to be responsible for the current global distribution of the disease, which is spread by rats on ships (15). Like many other

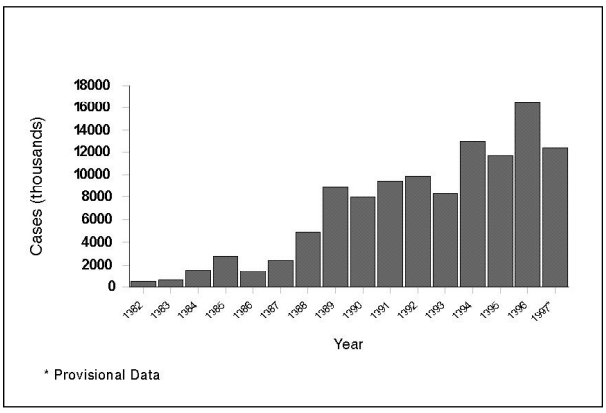


Figure 3. Reported cases of Lyme disease in the United States, 1982–1997.

vector-borne diseases, plague was controlled with antibiotics, insecticides, and rat control in the latter half of the 20th century. The number of cases reported to the World Health Organization decreased to an all-time low of 200 cases in 1981 (15). In recent years, however, epidemic plague has resurged, most notably in Africa, with an average of nearly 3,000 cases reported annually (approximately 65% from Africa) (15).

The decrease in plague incidence from 1950 to 1980 was followed by decreased financial support, lowered interest, and ultimately the deterioration of surveillance systems. Many countries were no longer capable of making a laboratory diagnosis of plague in the 1990s. For example, in 1994 when an outbreak of plague occurred in Western India (16) (which had reported its last case of plague in 1966), lack of laboratory capacity for diagnosis led to confusion as to the cause of the outbreak and panic within the population. An estimated 500,000 people fled Surat for other major cities, some of which subsequently reported secondary plague transmission (16).

Because effective epidemiologic investigation and an accurate laboratory diagnosis were not made in time, a relatively unimportant, focal public health event turned into an international public health emergency costing the Indian and the global economies billions of U.S. dollars (17). Plague can cause explosive epidemics when effective laboratory-based surveillance and prevention and control are not maintained in countries with enzootic disease. An important lesson learned from this incident was that laboratory-based international infectious disease surveillance is cost-effective.

Dengue

Dengue fever caused major epidemics from the 17th to the early 20th centuries (18). In most Central and South American countries, effective disease prevention was achieved by eliminating the principal epidemic mosquito vector, *A. aegypti,* during the 1950s and 1960s. In Asia, however, effective mosquito control was never achieved, and a severe hemorrhagic fever (DHF) emerged following World War II. During the 1950s, 1960s, and 1970s, this new form of dengue occurred as periodic epidemics in a few countries. During the 1980s, however, incidence increased dramatically, expanding distribution of the virus and the mosquito vector to the Pacific islands and tropical America (18). In the latter region, the *Ae. aegypti* eradication program had been disbanded in the early 1970s; by the 1980s, this species had reinfested most tropical countries of the region (Figure 4). Increased disease transmission and frequency of epidemics caused by multiple virus serotypes in Asia increased the movement of dengue viruses into these regions, resulting in a dramatic increase in epidemic dengue fever; hyperendemicity (the cocirculation of multiple virus serotypes); and the emergence of DHF in the Pacific Islands, the Caribbean, and Central and South America. Thus, in less than 20 years, both the American tropics and the Pacific Islands went from not having dengue to having an important dengue/DHF problem in 1998.

Globally, DHF has emerged as a major cause of hospitalization and death. The number of DHF cases reported from 1981 to 1995 is four times higher than that of the previous 30 years. In 1998, more than 2.5 billion persons live in areas of risk (Figure 5). Dengue is the second most important tropical disease (after malaria) with approximately 50 to 100 million cases of dengue fever and 500,000 cases of DHF each year.

Because of limited surveillance data and gross underreporting in most disease-endemic countries, the economic and public health impact of dengue is greatly underestimated.

Yellow Fever

Like dengue fever, yellow fever caused major epidemics from the 17th to the 20th centuries and was effectively controlled in the Americas by the *Ae. aegypti* elimination program in the 1950s and 1960s. Yellow fever is maintained in forest cycles involving monkeys and canopy-dwelling mosquitoes in both Africa and the Americas.

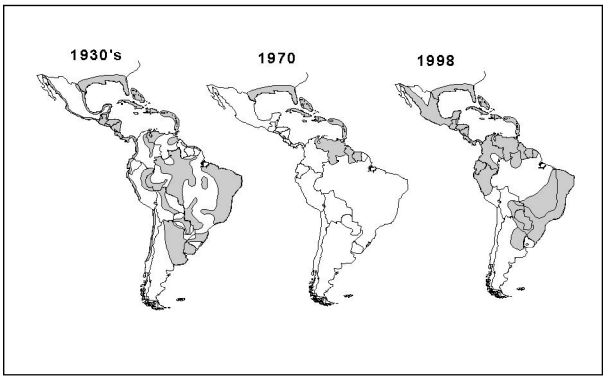


Figure 4. Geographic distribution of *Aedes aegypti* in the Americas, 1930s, 1970, and 1998.

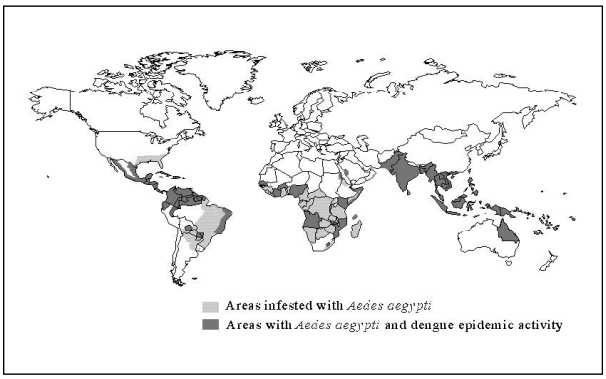


Figure 5. Global distribution of *Aedes aegypti* and of epidemic dengue, 1980–1998.

Human infections since the 1950s have been primarily in persons associated with the forest. Since the mid-1980s, however, epidemic yellow fever has resurged in West Africa, and for the first time in history, an outbreak occurred in Kenya in 1992 to 1993 (19).

Although the last urban epidemic in the Americas was in 1942 (20), urban epidemics may recur because nearly all major urban centers of the American tropics have been reinfested by *Ae. aegypti* in the past 20 years. Most persons in tropical American cities are at high risk for epidemic urban transmission because of low yellow fever immunity. Of added concern are the increasingly frequent reports of imported yellow fever to mosquito-infested urban areas (Figure 6). In the past 2 years, yellow fever cases have been imported to Santa Cruz, Bolivia; Manaus, Brazil; Villavicencia, Colombia; and Iquitos, Peru, all urban centers infested with *Ae. aegypti*. Moreover, two patients with yellow fever cases imported to the United States and Switzerland died; neither patient had been vaccinated.

Thus, the frequency of yellow fever moving from the American rain forest to tropical urban areas is increasing, and it is likely only a matter of time before an urban yellow fever epidemic will occur. The disease will then likely spread rapidly to other cities in the Americas and from there to cities in Asia and the Pacific, much as dengue has in the past 20 years (18). Because of the similarities in clinical expression between yellow fever and other common diseases such as dengue and leptospirosis and because the surveillance systems needed to detect yellow fever are very limited in most countries, widespread epidemic transmission would likely occur before the disease is detected. Emergency methods of controlling *Ae. aegypti* are ineffective (21,22); therefore, a major international public health emergency could occur.

These are only a few examples of emergent/resurgent vector-borne diseases, but there are many more that are causing increasingly frequent epidemics. Many go unreported because laboratory-based surveillance systems are not available in many countries.

Factors Involved in Vector-Borne Disease Emergence

The factors responsible for the emergence/resurgence of vector-borne diseases are complex. They include insecticide and drug resistance, changes in public health policy, emphasis on emergency response, deemphasis of prevention programs, demographic and societal changes,

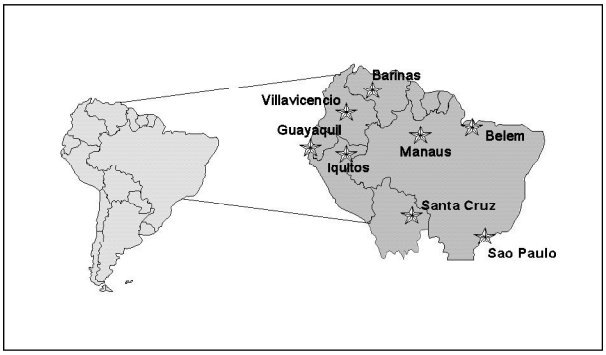


Figure 6. Major urban centers of South America recently infested with *Aedes aegypti* and at high risk for imported yellow fever.

and genetic changes in pathogens (10). Public health policy decisions have greatly decreased the resources for surveillance, prevention, and control of vector-borne diseases in the 1960s and 1970s, primarily because control programs had reduced the public health threat from these diseases. Those decisions, the technical problems of insecticide and drug resistance, as well as too much emphasis on insecticide sprays to kill adult mosquitoes, contributed greatly to the resurgence of diseases such as malaria and dengue. Decreased resources for infectious diseases in general resulted in the discontinuation or merger of many programs and ultimately to the deterioration of the public health infrastructure required to deal with these diseases. Moreover, good training programs in vector-borne diseases decreased dramatically after 1970. Thus, in 1998, we are faced with a critical shortage of specialists trained to respond effectively to the resurgence of vector-borne diseases (10,23). A related problem is the lack of preventive medicine training in most medical schools. The curative approach and emphasis on high-tech solutions to disease control have led most physicians, health officials, and the public to rely on "magic bullets" to cure an illness or control an epidemic (21).

Major global demographic and societal changes of the past 50 years have directly affected the emergence/resurgence of vector-borne and other infectious diseases (10,21,23,24). Unprecedented population growth, mostly in developing countries, resulted in major movements of people, primarily to urban centers. This unplanned and uncontrolled urbanization (inadequate housing, deteriorating water, sewage, and waste management systems) produced ideal conditions for increased transmission of mosquito-borne, rodent-borne, and water-borne diseases. The prospects for the future are not good; nearly all of the world's population growth in the next 25 years will occur in the urban centers of developing countries, many of them in tropical areas where vector-borne diseases occur most frequently (25).

Other societal changes, such as agricultural practices and deforestation (10), increase the risk for vector-borne disease transmission (Table 2). Many irrigation systems and dams have been built in the past 50 years without regard to their effect on vector-borne diseases. Similarly, tropical forests are being cleared at an increasing rate, and agricultural practices such as rice production have also increased.

Consumer products make ideal breeding sites for domesticated mosquitoes. Packaged in nonbiodegradable plastics, cellophanes, and tin, these products tend to be discarded in the environment where they collect rainwater. Discarded automobile tires, many in the domestic environment, make ideal mosquito breeding places as well as rat and rodent harborages. Container shipping and the global used tire industry have contributed to the increased geographic distribution of selected mosquito species that lay their eggs in used tires (26).

Finally, the jet airplane has had a major influence on global demographics (27). The airplane provides the ideal mechanism for transporting pathogens between population centers (10,18,21,23). The result is a constant movement of viruses, bacteria, and parasites among cities, countries, regions, and continents.

Climate change (e.g., global warming and El Niño Southern Oscillation) is often cited as the cause for the emergence/resurgence of vector-borne diseases, especially malaria, dengue, and

Table 2. Influences on emergent/resurgent vector-borne diseases

Urbanization	Deforestation	Agricultural Practices
Dengue fever	Loaiasis	Malaria
Malaria	Onchocerciasis	Japanese encephalitis
Yellow fever	Malaria	St. Louis encephalitis
Chickungunya	Leishmaniasis	West Nile fever
Epidemic polyarthritis	Yellow fever	Oropouche
West Nile fever	Kyasanur Forest disease	Western equine encephalitis
St. Louis encephalitis	La Crosse encephalitis	Venezuelan equine encephalitis
Lyme disease	Eastern equine encephalitis	
Ehrlichiosis	Lyme disease	
Plague		

yellow fever. While meteorologic factors such as temperature, rainfall, and humidity influence the transmission dynamics of vector-borne diseases, climate change has not yet been scientifically proven to have caused the emergence/resurgence of any of the vector-borne diseases described above.

The Future

Reversing the trend of emergent/resurgent vector-borne diseases is a major challenge. Vaccines, available for only a few diseases (yellow fever, Japanese encephalitis, tick-borne encephalitis, tularemia, plague), are not widely used. Vaccine prospects for major vector-borne diseases are not good. With the exception of malaria, few other vector-borne diseases have funding for vaccine research.

In the next decade, therefore, vector control will be required to interrupt transmission of most emergent/resurgent vector-borne diseases. Environmentally safe insecticides and research on alternative approaches (such as biological control) are needed. Integrated prevention strategies must be developed and implemented in endemic/enzootic-disease areas. In addition to economic support for research, human resources are needed to develop and implement sustainable prevention programs. Adequately trained personnel are lacking in most developing countries, as are academic institutions with the programs to train them. Policy changes must be made to support public health approaches to disease prevention. All these factors are needed to rebuild the public health infrastructure. Ultimately, however, demographic trends that have resulted in increased population pressure on urban centers and changes in agricultural practices must be reversed. Only then will we be able to effectively reverse the current trend of emergent/resurgent vector-borne disease in the 21st century.

Acknowledgment

The author is grateful to numerous colleagues around the world for contributing data and information used in preparation of this paper.

Dr. Gubler is director of the Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, CDC, in Fort Collins, Colorado. His research interests include field epidemiology, laboratory diagnosis, surveillance, prevention, and control of vector-borne diseases, with special emphasis on dengue/dengue hemorrhagic fever and other arboviruses.

References

1. Gubler DJ. Insects in Disease Transmission. In: Strickland GT, editor. Hunter tropical medicine, 7th edition. Philadelphia (PA): W. B. Saunders; 1991. p. 981-1000.
2. Philip CB, Rozenboom LE. Medico-veterinary entomology: a generation of progress. In: Smith RF, Mittler TE, Smith CN, editors. History of entomology. Palo Alto (CA): Annual Reviews Inc; 1973.
3. Gubler DJ. The global resurgence of arboviral diseases. Trans Roy Soc Trop Med Hyg 1996;90:449-51.
4. Krogstad DJ. Malaria as a reemerging disease. Epidemiol Rev 1996;18:77-89.
5. Bruce-Chwatt LJ. The Manson Oration, May 1979. Man against malaria: conquest or defeat? Trans Roy Soc Trop Med Hyg 1979;73:605-17.
6. Hammon WM. Dengue hemorrhagic fever—do we know its cause? Am J Trop Med Hyg 1973;22:81-91.
7. Pan American Health Organization. Dengue in the Caribbean, 1977. Scientific Publication No. 375. Washington: The Organization; 1979.
8. Reeves WC. Recrudescence of arthropod-borne diseases in the Americas. Washington: Pan American Health Organization; 1972. PAHO Scientific Publication No. 238. DC.
9. Groot H. The reinvasion of Colombia by *Aedes aegypti*: aspects to remember. Am J Trop Med Hyg 1980;29:330-8.
10. Lederberg J, Shope RE, Oaks SC Jr, editors. Emerging infections: microbial threats to health in the United States. Washington: National Academy Press; 1992.
11. The World Health Report 1996: fighting disease, fostering development. Geneva: World Health Organization; 1996.
12. Zucker JR. Changing patterns of autochthonous malaria transmission in the United States: a review of recent outbreaks. Emerg Infect Dis 1006;2:37-43.
13. Molyneux DH. Current public health status of the trypanosomiases and leishmaniases. In: Hilde G, Mottram JC, Coombs GH, Holmes PH, editors. Trypanosomiasis and leishmaniasis. London: CAB International; 1997. p. 39-50.
14. Dennis DT. 1998. Epidemiology, ecology, and prevention of Lyme disease. In: Rahn DW, Evens J, editors. Lyme disease. Philadelphia (PA): American College of Physicians; 1998. p. 7-34.
15. Dennis DT. Plague as an emerging disease. Emerging Infections II. In press 1998.
16. Ramalingaswami V. The plague outbreaks of India, 1994–a prologue. Current Science 1996;71:781-806.
17. Plague—India 1994: economic loss. Geneva: World Health Organization; 1997. p. 14.
18. Gubler DJ. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. In: Gubler DJ, Kuno G, editors. Dengue and dengue hemorrhagic fever. London: CAB International. p. 1-22.
19. Sanders EJ, Tukei PM. Yellow fever: an emerging threat for Kenya and other East African countries. East Afr Med J 1996;73:10-2.
20. Monath TP. Yellow fever. In: Monath TP, editor. The arboviruses: epidemiology and ecology. Boca Raton (FL): CRC Press; 1988. p. 139-231.

21. Gubler DJ. *Aedes aegypti* and *Aedes aegypti*-borne disease control in the 1990s: top down or bottom up. Am J Trop Med Hyg 1989;40:571-8.
22. Reiter P, Gubler DJ. Surveillance and control of urban dengue vectors. In: Gubler DJ, Kuno G, editors. Dengue and dengue hemorrhagic fever. London: CAB International; 1997. p. 425-62.
23. Gubler DJ. Epidemic dengue and dengue hemorrhagic fever: a global public health problem in the 21st century. In: Scheld WM, Armstrong D, Hughes JM editors. Emerging infections 1. Washington: ASM Press; 1997. p. 1-14.
24. Addressing emerging infectious disease threats: a prevention strategy for the United States. Atlanta (GA): Centers for Disease Control and Prevention; 1994.
25. World resources 1996-97. A guide to the global environment. The urban environment. New York: Oxford University Press: 1996.
26. Reiter P, Sprenger D. The used tire trade: a mechanism for the worldwide dispersal of container breeding mosquitoes. J Am Mosq Ctrl Assoc 1987;3:494-501.
27. Gubler DJ. Arboviruses as imported disease agents: the need to increased awareness. Arch Virol 1996;11:21-32.

Global Climate Change and Infectious Diseases

R. Colwell,* P. Epstein,† D. Gubler,‡ M. Hall,§ P. Reiter,‡ J. Shukla¶, W. Sprigg,# E. Takafuji, and J. Trtanj§**

*University of Maryland Biotechnology Institute, College Park, Maryland, USA; †Harvard Medical School, Boston, Massachusetts, USA; ‡Centers for Disease Control and Prevention, Atlanta, Georgia, USA; §National Oceanic and Atmospheric Administration, Washington, D.C., USA; ¶Institute of Global Environment and Society, Inc., Calverton, Maryland, USA; #National Research Council, Washington, D.C., USA; **Walter Reed Army Institute of Research, Washington, D.C., USA

Climate change, if it occurs at the level projected by current global circulation models, may have important and far-reaching effects on infectious diseases, especially those transmitted by poikilothermic arthropods such as mosquitoes and ticks. Although most scientists agree that global climate change will influence infectious disease transmission dynamics, the extent of the influence is uncertain. This conference session provided an overview of the issues associated with climate change as it relates to the emergence and spread of infectious diseases.

Two papers set the stage by reviewing data that support or do not support the conclusion that climate change has already influenced transmission of infectious diseases. Some studies support such conclusions as warming at higher elevations, including the retreat of tropical summit glaciers, upward plant displacement, elevational shifts in insect populations and vector-borne diseases, and upward shift of the freezing isotherm (150 m, which is equivalent to 1°C warming) since 1970. Other studies, however, point out that in centuries past, vector-borne diseases such as malaria, dengue, and yellow fever occurred regularly in temperate regions in epidemic form during the summer months. The diseases were eliminated from Europe and North America, and although many areas still have the mosquito vectors, epidemic disease transmission has been prevented by improved living conditions and effective mosquito control. Also, since malaria has historically occurred at elevations of 2,400 m to 2,600 m, its current transmission at high altitudes does not necessarily prove that transmission at these high altitudes is the result of climate change.

The second set of papers provided current evidence of global climate change and described how climatologic data might be used to understand geographic spread and transmission dynamics of an important emerging infectious disease such as cholera. The speakers concluded that global warming is occurring and that weather events appear to be associated with the emergence and spread of cholera in the Americas between 1991 and 1998.

Speakers then focused on the research that will be required to answer the many questions relating to climate change and infectious diseases. They described an effort initiated by the National Oceanic and Atmospheric Administration to take advantage of the strong El Niño Southern Oscillation (ENSO) signal in 1997 to 1998 to study the effect of ENSO on vector-borne diseases. The hypothesis was that ENSO-related changes in precipitation, temperature, and other environmental variables have both direct effects (through drought, flood, and extreme weather events) and indirect effects (through changes in transmission and outbreaks of infectious diseases, particularly diseases transmitted by mosquitoes, rodents, or water) on human health. Diseases studied in the ENSO experiment include cholera in Bangladesh and Peru, cryptosporidiosis in the United States, waterborne and water-related diseases in Florida, marine ecologic disturbances in the eastern United States, dengue in different parts of the world, malaria in Africa, domestic arboviral encephalitides in the United States, and hantavirus pulmonary syndrome in the United States. The National Academy of Sciences and

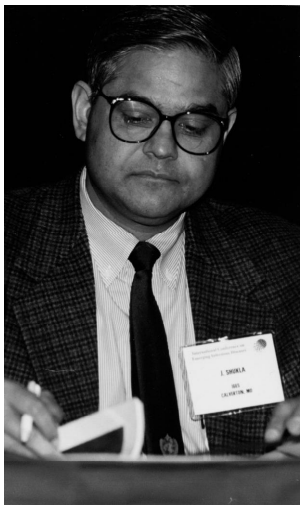
Institute of Medicine plan to appoint a committee to review critically the published work on this topic and make recommendations for a national research agenda. A number of U.S. government agencies will support this committee financially.

The final presentation addressed the need for cooperation and partnerships in implementing this research agenda. The government agencies involved have unique expertise and perspectives that can be brought to bear on the problem of climate change. Emphasis must be placed on public health intervention measures that are properly implemented and can mitigate the effect of global climate change on infectious disease incidence and geographic spread.

Suggested Bibliography

1. Epstein PR, Diaz HF, Elias S, Grabherr G, Graham NE, Martens WJM, et al. Biological and physical signs of climate change: focus on mosquito-borne disease. Bulletin of the American Meteorological Society 1998;78:409-17.

Jagadish Shukla
Institute of Global Environment and Society, Inc., Calverton, Maryland, USA



Ernest Takafuji
Walter Reed Army Institute of Research, Washington, D.C, USA

Emerging Zoonoses

James Childs,* Robert E. Shope,† Durland Fish,‡ Francois X. Meslin,§ Clarence J. Peters,* Karl Johnson,¶ Emilio Debess,# David Dennis,* and Suzanne Jenkins**

*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; †University of Texas Medical Branch at Galveston, Galveston, Texas, USA; ‡Yale School of Medicine, New Haven, Connecticut, USA; §World Health Organization, Geneva, Switzerland; ¶University of New Mexico, Albuquerque, New Mexico, USA; #National Association of Public Health Veterinarians; and **Council of State and Territorial Epidemiologists

Zoonotic pathogens cause infections in animals and are also transmissible to humans; knowledge of the extrahuman reservoirs of these pathogens is thus essential for understanding the epidemiology and potential control of human disease. Zoonotic diseases are typically endemic and occur in natural foci. However, ecologic change and meteorologic or climatic events can promote epidemic expansion of host and geographic range. For practical reasons, surveillance of zoonotic agents too often relies on the identification of human cases. Surveillance in natural hosts may be difficult because of the ecologic complexity of zoonoses; multidisciplinary teams of ecologists, mammalogists, ornithologists, and entomologists, as well as physicians and epidemiologists, may be required for successful investigations. A recent trend in studying zoonoses that have strong environmental correlates includes geographers and mathematical modelers, who integrate satellite remote sensing and geographic information systems to predict outbreaks. Understanding extrahuman life cycles and predicting zoonotic disease outbreaks may permit control activities targeted at several points in the cycle of pathogen maintenance before human infection begins. These control efforts are important because most zoonoses are not amenable to eradication, except perhaps those in areas where animal reservoirs are targeted for vaccination, e.g., fox rabies in Europe.

In the United States, tick-borne zoonoses have emerged at the relatively constant rate of one per decade over the past 100 years. However, the incidence of human tick-borne disease has increased exponentially over the past two decades—primarily because of ecologic change caused by reforestation. Large-scale reforestation of the northeastern coastal states since the early part of this century precipitated a natural succession of ecologic changes that included increased deer density, expansion of the natural range of the deer-dependent tick *Ixodes scapularis*, and increased transmission rates of tick-borne pathogens. *I. scapularis* is a competent vector of at least four enzootic tick-borne pathogens *(Borrelia burgdorferi, Babesia microti, Ehrlichia phagocytophila*, and a Powassanlike encephalitis virus). Because of its anthropophilic nature, *I. scapularis* is also an excellent bridge vector for transmission of these pathogens to humans. This dramatic expansion in the distribution of *I. scapularis* in the northeastern United States has caused the current epidemic of Lyme disease and has increased the range of human babesiosis in New England. However, the recent emergence of human granulocytic ehrlichiosis resulted from the recognition of human cases caused by a zoonosis already well established within *I. scapularis* populations. As *I. scapularis* continues to expand its range bringing more people in contact with novel enzootic tick-borne pathogens, additional tick-borne diseases may emerge as new public health threats.

Since the unprecedented impact of bovine spongiform encephalopathy (BSE) and new variant Creutzfeldt-Jakob disease (nvCJD) on animal health and national politics and economies, this new zoonosis has prompted many questions in the field of foodborne disease control and prevention. BSE and nvCJD, caused by an unconventional agent, the nature of which remains controversial, are invariably fatal. The threat to human health is compounded because the causative agent is resistant to conventional physical and chemical methods of decontamina-

tion and cannot be fully inactivated by any of the current food technologies. A preclinical diagnostic test remains elusive. Traditional food safety programs cannot prevent infection to consumers once the agent has entered the food chain. New and reemerging conventional or unconventional foodborne pathogens of animal origin must be better addressed, and food safety programs with emphasis on the preharvest and harvest food stages must be developed. Control is best achieved at the feed preparation and farm level and at the harvest stage. Consumer health takes precedence over market concerns, and when data are incomplete, a conservative response is warranted until the risk can be accurately assessed.

Diseases of humans caused by rodent-borne viruses in the families *Bunyaviridae* and *Arenaviridae* include the newly recognized hantavirus pulmonary syndrome and the South American hemorrhagic fevers. Many of these diseases present control challenges—because vaccines may not be developed, because of characteristics of the exposed population, or because control of rodent reservoirs in the affected areas is impractical or unachievable. Many of these diseases may be increasing in frequency as humans modify forest and natural savannah environments for agriculture, inadvertently promoting human-rodent contact and increasing the number of suitable habitats used by rodents, which are habitat generalists and also virus reservoirs. Serious gaps in our understanding of the natural history of these viruses and their hosts limit a targeted intervention. The prevalence of virus infection in rodent populations may be less important than the absolute number and demographic characteristics of infected mice. A single, newly infected, subadult mouse may shed in its urine and feces the high quantities of virus needed to infect a person by the aerosol route. However, effective maintenance of virus may hinge on persistent infections in older, dominant, male rodents that survive over extended periods and have the highest prevalence of infection but only shed sufficient virus in their saliva to perpetuate rodent-to-rodent transmission through intraspecific aggressive encounters. Recent developments in remote sensing and geographic information systems, coupled with longitudinal studies of virus activity and rodent population dynamics, hold promise for developing models predictive of when and where outbreaks of rodent-borne zoonoses could occur.

Surveillance of the unknown appears to be a thankless task, and it is probable that we will learn of an "Andromeda" event after an urban population is struck, although the agent is most likely to arise in a rural, tropical setting. The health and safety of future generations may depend on our ability to rapidly detect, monitor, and control disease caused by novel zoonotic agents. Uniform surveillance definitions, reliable specimen collection, shipping and handling, and means for rapid communication will be critical.

New Approaches to Surveillance and Control of Emerging Foodborne Infectious Diseases

Robert V. Tauxe
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Each year in the United States, foodborne diseases affect millions of persons, who become ill after exposure to any of a growing spectrum of identified agents and toxins. Typhoid fever and other foodborne diseases common a century ago have been controlled by measures that prevent contamination of food and water with human sewage and by technologies (such as milk pasteurization) that eliminate any remaining pathogens. Many recently identified foodborne diseases are caused by contamination with animal feces and can be prevented by measures that reduce contamination and eliminate residual pathogens. In the future, growing attention will need to be directed to the safety of the food and water the animals themselves consume.

New foodborne diseases emerge for many reasons, including changes in the pathogens themselves, increasingly centralized and concentrated food production, globalization of the food supply, and increases in populations at higher risk. Better surveillance and investigation now detect outbreaks that a few years ago would have been missed. The continuing challenges are to identify new pathogens as they emerge, understand how foodborne pathogens contaminate food and cause illness, and define and implement the best prevention strategies.

Many efforts are now under way to improve food safety in the United States. In 1997, the National Food Safety Initiative outlined an interagency effort to enhance foodborne disease surveillance, research, and prevention. The Centers for Disease Control and Prevention (CDC) and state and local health departments have begun to implement improved surveillance strategies, including additional resources for basic surveillance and investigation, an active surveillance network called FoodNet, surveillance for antimicrobial resistance, and a network for molecular subtyping called PulseNet. Basic research at the National Institutes of Health (NIH) is clarifying virulence mechanisms and developing prevention tools. Dennis Lang, National Institute of Allergy and Infectious Diseases, emphasized that NIH-supported investigators who study the organisms responsible for foodborne illness represent a national resource that can be used to address food safety questions more effectively. New approaches to prevention are now being implemented by the food regulatory agencies, and more approaches, including irradiation, have been approved for industry use.

Barbara Herwaldt, CDC, reported that *Cyclospora cayetanensis* is an archetypical emerging foodborne pathogen. This recently described parasitic pathogen sprang to national attention in nationwide outbreaks in 1996, which were traced to raspberries imported from Guatemala. Outbreaks recurred in 1997, leading to a suspension of importation, despite efforts of the Guatemalan raspberry industry to reduce potential contamination. With improved surveillance, other outbreaks were detected, investigated, and traced to mesclun lettuce and basil. CDC investigation has now documented *C. cayetanensis* as a common cause of springtime diarrhea among children in Guatemala. Critical gaps in our understanding of the biology and epidemiology of this parasite, particularly in the raspberry farm environment, need to be closed before effective control measures can be developed.

B. Swaminathan, CDC, described a new subtyping strategy for public health surveillance of *Escherichia coli* O157:H7 that will become available electronically later this year. This strategy depends on standardized molecular fingerprinting in public health and food regulatory agency laboratories by pulsed-field gel electrophoresis (PFGE). With standardized methods and equipment, excellent interlaboratory comparability of DNA fingerprint patterns has been achieved. Twenty-four states, the U.S. Department of Agriculture, and the Food and Drug Administration are now equipped to use CDC's PFGE method for *E. coli* O157:H7. These

laboratories are being linked to form a collaborative network for molecular subtyping, PulseNet, which will permit rapid comparison of identified PFGE profiles with the national database at CDC. Efforts are also under way to apply the same strategy to other foodborne pathogens. In 1997, PFGE results were already critical to epidemiologic investigations of several outbreaks of *E. coli* O157:H7 infections. These included a Colorado outbreak traced to ground beef and a multistate outbreak related to alfalfa sprouts.

Alison O'Brien, Uniformed Services University of the Health Sciences, described a new approach to prevention, based on an attachment protein present in enteropathogenic *E. coli* as well as *E. coli* O157:H7. This protein, intimin, permits the bacteria to attach to mucosal cells and produce a characteristic pathologic change. In a calf model, *E. coli* O157:H7 can cause diarrhea and this change; the change does not occur if the *E. coli* lack the gene for intimin. Intimin is highly antigenic and acid stable, and antibodies raised to it block adherence in vitro. The intimin gene has been introduced into plants, where it is produced in the leaves. This means that an antitransmission vaccine based on intimin can be produced cheaply in plants and be given to calves. The vaccine could even be fed to animals if intimin were produced in fodder plants. In the future, we may be able to prevent *E. coli* O157:H7 in humans by vaccinating the bovine reservoir.

Henrik Wegener, Danish Zoonosis Center, described the emergence of vancomycin-resistant enterococci (VRE) in northern Europe, linking it to the use of a related glycopeptide antibiotic, avoparsin, in food animals. VRE were common in poultry flocks and swine herds exposed to this antibiotic, and 5% of healthy carnivorous humans were carriers of VRE. Sequencing the resistance gene showed that one genotype was present in poultry, a second was present in swine, and both were present in humans. Thus, VRE are unlikely to have spread from animals to humans rather than vice versa. After avoparsin was withdrawn in Denmark in 1995, the prevalence of VRE in chickens dropped; the European Union banned the agent in 1997. In some countries, amplification of VRE in hospitals where vancomycin use is frequent may follow introduction of resistance strains from food sources. Other antibiotics being developed for human use (e.g., streptogramins) have analogues used in agriculture for years, to which resistance may already have emerged. Integrated resistance surveillance systems, data on antibiotic use in humans and in agriculture, and prudent agricultural use policies are critical to managing the growing challenge of antibiotic resistance related to foods and food animals.

FoodNet and Enter-net: Emerging Surveillance Programs for Foodborne Diseases

Samantha Yang
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

The public health challenges of foodborne diseases are changing as a result of newly identified pathogens and vehicles of transmission, changes in food production and distribution, and an apparent decline in food safety awareness. Response to these new challenges requires new surveillance strategies to monitor the incidence of human illness and provide data for developing effective prevention strategies.

FoodNet

The Foodborne Diseases Active Surveillance Network (FoodNet), the principal foodborne disease component of the Centers for Disease Control and Prevention's (CDC) Emerging Infections Program (EIP), is a collaborative project with participating EIP sites, the U.S. Department of Agriculture (USDA), and the U.S. Food and Drug Administration (FDA). To determine more precisely the incidence of foodborne illness in the United States, FoodNet was established in five locations (selected counties in California, Connecticut, and Georgia, and the entire states of Minnesota and Oregon) in 1995 and was expanded to selected counties in Maryland and New York in 1997. The population of these seven FoodNet sites in 1997 was 20.3 million (7.7% of the U.S. population).

The objectives of FoodNet are to 1) describe the epidemiology of new and emerging bacterial, parasitic, and viral foodborne diseases of national importance; 2) more precisely determine the frequency and severity of foodborne diseases in the United States; and 3) determine the proportion of foodborne disease caused by eating specific foods. By monitoring foodborne disease incidence over time, FoodNet will document the effectiveness of new food safety initiatives, such as the USDA Food and Safety Inspection Service's Pathogen Reduction and Hazard Analysis and Critical Control Points (HACCP) Rule, in decreasing the number of cases of foodborne disease in the United States each year. To address these objectives, FoodNet conducts active surveillance and related studies: a population survey, a physician survey, and a case-control study of *Escherichia coli* O157:H7 infections.

Population Survey

Duc Vugia, California Department of Health, reported the results of the FoodNet population survey, which was conducted between July 1, 1996, and June 30, 1997, in selected counties of California, Connecticut, Georgia, and the entire states of Minnesota and Oregon. This survey provided an estimate of the prevalence of acute diarrhea and the frequency with which patients with acute diarrhea sought medical care. In 9,003 interviews, 11% of persons reported acute diarrhea in the 4 weeks before the interview, 12% of persons with acute diarrhea called a health-care provider as a result of this illness, and 8% sought medical care. These data indicate an estimated 1.4 acute diarrheal episodes per person each year in the United States, with 1% of the population seeking medical care because of acute diarrhea.

Physician Survey

In 1996, to obtain information on stool culturing practices, physicians in California, Connecticut, Georgia, Minnesota, and Oregon were surveyed. Results were reported by Thomas Hennessy, CDC. Of the 1,783 physicians responding to this survey, 44% reported requesting a stool culture from the most recent patient they remembered seeing who had acute diarrhea. Patient factors significantly associated with stool culture requests included bloody stools, a diagnosis of AIDS, diarrhea lasting longer than 3 days, travel to a developing country, and fever. Stool culture ordering practices differed by physicians' specialties and geographic site; however, culturing practices did not differ by payment plan, such as managed care or fee-for-service.

Variability in culturing practices by specialty and geographic location suggests a need for clinical diagnostic guidelines for diarrheal illnesses.

Case-Control Study

A case-control study was conducted at FoodNet sites to identify risk factors for sporadic *E. coli* O157:H7 infections and to explain variations in the incidence of *E. coli* O157:H7 incidence among FoodNet sites. Heidi Kassenborg, Minnesota Department of Health, presented results from this study, which was conducted between March 1, 1996, and April 30, 1997, in selected counties of California, Connecticut, Georgia, and the entire states of Minnesota and Oregon. Data were obtained from 200 nonoutbreak case-patients and 380 controls, matched by age and telephone exchange. For all sites combined, illness was associated with eating pink hamburgers or pink ground beef and with visiting a farm. Regional variation in beef processing and in exposure to farms may have contributed to the regional variability of *E. coli* O157:H7 infections.

Enter-net

Funded by the European Commission, Enter-net (formerly Salm-Net) is an international surveillance system for *Salmonella* infections (including data on antibiotic resistance) and *E. coli* O157 infections. Microbiologists and epidemiologists responsible for national laboratory-based surveillance of these pathogens in 15 European countries form the Enter-net network. Ian Fisher, Communicable Disease Control Centre, United Kingdom, described Enter-net and highlighted international outbreaks recognized by the network. Investigations of these outbreaks led to public health interventions and product recalls in Europe. The international database of laboratory-confirmed cases of salmonellosis produced by Enter-net also allows trends in this illness to be observed over several years. For example, Enter-net is documenting the continuing problem of *Salmonella* serotype Enteriditis in western Europe. Enter-net represents a working model of how focused infection-specific international surveillance involving key public health professionals can help monitor and detect international outbreaks of foodborne infection.

Carol Hardegree
Food and Drug Administration,
Washington, D.C., USA

Enhancing State Epidemiology and Laboratory Capacity for Infectious Diseases

Deborah A. Deppe
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

The Epidemiology and Laboratory Cooperative is an agreement that provides state and large local health departments with resources to strengthen and enhance basic capacity for public health surveillance and response for infectious diseases. The funding is used to implement new technology, upgrade systems, train staff, and purchase office and laboratory equipment. Six of the 30 sites reported on their programs.

The Vermont Department of Health has undertaken statewide efforts to improve communicable disease reporting through legislation. Before 1997, public health law required physicians, nurses, hospital administrators, and school and town health officials to report communicable disease (defined by regulation) to the Commissioner of Health. Legislation proposed and passed in 1997 required health maintenance organizations and managed care organizations to report as well. This model law defines such information as "confidential and privileged" and extends protection to investigations and studies of disease outbreaks.

The Kansas Department of Health and Environment is building epidemiology and laboratory capacity by expanding electronic surveillance and analysis, enhancing surveillance for diarrheal disease, and integrating information from other sources into the existing surveillance system. The new programs are flexible so they can meet the challenges posed by new and emerging infectious diseases as well as changing needs within public health.

The New York State Department of Health uses electronic communications to enhance rapid reporting of communicable disease epidemiologic and laboratory data within the state. In July 1997, seven counties began pilot testing the New York Health Information Network (HIN), a secure Intranet site. Web forms were designed for designated county personnel to submit confidential case and supplemental information on 61 reportable communicable diseases on the HIN. Since implementation, reports from local health departments to the state have been more timely. Counties can easily update and query their own data and can access statewide reports generated by the state and posted on the HIN.

The Los Angeles County Department of Health Services has used pulsed-field gel electrophoresis (PFGE) for outbreak investigations for 2 years. Bacteria caused 140 (24%) of 576 reported outbreaks, 36% of health facility outbreaks, but only 19% of community outbreaks. PFGE was used in 32 investigations, of which 29 (91%) were nosocomial. The most common organisms were staphylococcus and enterococcus. In contrast, PFGE was used in only 3 (4%) of 77 investigations of community bacterial outbreaks. Health departments should consider the number, setting, and causes of outbreaks that they investigate to determine if PFGE will be useful in their investigation arsenal.

The Washington State Department of Health has developed a pilot electronic laboratory-based reporting mechanism to route infectious disease reports from a large managed care organization to local health agencies. The overall goal is development of a generic electronic reporting mechanism. For this project, Health Level 7 was identified as a common ground for sharing data. Preliminary data suggest that timeliness and completeness of reporting will improve. Adhering to nationally recognized standards and codes may help reduce problems associated with transferring data; however, no public health software or implementation package was available, so resource-intensive customization was necessary.

The Maine Bureau of Health is attempting to characterize statewide hepatitis C (HCV) prevalence through a review of existing databases (hospital discharges, deaths, Medicaid registry, blood donor screening), mandatory laboratory

reporting with physician questionnaire follow-up, a blinded seroprevalence survey in sexually transmitted disease clinics, and a survey of gastroenterologists. Initial data indicate dramatic increases in HCV-related hospitalization and in Medicaid expenditures during the mid-1990s and an unexpectedly high proportion of patients with injection drug–associated risk histories. Local surveillance data are useful for public policy decisions and as educational tools for physicians and the public.

International Cooperation

James LeDuc
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Experts from the World Health Organization (WHO), the European Union (EU), the U.S. Department of Defense (DoD), and other organizations summarized existing and planned collaborations on emerging infectious diseases. The session was chaired by David Heymann, WHO, and James LeDuc, CDC.

One speaker, V. Ramalingaswami, All India Institute of Medical Sciences, India, summarized lessons learned from the plague outbreak in Surat, India. The plague outbreak, the first in many years, found the country ill-prepared to diagnose this disease, and young clinicians lacked experience in recognizing or managing plague-infected patients.

Two speakers examined regional collaborations. Christopher Bartlett, Public Health Laboratory Service, London, summarized the development of international surveillance within EU. The Maastricht Treaty provided the political will to underpin these activities; since the treaty, the heads of European institutes with responsibility for national surveillance have met regularly to assist in strategic development of surveillance activities. Disease-specific networks have been established, each with an operational protocol that sets out agreed case definitions and standard methods and use of information obtained. High quality and timely information is now being provided on a steadily increasing spectrum of infectious diseases through weekly electronic and monthly surveillance bulletin publications. Oyewale Tomori, regional virologist for Africa from WHO, explained that despite substantial advances in disease prevention and control, communicable diseases still constitute a major health problem for Africa. Concern about the deplorable and worsening state of disease control in Africa has led ministers of health in the region to pass several resolutions on prevention of epidemic infectious diseases, yet frequent epidemics continue. Apart from the development of a few disease-specific laboratory diagnostic networks, laboratory services in Africa remain rudimentary and underdeveloped. The WHO Regional Office for Africa has recently formulated a strategic plan for integrated disease surveillance and an action plan to strengthen laboratory capacity in Africa; international support is urgently needed to implement these plans.

Global collaborations were also discussed. Nils Daulaire, U.S. Agency for International Development (USAID), described the recently announced $50 million initiative to address infectious diseases globally. These funds will be used to focus activities on tuberculosis, malaria, antimicrobial resistance, and surveillance, both in countries with USAID missions, as well as regionally. Michael McCarthy, DoD, summarized the new DoD initiative on emerging infectious diseases and explained how the overseas laboratories in Thailand, Kenya, Peru, Brazil, Egypt, and Indonesia will work closely with their host nations and DoD scientists to address emerging endemic disease threats. Maria Neira, WHO, described recent activities to address cholera and other diarrheal diseases of epidemic potential globally. These activities included the secondment of a CDC medical epidemiologist to the WHO subregional office for southern Africa, where in the past several years a plan to improve recognition and response to epidemic diarrheal diseases has been developed and implemented. Included in the program are training on improved patient management, strengthened laboratory capacity, and better communication both within and between countries of southern Africa.

Public Health Surveillance and Information Technology

Robert W. Pinner
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Applying Modern Information Technology to Reporting for Public Health—the Role of Standards

Clement McDonald, Indiana University School of Medicine, discussed the role of standards in the application of modern information technology to public health reporting. He pointed to the rich data sources stored electronically in clinical laboratories, pathology and cytology reporting systems, pharmacies, and hospitals, and emphasized the trend toward increasing automation.

Interest and demand for electronic delivery of data come from many interested parties—3rd party payers, researchers, physicians, and public health officials. However, substantial barriers to smooth electronic flow of this information include the storage of data in isolated areas, varying internal structures among information systems, and considerable variation in codes (e.g., for laboratory tests and results). Overcoming these barriers requires defining, adopting, and implementing standards for messages, codes, identification (e.g., persons, providers, places), and security.

Messages

Health Level Seven (HL7) is a message standard that defines messages for laboratory and other clinical results, immunization reporting, drug usages, patient registration, and clinical trials. HL7 provides standards for the structure and organization of clinical messages, defining data types, and structure of the "records" in the message. A 1997 Healthcare Information Management System Societies/Hewlett-Packard Leadership Survey found that HL7 was the most important health informatics standard. HL7 is an American National Standards Institute (ANSI)–approved clinical message standard used widely in the United States and internationally. Additional information can be found at the HL7 Internet web site: http://www.mcis.duke.edu/standards/HL7/hl7.htm.

Codes

Code standards include Logical Observations Identifiers Names and Codes (LOINC), a code standard that identifies clinical questions, variables, and reports; Systematized Nomenclature of Medicine (SNOMED), which identifies procedures and possible answers to these questions, such as test results; Current Procedural Terminology, Version 4 (CPT4), which identifies procedures; and the National Library of Medicine's Unified Medical Language (UMLS), a metathesaurus of most code systems.

LOINC comprises a database of 15,000 variables with synonyms and cross-mappings and covers a wide range of laboratory and clinical subject areas (e.g., blood bank, chemistry, hematology, microbiology, vital signs, body measurements, obstetric ultrasound, and electrocardiograms). LOINC's formal naming structure has six parts: component (analyte); property measured; time aspect; system (specimen, organ), precision, method. LOINC is being adopted by several large reference laboratories, and it has been incorporated into UMLS. Additional information about LOINC can be found at http://www.mcis.duke.edu/standards/termcode/loinc.htm.

SNOMED defines code standards in a variety of clinical areas, called coding axes: topography; morphology; function; living organisms; chemicals, drugs, and biologic products; physical agents, activities, and forces; occupations; social context; diseases/diagnoses; procedures; general linkages/modifiers.

Security and Privacy

Privacy issues include both information technology and policy considerations. For example, security can be addressed by encryption techniques; policies that strongly discourage sharing of passwords are also required for adequate privacy and security.

The public health system has been working to adopt needed standards for immunization data transactions using HL7, data elements for emergency department systems, and an approach for piloting electronic reporting from clinical laboratories (which defines an HL7 message with LOINC codes for identifying tests and SNOMED for identifying results, and a set of tables that define reportable diseases in terms of specific tests and results) (1).

The "rules" for achieving public health goals for electronic clinical data are as follows. 1) Take advantage of the momentum of the existing standards in hospitals and laboratories. 2) Recognize that this is difficult and will take a long time. 3) Consider the source system data structures when defining data needs.

Opportunities and Pitfalls for Surveillance

William Braithwaite, Department of Health and Human Services, described the Administrative Simplification provision of the Health Insurance Portability and Account Act of 1996 (HIPAA), which is intended to standardize the electronic data interchange of certain administrative and financial transactions while protecting the security and privacy of transmitted information. The act mandates nine transaction standards (e.g., claims, encounters, enrollment) including code sets; coordination of benefits information; unique identifiers (including defining allowed uses) for individuals, employers, health plans, and health-care providers; and security, confidentiality, and electronic signatures. Once standards are adopted, all health plans, clearinghouses, and those providers who choose to conduct transactions electronically will be required to implement them. The time line for implementation calls for adoption by the Secretary of Health and Human Services (HHS) during 1998 of all standards except claim attachments. ("Claim attachments" refers to information requested by an insurance payer from a health-care provider to justify submitted charges and is difficult to standardize because of the diversity of requests.) The Secretary will look first to industry for a consensus standard developed by an ANSI-accredited standards development organization and will rely upon advice of the National Committee on Vital and Health Statistics. The HHS implementation strategy involves a three-tiered approach. 1) The HHS Data Council, a senior level policy guidance and decision-making group, is the contact for the National Committee on Vital and Health Statistics. 2) The Data Council's Health Data Standards Committee provides management of the standards activities. 3) Implementation Teams provide research, analysis, and development of standards and implementing regulations. The HHS adopts a standard by publishing in the Federal Register a Notice of Intent to gather information when no consensus exists and a Notice of Proposed Rule Making, which provides a draft final rule. Publication of a Final Rule marks the "adoption" by HHS of a particular standard.

Standards proposed for adoption include X12N Version 4010 for all transactions except pharmacy claims, for which the National Council for Prescription Drug Program Version 3.2 is proposed. Coding standards proposed for adoption include ICD-9-CM, followed by ICD-10-CM in 2001 for diagnoses, and ICD-9-CM Vol. 3 and Health Care Financing Administration Common Procedure Coding System (HCPCS) for procedures. Proposed identifier standards are the National Provider Identifier Health Care Financing Administration (HCFA) for providers, the PAYERID (HCFA) for health plans; and the Employer Identification Number (Internal Revenue Service) for employers. A Notice of Intent will be published to seek input regarding the individual identifier.

Important issues for public health surveillance in the next phases include participating in development of the data content of these standards, the standard for claim attachments, and the electronic medical records standards, and developing health information privacy that maintains appropriate access to data for public health purposes. Additional information about the Administrative Simplification provisions of HIPAA can be found at http://aspe.os.dhhs.gov/admnsimp/.

Public Health Surveillance for the 21st Century

Paul Stehr-Green, Washington Department of Health, emphasized public health surveillance as the foundation of public health practice. Public health surveillance needs to adapt to changing health practice, such as requiring assessment of the risks for new and reemerging infectious diseases or environmental hazards.

Public health should use the array of new information tools available. The Blueprint for Surveillance is a document prepared by the Council of State and Territorial Epidemiologists; it outlines the National Public Health Surveillance System. This conceptual framework approaches surveillance for not only reportable diseases, but also for a variety of health outcomes, costs, and risk factors important to public health. The National Public Health Surveillance System would involve other approaches (taking into account available funding levels and the particular goals of surveillance at each level of the public health system) in addition to the traditional reportable diseases surveillance model. The primary goals of the National Public Health Surveillance System include 1) coordinating new and existing public health surveillance systems and linking them to facilitate the exchange of data; 2) encouraging partnerships of federal, state, and local public health professionals in decision-making about surveillance activities; 3) reviewing existing surveillance (and other data collection efforts that have a surveillance component) and making decisions about new surveillance efforts and changes in existing systems; 4) monitoring the adequacy of methods and processes involved in current surveillance systems; and 5) developing a comprehensive description of conditions under surveillance to bring attention to public health surveillance activities and justify the need to support these activities. Recent accomplishments include an effort coordinated by the Centers for Disease Control and Prevention's (CDC) Health Information and Surveillance Systems Board to integrate a number of current surveillance systems; updating the agency's inventory of surveillance systems; developing a policy to monitor and evaluate proposals to develop new or to substantially modify existing surveillance systems; developing an investment analysis policy, which may allow the use of some portion of funds to support the development and maintenance of integrated surveillance and information systems by state health departments; and developing resources that have been made available to state health departments for enhancing infectious diseases surveillance capacity. Washington State is formally reviewing the regulatory foundation for surveillance and is developing and piloting electronic systems for the collection, management, analysis, and dissemination of surveillance data and information, including a collaboration between the health department and Group Health Puget Sound Cooperative to electronically send selected laboratory data to the department of health for surveillance. State and local health departments should commit to changing from old to modern ways of approaching surveillance, and CDC should provide leadership to bring together disparate stakeholders and to provide flexible resources to help state and local health departments effect modernization and integration of surveillance.

Reference

1. McDonald CF, Overhage JM, Dexter P, Takesue BY, Dwyer DM. A framework for capturing clinical data sets from computerized sources. Ann Intern Med 1997;127:675-82.

Innovative Information-Sharing Strategies

Bradford A. Kay,* Ralph J. Timperi,† Stephen S. Morse,‡ David Forslund,§ Julie J. McGowan,¶ and Thomas O'Brien#

*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; †The Massachusetts State Laboratory Institute, Jamaica Plain, Massachusetts, USA; ‡Defense Advanced Research Projects Agency, Arlington, Virginia, USA; §Los Alamos National Laboratory, Los Alamos, New Mexico, USA; ¶University of Vermont, Burlington, Vermont, USA; and #Brigham and Women's Hospital, Boston, Massachusetts, USA

National and global health issues accentuate the need for health professionals to rapidly and effectively acquire and disseminate information. This session highlighted four innovative systems for communicating health information.

ProMED

Many experts, both within and outside government, have warned of the need to improve capabilities for dealing with emerging infectious diseases; development of an effective global infectious disease surveillance system has been the primary recommendation. ProMED, a project of the Federation of American Scientists, was inaugurated in 1993 at a conference in Geneva as a vehicle for developing, coordinating, and promoting plans for a global program to identify and respond to emerging infectious diseases. Members of the ProMED Steering Committee include (among others) representatives of the Centers for Disease Control and Prevention, the National Institutes of Health, the World Health Organization (WHO), the Pan American Health Organization, and the International Office of Epizootics.

In 1994, in cooperation with SatelLife/HealthNet, ProMED developed an e-mail conference system, ProMED-mail, on the Internet. Originally developed to allow worldwide scientist-to-scientist communications on emerging infectious diseases, the system rapidly evolved into a prototype for an open-architecture, real-time outbreak reporting system intended to complement official surveillance systems. Today, with more than 10,000 subscribers from more than 125 countries, ProMED-mail is increasingly providing the first reports of infectious disease outbreaks. All items are read by scientists before posting. Reporting of incidents or outbreaks, infectious disease problems of emerging interest, and discussions on how to improve surveillance and response capabilities are especially encouraged. To subscribe to the ProMED-mail electronic conference, send an e-mail message to majordomo@usa.healthnet.org, and write "subscribe promed" in the text space.

TeleMed

The Advanced Computing Laboratory at Los Alamos National Laboratory, Los Alamos, New Mexico, developed TeleMed, an electronic medical record for managing tuberculosis patients through a collaboration with the National Jewish Center for Immunology and Respiratory Medicine in Denver, Colorado. TeleMed provides a snapshot of patient data, presented chronologically with access to laboratory test results, clinical history, radiology images, reports, and treatment history. A particularly valuable feature allows physicians to annotate the medical record, either orally or in writing, for collaborating physicians to retrieve. Medical expertise can also be exchanged in real time, with both users sharing the same screen and with each having the capability to drive the mouse-pointer. TeleMed, now available on the Internet using Java-based technology, enables physician specialists to support primary care providers in the management of complex medical problems. The technology creates a "virtual patient record" that allows the integration of databases from multiple clinics and multiple providers across geographically separated areas. This permits individual health-care facilities to continue to own and manage their own data while making the data accessible to others treating the same patient. TeleMed provides a time-oriented record of the patient's medical history but only retrieves the actual data on demand, thereby minimizing the bandwidth requirements of the

networking capabilities. Distributed ownership of the data means that only one copy of the data exists, and that copy remains where it was created. Location of the data is obtained from a master patient index that provides pointers to the data. Security and access to the data are controlled and protected with encryption technology.

VTMedNet

Vermont MedNet has been described as the "first comprehensive statewide health information network in the nation." VTMedNet was developed to provide timely access to medical information in support of health-care delivery across the state of Vermont. The system was unique, not because it used advanced technology, but because it used basic technology. VTMedNet Plus, the network's evolution into voice, image, and video, has already garnered national recognition for its initiatives in the area of telemedicine. The network's home page has become a primary resource for the dissemination of information about Vermont's health-care community and information for Vermont's health-care consumer. It is also being used to collect data for research and public health reporting and to distribute aggregate information to improve health-care delivery in one of the nation's most rural states. VTMedNet is the culmination of a partnership among all major health-care organizations in Vermont, including the University of Vermont, Fletcher Allen Health Care, Vermont State Medical Society, and the Vermont Hospital Association. To access the network, users must have their own computers and modems. A simple, configured, shareware communications script is provided to those who request it. VTMedNet is primarily an intranet and provides e-mail and Internet access for the state's health-care providers and access to health information from around the world. It is also designed to serve as a "virtual colleague," encouraging communication among all of Vermont's health-care providers through targeted listservs.

WHONET

WHONET is database software for the management of routine microbiologic test results. Its primary goals are to enhance local capabilities for analysis and to facilitate the exchange of microbiologic data between centers. WHONET is a DOS-based application that may be used alone on personal computers or in conjunction with existing mainframe- or minicomputer-based clinical information systems. Data conversion ("downloading" or "translating") from hospital systems or commercial automated susceptibility test machines can usually be accomplished with BACLINK software, also available free of charge from WHO. WHONET is not a complete laboratory management system but can be used for simple clinical reporting of results. Software development has concentrated on data analysis, particularly of the results of antimicrobial susceptibility tests. The analytic tools aid the selection of antimicrobial agents, the identification of hospital outbreaks, and the recognition of quality control problems in the laboratory. Review of antimicrobial results also permits characterization of resistance mechanisms and the epidemiology of resistant strains.

The software consists of three sections. 1) Data Entry. In addition to the routine entry of susceptibility test results (disk diffusion, MIC, and/or E-test), this program permits printing, retrieval, and correction of clinical records as well as immediate feedback on test results. If data are converted from an existing laboratory system, for example with BACLINK, direct entry of data into WHONET is unnecessary. 2) Data Analysis. Currently supported analyses include listings and summaries of isolates by user-defined criteria; tabulation of the percentages of resistant, intermediate, and susceptible isolates by species; zone diameter and MIC histograms; scatterplots of zone diameter versus zone diameter or MIC versus MIC; scatterplots of zone diameter versus MIC scatterplots and the calculation of zone diameter/MIC regression curves; listings and summaries of isolates by resistance profile; and automated screening of the data for unusual isolates. 3) Configuration Program. This program permits the user to enter and modify laboratory-specific information such as patient-care areas, antibiotics and interpretive breakpoints, language, and hardware.

Getting the Handle off the Proverbial Pump: Communication Works

Lela F. Folkers,* Maria Teresa Cerqueira,† Robert E. Quick,* James Kanu,* and Gauden Galea‡
*Centers for Disease Control and Prevention, Atlanta, Georgia, USA;
†Pan American Health Organization, Washington, D.C., USA; and
‡University of Malta, Zebbug, Malta

Health Promotion, Communication, Education, and Community Participation: A Theory-Based Framework

During the last decades of this century, we have come to recognize that human and social development are affected by the health status of the population and that medical care alone cannot fully address all the determinants of health. Health promotion strengthens primary health care and contributes to public health by enabling people to become involved in community action for health while working to maintain healthy lifestyles and behavior. Health promotion is part of the communications effort involved in the prevention and control of emerging infections.

Health promotion, as defined by various international and regional health promotion conferences (Ottawa 1986, Adelaide 1988, Sundsvall 1991, Bogota 1992, Port of Spain 1993, and Jakarta 1997), enhances intersectoral action by increasing the focus on community involvement and action for health, placing healthy public policy on the agenda, creating supportive environments, and developing personal health skills. Health promotion is one of five policy directives of the Pan American Health Organization (PAHO) Strategic and Programmatic Orientations for 1995-1998. The PAHO/World Health Organization (WHO) Regional Plan of Action for Health Promotion includes the following objectives: 1) promote social development based on principles of equity and the right of citizens to health and well-being; 2) strengthen the concept of a health culture based on healthy environments, behavior, and lifestyles; and 3) develop the health sector's capacity to recognize, support, and lead intersectoral processes for promoting health.

To fully meet the goals of health promotion and disease prevention, programs must inform and guide policies, plans, and activities for health. Health education, communication, and community participation have a wide range of theoretical frameworks. Among the more important are 1) participatory community development political theories that explain capacity building, democratic organization, and management styles; 2) community-based social support networks that facilitate interpersonal communication and consensus around healthy lifestyles; 3) learner-centered cognitive theories that describe and explain the process of acquiring and updating values, knowledge, and skills; and 4) the behavior change framework, especially persuasion theories that describe and explain the process of adoption of healthy lifestyles, both individually and collectively. These theories create supportive environments, strengthen community action, develop personal health skills, and sustain positive behavior change.

Diarrhea Prevention through Point-of-Use Disinfection and Safe Storage of Water: The Need for Innovative Interventions to Change Behavior

In many parts of the developing world, drinking water is collected from unsafe sources and is further contaminated during storage in household vessels. Simple, inexpensive disinfectant generators, better storage vessel designs, and community education allow families to disinfect drinking water immediately after collection and to store treated water in narrow-mouth, lidded vessels designed to prevent recontamination. This three-component prevention strategy has been field tested in Bolivia and Guatemala with remarkable success. Urban and rural families readily accepted the vessels and disinfectant, operated disinfectant generators, reliably obtained adequate levels of free chlorine in stored water, and produced from contaminated

sources potable water that met WHO standards for microbiologic quality. One study showed that the intervention reduced diarrheal disease episodes in children and infants by 44%. Guatemalan street vendors added a soap dish beside the water vessel to produce safer drinks and attract more customers. In Bolivia, water vessels and disinfectant are now commercially produced and marketed. Although the intervention costs less than US$1.00 per person per year and water vessels have been well accepted, in several projects the use of chlorine disinfectant has decreased over time. Health communication and initial adoption of water vessels alone has not changed the long-term water treatment behavior in a large percentage of the population. Formative research is needed prior to implementing these projects, and innovative behavioral techniques are needed to motivate and sustain behavioral change.

Lassa Fever Prevention In Endemic and Epidemic Situations—Sierra Leone

Lassa fever, a viral disease prevalent in West Africa, was first described in a village called Lassa in northern Nigeria. The disease affects healthy persons of all ages and both sexes and results in severe acute illness with a 16% death rate. The virus is transmitted from rodents to humans and from person to person. The disease is a major cause of illness and death in disease-endemic areas in Sierra Leone.

In 1976, the Lassa Fever Research Project was established as a collaborative effort between the Centers for Disease Control and Prevention and the Ministry of Health in Sierra Leone. The mandate was to study all aspects of the disease including epidemiology, diagnosis, treatment, prevention, and control. In the intervening years much has been learned about the virus and the disease it causes. Ribavirin, a drug effective against the disease, is not easily accessible, and no vaccine is available; therefore, prevention of endemic Lassa fever is vital. A multidisciplinary strategy for prevention and control has been developed and includes three components: clinical therapy, public education, and rodent control. Physicians at regional hospitals and village health workers have been trained to recognize the disease and its symptoms and to isolate and treat Lassa fever patients. Public education and communication activities have helped the general population recognize the disease and prevent transmission. Additional education and training provided information on how to reduce contact between humans and rodents. These promising approaches were disrupted by civil war.

A January 1996 outbreak lasted until April 1997. Of 664 reported cases, 427 were confirmed Lassa fever cases; 82 patients died. In response to the outbreak, an isolation and referral network was established and an emergency training workshop on surveillance, case management, prevention, and control of Lassa fever was organized for 40 health-care workers in two districts in Eastern Province. Lassa fever continues to be a major health problem in Eastern Province of Sierra Leone.

Healthy Islands and Emerging Infectious Diseases

In March 1995 in Yanuca Island, Fiji, the Western Pacific Regional Office of WHO introduced the Healthy Islands program. This program recognizes the peculiar character of island settings and seeks to reorient health and developmental planning in a manner that addresses this character. This approach to health promotion, which takes into account the setting of a particular health problem, has become prevalent since the Ottawa Health Promotion Conference in 1986.

Island states have contributed to the epidemiologic study of infectious diseases in areas such as the delineation of area-species or population-disease relationships and the history of infectious disease. This contribution stands to repeat itself as many modern island nations exhibit the factors that have been linked to the emergence of infectious disease, including economic vulnerability; unsustainable resource use; substantial internal migration; breakdown of water, sanitation, and public health services (especially in areas of rapid urbanization); and large inflows of tourists. Healthy Islands projects aimed at holistic solutions to these problems have addressed, for example, malaria control in the Solomon Islands, environmental health protection in Fiji, and water supply and sanitation in Tonga. These projects have included health communication with community development approaches directed at the peculiar problems of the island setting.

The Internet helps promote some of the principles of the Healthy Islands approach. The Internet promises to be an excellent tool for

overcoming professional isolation, a major shortcoming of the island setting, and providing authoritative and timely access to information. Two projects demonstrate this approach: The SYNAPSE is a network of health-care professionals in the Mediterranean island of Malta, and Pacnet is an electronic mailing list run by the Secretariat of the Pacific Community as a forum for public health practitioners with an interest in the Pacific. The Internet also serves to illustrate the "small-scale syndrome." The logistic problems that render small economies vulnerable increase the cost of bandwidth per head of population in small isolated communities. The skills to effectively use the technology are scarce and the cost of introduction great. Solutions like the Internet, appropriate in larger countries, are relatively costlier and may be less appropriate in smaller contexts.

The Internet is a good metaphor for Healthy Islands programs that seek to apply technology imported from larger countries and adopt it within cost-effective, holistic frameworks for health promotion. These solutions are relevant to the small isolated context, address issues that cut across sectoral and health service boundaries, and tend to be potentiated by concerted regional action.

Communicating Infectious Disease Information to the Public

Elias Abrutyn
Allegheny University of the Health Sciences Philadelphia,
Pennsylvania, USA

At one time, information about the science of medicine was almost the sole purview of physicians and scientists, and the vehicle of communication was predominantly the scientific journal. Today, a broad audience is interested in the results of scientific investigations, which are disseminated widely in a variety of media. This session sought to provoke discussion about scientific communication in the broadest sense and to describe the roles and perspectives of science writers and journalists.

Robin Cook, a science fiction writer, described two experiences during his medical training that prompted him to become an author: He realized that medicine involved high drama with star quality, and he noted a tremendous gulf between what physicians knew and what the public knew about medicine.

Physicians and scientists need to recognize basic differences between the goals of medical professionals and the goals of the media. Physicians and scientists seek to transmit information; the media, on the other hand, seek to entertain in addition to transmitting information. Fiction is a powerful tool because it places information in an emotional context that people remember, and its message has lasting influence.

Nichols Fox, a free-lance writer, discussed her interest in foodborne infectious diseases, particularly *Escherichia coli* diarrheal disease. Sometimes, in their research, reporters arrive at conclusions that are not entirely objective. The following are some of the conclusions Nichols Fox shared with the panel. 1) When you close the door on one microbe, you open the door for another. 2) Measures that make food more affordable may also increase disease risk. 3) Efficiency may not be the most important issue in food production, and in a cost-benefit analysis, the people benefitting are not always the ones sharing the cost. 4) Treatment of food animals and risk for disease are related. 5) Recycling food animals, particularly diseased animals, into animal feed, can cause problems.

Laurie Garrett, science and medical writer for Newsday magazine, highlighted the ability to place events within a historical perspective, discussed reasons for differing viewpoints of the same events (particularly differences between journalists and scientists), and suggested ways in which journalists and scientists can broaden public perspective.

Paraphrasing Barbara Rosenberg of the Harvard School of Public Health, Laurie Garrett noted public health professionals cannot see their work in a historical light. At the same time, seeing events in such light may not be possible. Further, each person's perspective is determined by cultural, educational, and other factors; therefore, alternative views of the same event should be allowed.

Like public health professionals, journalists need to consider the historical perspective as they deal with the task of reporting daily events. Journalists and scientists should gauge the current and future import of an event and examine how it reflects on events of the past. The Heisenberg principle of uncertainty also applies to epidemiology. When you see an event, you alter it—in particular, you bring your cultural perspective to it. Cultural perspective and scientific training affect interpretation of events and should be taken into account when making observations.

Journalists and authors of science fiction may make the scientific community uncomfortable with probing questions. Sometimes they simply reflect a different point of view or perspective; sometimes they make historical connections not plainly obvious to everyone. With a broad view in mind, scientists and journalists can bring a larger perspective to the public.

Patricia Cornwell, a crime novelist, noted that two sayings are wellknown in the morgue:

the case is only as good as the evidence (a book is only as good as the existing research), and as forensic pathologists say, people often die in the way that they lived—a saying not true about infectious diseases and bioterrorism in which the randomness is striking.

The session's message was that scientists should view science writers as the scribes who can disseminate a story to the public by translating technical language into accessible terms. Scientists, like science writers, should cultivate good sources and pick stewards who will communicate the information accurately. The world wants to know about emerging threats to health, and writers can help.

APEC Emerging Infections Network: Prospects for Comprehensive Information Sharing on Emerging Infections within the Asia Pacific Economic Cooperation

Ann Marie Kimball,* Carrie Horwitch,* Patrick O'Carroll,† Sumarjati Arjoso,‡ Chaiyos Kunanusont,§ Ya-Shin Lin,* Clifford Meyer,* Laura Schubert,* and Phillip Dunham*

*University of Washington, Seattle, Washington, USA; †The Centers for Disease Control and Prevention, Atlanta, Georgia, USA; ‡Ministry of Health, Indonesia; and §Ministry of Public Health, Thailand

Trading blocs realize the strategic importance of and threats from emerging infections, particularly those related to travel and food. Like the European Union, the Asia Pacific Economic Cooperation (APEC) is undertaking an initiative in emerging infections.

The APEC Emerging Infections Network project builds on an existing Internet-based educational network (APEC EduNet), created to help link APEC "study centers" at designated universities. Use of collaborative tools, such as e-mail and the World Wide Web, helps bridge the broad geographic expanse and diversity of APEC economies, permitting scientists and policy makers to share information and more effectively combat emerging infectious disease through surveillance, prevention, research, and control measures.

In the project's first year, staff made site visits to Thailand, Indonesia, the Philippines, and Canada, and compiled information regarding Internet access in these selected economies. Multidrug-resistant tuberculosis (MDRTB) was selected as a disease priority by the partner economies. Accurate, prospective surveillance data on MDRTB are not generally available. Information sharing by e-mail and automated e-mail lists has been successful, and feedback suggests these strategies will become increasingly useful. The Emerging Infections Network (EINet) Web site includes project information, surveillance data, policy discussion, prevention guidelines, and distance learning resources about emerging infections.

Human networking is as important as technology-based networking in addressing emerging infections. Technology is adequate to support communications if a comprehensive telecommunications strategy is used. APEC, unlike the European Union, does not have the treaty basis to support this intercountry collaboration, so memoranda of understanding are needed to facilitate sustainable surveillance information flow and scientific cooperation. Numerous member economies are eager to be included in project activities. In the second year the project is expanding both in terms of breadth of information and geography of economies.

Additional Information

1. Kimball AM. Pacific Rim economic ties spur emerging infections network. Washington Public Health 1997;22.
2. Lance CR, Joseph CA, Bartlett CLR. European surveillance of travel-associated Legionnaires disease. Slide presentation at the International Conference on Emerging Infectious Diseases, Atlanta.
3. WHO/IUATLD. Global project on anti-tuberculosis drug resistance surveillance, 1994-1997. Geneva: World Health Organization;1997. p. 1-227.

Controversies in the Prevention and Control of Antimicrobial Drug Resistance

David Bell
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

In Hospitals

William Jarvis, Centers for Disease Control and Prevention (CDC), discussed antimicrobial resistance related to hospitalization. Two major factors contribute to the emergence and spread of antimicrobial resistance in hospitals: a high rate of antimicrobial drug use and inadequate infection control practices. Much antimicrobial drug use in hospitals is inappropriate (e.g., the use of vancomycin to treat a staphylococcal infection susceptible to methicillin, or the continuation of perioperative prophylaxis beyond 24 to 48 hours). Educational efforts on antimicrobial drug use in hospitals have had mixed success. More aggressive and controversial approaches to improve the use of these drugs have been proposed; for example, excluding certain drugs (such as vancomycin) from the routine reporting of susceptibility results; monitoring antimicrobial use with feedback to physicians concerning inappropriate use; antibiotic-use audits targeting problem areas (e.g., no diagnostic test done, more than four drugs used during one hospitalization, use for more than 3 weeks continuously); regulating drug promotion; requiring justifications for use; using computer-generated stop orders; and developing formularies, restrictions, and protocols by a multidisciplinary team.

In Communities

Keith Klugman, South African Institute of Medical Research, spoke on community-acquired infections, focusing on respiratory pathogens. One controversial area concerns the extent to which drug resistance identified in the microbiology laboratory correlates with clinical failure. Since clinical trial data are frequently unavailable, assessment of drug efficacy is often based on pharmacodynamics; i.e., a drug is believed efficacious if its concentration at the site of infection exceeds the organism's MIC. Otitis media and meningitis studies support this approach. In Pakistan, laboratory data indicate that 78% of pneumococci are resistant to cotrimoxazole, yet the clinical treatment failure rate is only 15%. The reasons for this discrepancy are unknown, but the issue is important because alternative drugs are more expensive. Standardization of laboratory methods and appropriate surveillance methods are essential.

Another controversial area involves antibiotic use and how to improve it. For many infections, the optimal dose and duration of therapy are unknown. Antibiotics are often prescribed inappropriately because physicians are uncertain when antibiotics are indicated and patients demand them; educating both of these groups is a challenge. Better diagnostics to reduce empiric therapy would be helpful. Other areas of uncertainty include the use of vaccines to decrease colonization and infection with resistant organisms and the extent to which antibiotics given for a specific indication might lead to resistance in different organisms.

In Veterinary Medicine

Klaus Stoehr, World Health Organization (WHO), addressed controversies related to use of antibiotics (preventive, therapeutic, growth-promoting) in food animals. Some antibiotic use contributes to the pool of resistant human pathogens. Both medical and nonmedical uses of antibiotics should be reduced. More scientific data are needed to address issues related to antibiotic use in food animals, including elucidating the human health impact, e.g., the percentage of resistance genes or resistant organisms originating in animals and the extent to which therapy of zoonotic bacterial infections in humans has been compromised because of resistance. The economic benefits of subtherapeutic antimicrobial use for growth promotion are also controversial; one study estimates that production costs without such use would increase by up to 8%, but recent experience

in Sweden indicates that meat produced without growth promotants can be priced competitively. A WHO meeting in 1997 on the medical impact of antibiotic use in livestock production recommended antimicrobial resistance monitoring and prudent use of antibiotics in food animals.

In Developing Countries

Antonio C. Pignatari, Escola Paulista da Medicina, São Paulo, Brazil, discussed antibiotic resistance issues in developing countries. More than two thirds of the world's population lives in developing countries, where the contrast between wealth and poverty is extreme. Infectious diseases represent the main public health problem. Because of inadequate resources for surveillance, control, and treatment, antimicrobial-resistant infections have become a major problem with serious implications for the health system and the economy. The main problems with drug resistance are seen in the treatment of diarrheal diseases, sexually transmitted diseases, pneumococcal infections, tuberculosis, nosocomial infections, and malaria. Restrictive antimicrobial use policies (which are controversial) can be effective in the hospital but are difficult to implement in the community. In many areas, the availability of medical care is limited; thus, laws requiring a physician's prescription for antibiotics are difficult to enforce. Pharmacies provide an important service in dispensing medications, yet most developing country pharmacists have limited training. The use of antimicrobial drugs in food animals is also a problem in developing countries, and no controls are in place to address it. Control of antimicrobial resistance and emerging infections in developing countries cannot be achieved without addressing closely related social and economic issues.

In Clinical Laboratories

Fred Tenover, CDC, addressed antibiotic resistance issues in the microbiology laboratory. Laboratorians must move from susceptibility testing to finding resistance. A common misconception is that new resistance mechanisms are easily identified because they result in high MICs and low zone sizes. However, many new resistance mechanisms lead to MICs close to the breakpoint for resistance. More sensitive screening tests are being introduced to detect resistance, but they cannot replace MICs; therefore, laboratory workload is increasing in an era of downsizing.

Several "drug-bug" combinations are problematic and require new approaches. For detecting nonsusceptibility (intermediate or full resistance) of staphylococci to vancomycin, the traditional method of disk diffusion testing is not reliable. Acceptable methods include the Brain Heart Infusion vancomycin agar screening test developed for enterococci or broth microdilution tests held 24 hours. For pneumococci, testing for susceptibility to both penicillin and extended spectrum cephalosporins is important because resistance to these drugs is becoming more common. For invasive isolates where the need to detect resistance is urgent, the oxacillin screening test should not be used; MIC methods should be used directly. For gram-negative bacilli, traditional methods to detect resistance to extended spectrum beta-lactam drugs are inadequate, although the latest National Committee for Clinical Laboratory Standards guidelines present effective screening tests. Finally, sensitivity must be determined for clinically important isolates treated with fluoroquinolones, since selective pressure for resistance is increasing as a result of widespread use.

Infectious Causes of Chronic Inflammatory Diseases and Cancer

Gail H. Cassell
Lilly Research Laboratories, Indianapolis, Indiana, USA

Powerful diagnostic technology, plus the realization that organisms of otherwise unimpressive virulence can produce slowly progressive chronic disease with a wide spectrum of clinical manifestations and disease outcomes, has resulted in the discovery of new infectious agents and new concepts of infectious diseases. The demonstration that final outcome of infection is as much determined by the genetic background of the patient as by the genetic makeup of the infecting agent is indicating that a number of chronic diseases of unknown etiology are caused by one or more infectious agents. One well-known example is the discovery that stomach ulcers are due to *Helicobacter pylori.* Mycoplasmas may cause chronic lung disease in newborns and chronic asthma in adults, and *Chlamydia pneumoniae*, a recently identified common cause of acute respiratory infection, has been associated with atherosclerosis. A number of infectious agents that cause or contribute to neoplastic diseases in humans have been documented in the past 6 years. The association and causal role of infectious agents in chronic inflammatory diseases and cancer have major implications for public health, treatment, and prevention.

The belief that infectious agents are a cause of chronic inflammatory diseases of unknown etiology and of cancer is not new. Approximately 100 years ago, doctors noted a connection between cervical cancer and sexual promiscuity that transcended mere coincidence (1). By 1911, a connection between viruses and cancers in animals had become well established (2). As early as the 1930s, mycoplasmas were proposed as a cause of rheumatoid arthritis in humans, and shortly thereafter, they were proven to be the most common cause of naturally occurring chronic arthritis in animals (3). Proof of causality of cancer and arthritis in humans was more difficult. When searches for infectious agents in cancer and arthritis found none, research began to focus on mechanisms of inflammation, tumorogenesis, and drug discovery. More recently, however, scientists have renewed searches for infectious agents.

Advances in molecular biology and medical devices have revolutionized our ability to detect very low numbers of infectious agents in specimens collected directly from the affected site. HIV has demonstrated the ability of infectious agents to produce slowly progressive, chronic disease with a wide spectrum of clinical manifestations and disease outcomes. Increased understanding of the body's defense mechanisms and the demonstration that final outcome of infection is as much determined by the genetic background of the host as by the genetic makeup of the infecting agent suggest that a number of chronic diseases of unknown etiology may be caused by an infectious agent.

Recent data suggest a role for one or more infectious agents in the following chronic diseases: chronic lung diseases (including asthma), cardiovascular disease, and cancer. Many of the agents implicated are commonly transmissible and are either treatable with existing antibiotics or are potentially treatable with antiviral drugs. Thus, proof of causality in any one of these diseases would have enormous implications for public health, treatment, and prevention. Few areas of research hold greater promise of contributing to our understanding of infectious diseases and the eventual relief of human suffering.

The intent of this paper is not to provide a comprehensive review of chronic inflammatory diseases of unknown etiology and the agents implicated but rather to utilize several models to discuss available data and to illustrate the difficulty in proving causality in chronic inflammatory diseases. The discussion is based

Address for correspondence: Gail H. Cassell, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285, USA; fax: 317-276-1743; e-mail: Cassell_Gail_H@Lilly.com.

upon the following assumptions. Most chronic inflammatory diseases are likely multifactorial. Heredity, environment, and nutrition are critical determinants of disease expression with heredity being the most important.

Theoretically, chronic inflammatory diseases currently of unknown etiology could result from three different types of pathogens: 1) those that are fastidious and previously recognized but because of their fastidiousness or lack of appreciation of their disease-producing potential are not included in the differential diagnosis, and 2) infectious agents previously not recognized that therefore go undetected. Infection with either group can result in misdiagnosis and lack of treatment. Depending upon the biology of the organism and intrinsic and extrinsic factors of the host the organism can persist, resulting in chronic inflammation. The third group of pathogens would be those that elicit an autoimmune response resulting in persistent inflammation without the persistence of the inciting agent. Examples of the first two groups of pathogens will be discussed here using mycoplasmas to typify the first group and *Chlamydia pneumoniae* the second. Finally, recent advances in our understanding of the role of infectious agents in cancer will also be summarized.

Chronic Lung Diseases

Murine Chronic Respiratory Disease as a Model System

The difficulty in establishing the infectious etiology of a chronic obstructive lung disease is well illustrated by *Mycoplasma pulmonis* and murine chronic respiratory disease. Proof that *M. pulmonis* can cause this disease took nearly 50 years and required inoculation of germ-free animals (4). Chronic bronchopneumonia in rats was first described in 1915 when this species came into general use for experimental purposes (5). In approximately 1940, a Mycoplasma, later identified as *M. pulmonis*, was recognized as a possible cause (6), but the ubiquity of the organism and its frequent isolation from healthy as well as diseased rats and mice (even from trachea and lungs) soon gave it the reputation of being a commensal with little pathogenic potential. The failure of pure cultures of this organism to consistently produce disease of the lower respiratory tract also precluded its acceptance as the etiologic agent. Only in the early 1970s was *M. pulmonis* alone shown to consistently reproduce all of the characteristic clinical and pathologic features of the natural respiratory disease when inoculated into animals maintained under germ-free conditions (7). Subsequent studies provided explanations for previous difficulties in reproducing the disease.

The respiratory disease caused by *M. pulmonis* is slow to begin and long-lasting. Consequently, the disease has various stages of pathologic lesions and a lack of uniform lesions, even among animals in the same cages (due partly to variables that can affect development of the disease in the lower respiratory tract, such as intracage ammonia produced by bacterial action on soiled bedding, synergy with murine respiratory viruses and other bacterial pathogens, and nutritional factors) (7). However, comparison of animals matched for age, sex, and microbial and environmental factors indicates that heredity is the most critical determinant of susceptibility, lesion character, and disease severity. Susceptibility among animal species and among strains of the same species differ dramatically (8-11).

Intranasal inoculation of *M. pulmonis* produces markedly different lesions in F344 rats and in CD-1 mice, even when the dose is comparable on the basis of lung and body weight. In rats the lesions progress slowly from the upper respiratory tract distally, with alveolar involvement occurring days to months following inoculation, whereas in mice, alveolar lesions develop within hours after infection and are responsible for acute alveolar disease and death within 3 to 5 days. Depending on their genetic background, mice that survive the acute disease develop chronic lung disease characterized by bronchiectasis that persists for up to 18 to 24 months or the lifetime of the animal.

Studies of naturally occurring and experimentally induced disease indicate that *M. pulmonis* also causes a slowly progressing upper genital tract disease in LEW and F344 rats (18). Pups can become infected in utero, at the time of birth due to cervical and vaginal infection of the dams, or via aerosol from dams shortly after birth. Even though the organisms can be shown to colonize the ciliated epithelium of the upper and lower respiratory tracts of pups, microscopic lesions are not detectable for 2 to 6 months depending on the strain of rat. Development of obstructive lung disease can require as long as 12 to 18 months.

Differences in severity and progression of the lung lesions due to *M. pulmonis* in LEW and F344 rats are related to differences in the degree of nonspecific lymphocyte activation in the two strains or an imbalance in regulation of lymphocyte proliferation in LEW rats (12). *M. pulmonis* possesses a potent B cell mitogen, and, in addition, the organism is chemotactic for B cells (13). Interestingly, LEW rats are also more susceptible to other chronic inflammatory diseases, including streptococcal cell-wall induced arthritis, adjuvant-induced arthritis, and allergic encephalomyelitis (12).

Ureaplasma urealyticum as a Cause of Pneumonia in Newborns and Its Association with Chronic Lung Disease (CLD) in Premature Infants

Respiratory dysfunction represents the most common life-threatening problem in premature infants and one of the largest costs of neonatal intensive care (14). Infants weighing less than 1,000 g at birth are more likely than those with greater birth weights to die within the first few days of birth of respiratory-related problems; those who survive are at an increased risk of CLD (15). Approximately 20% of stillborn babies and infants dying within 72 hours of delivery have histologic evidence of pneumonia (16). Yet the true incidence of lower respiratory infection acquired either in utero or at the time of delivery and its contribution to death or development of CLD are unknown. The cause of lower respiratory disease in newborn babies is a diagnostic dilemma because pneumonia in early neonatal life is usually clinically and radiologically indistinguishable from surfactant-deficiency syndrome (17). Furthermore, meaningful cultures from the lung are not easily obtained, whereas cultures of the throat, nasopharynx, and blood are unrevealing or misleading.

Pneumonia

The mycoplasma *U. urealyticum*, a common commensal of the lower female genital tract, has recently been shown to cause respiratory disease in newborn infants. Retrospective (18) and prospective (19-21) studies indicate an association of *U. urealyticum* with congenital pneumonia. Case reports also provide evidence that *U. urealyticum* is a cause of pneumonia in newborn infants (22-23). The organism has been isolated from affected lungs in the absence of chlamydiae, viruses, fungi, and bacteria and in the presence of chorioamnionitis and funisitis (40) and has been demonstrated within fetal membranes by immunofluorescence (24) and in lung lesions of newborns by electron and immunofluorescent microscopy (20). The specific immunoglobulin (Ig) M response in several cases of pneumonia in newborns further documents in utero infection (20).

We have found that *U. urealyticum* is the single most common microorganism isolated from endotracheal aspirates of infants who weigh ≤2,500 g and who require supplemental oxygen within the first 24 hours after birth (19). Infants weighing ≤1,000 g and from whom *U. urealyticum* is isolated from the endotracheal aspirate are twice as likely to die as infants of similar birth weight but who are uninfected or infected infants ≥1,000 g. These findings support the hypothesis that only a select group of infants, i.e., those with very low birth weights, is subject to disease due to *U. urealyticum*. This fact may account for the seeming disparities in conclusions regarding the role of *U. urealyticum* in neonatal respiratory disease reached in earlier prospective studies that failed to distinguish this subpopulation at high risk from the whole (25,26).

That endotracheal isolations of *U. urealyticum* represent true infection of the lower respiratory tract is supported by initial isolation of ureaplasmas in numbers exceeding 1,000 CFUs (and sometimes exceeding 10,000 CFUs) and repeated isolations of the organism from tracheal aspirates for weeks and even months in some infants that continue to require mechanical ventilation. That the tracheal isolates are not merely a reflection of contamination from the nasopharynx is supported by the discrepancy in isolation rates between the two sites and recovery of *U. urealyticum* in pure culture from endotracheal aspirates in more than 85% of the infants (19). Concomitant recovery of the organism from blood of up to 26% of those with positive endotracheal aspirates and from cerebrospinal fluid (CSF) of some infants indicate that in some infants the organism is invasive (19). Fourteen percent of *U. urealyticum* endotracheal isolates were from infants born by cesarean section with intact membranes, indicating that in utero transmission occurs rather commonly, at least in premature infants.

In a study of 98 infants, respiratory distress syndrome, the need for assisted ventilation, severe respiratory insufficiency, and death were

significantly more common among those infants <34 weeks gestation from whom *U. urealyticum* was recovered from endotracheal aspirates at the time of delivery than among uninfected infants (27). *U. urealyticum* was isolated from 34% of blood cultures and also from four of six CSF samples and in 6 of 11 postmortem brain and lung biopsy pecimens. Eighty-two percent of the ureaplasma isolates were present in pure culture, and 48% of infants born by cesarean section with intact membranes had ureaplasmas isolated from one or more sites.

U. urealyticum can induce ciliostasis and mucosal lesions in human fetal tracheal organ cultures (20). Furthermore, we have shown that ureaplasmas isolated from the lungs of human infants with congenital and neonatal pneumonia produce a histologically similar pneumonia in newborn mice (28). Even in this mouse model, age is a critical determinant of disease. Newborn mice are susceptible to colonization of the respiratory tract and development of pneumonia; 14-day-old mice are resistant.

We have shown that endotracheal inoculation of premature baboons (well-established models of premature human infants) with *U. urealyticum* isolated from human infants results in the development of pathologically recognizable pulmonary lesions, including acute bronchiolitis with epithelial ulceration and polymorphonuclear infiltration, which is distinguishable from hyaline membrane disease (29). *U. urealyticum* can be isolated from blood, endotracheal aspirates, and pleural fluid and lung tissue from some of these animals 6 days after infection.

The available evidence provides a strong argument that *U. urealyticum* is a common cause of pneumonia in newborn infants, particularly those born before 34 weeks of gestation. The organism can be isolated from endotracheal aspirates in up to 34% of infants weighing <2,500 g; radiographic evidence of pneumonia is twice as common in these infants as in *U. urealyticum* negative infants (30% vs. 16%, p = .03) (30). Many of these infections develop as a result of in utero exposure. Cases of ureaplasmal pneumonia occur much less frequently in term infants. These findings in infants are consistent with the fact that *U. urealyticum* infection of the chorioamnion is also much more common before 34 weeks of gestation. Lack of transplacental passage of immunoglobulin prior to 32 weeks gestation (31) may partially explain these findings. Experience from mycoplasmal respiratory diseases of animals indicates that preexisting antibody is protective, whereas antibody in the presence of an established infection is rarely effective in elimination of the organism (32).

CLD in Premature Infants

Some, but not all, studies (33-36) show an association between isolation of *U. urealyticum* from the respiratory tract of newborn infants and the development of CLD (33). Differing results may be obtained because some studies do not limit culture isolation to the affected site (the lower respiratory tract), do not limit their patient population to those at greatest risk (birth weight <1,000 g); or do not limit culture isolation to within 12 hours of delivery, i.e., most likely infected in utero. Several facts suggest that infants who acquire *U. urealyticum* in utero may be at greatest risk for development of CLD. Dyke et al. (34) found *U. urealyticum* in the gastric aspirates of infants ≤1,000 g was associated with a significantly increased risk of CLD in those infants delivered by cesarean section but not in those delivered vaginally. This could result from a longer exposure to *U.urealyticum* as a result of in utero exposure, or it may be a reflection of differences in the virulence of those organisms found only in the cervix versus those that have invasive potential and that can cause an ascending infection from the vagina into the uterus. Along these lines it is of interest that a recent study of 49 preterm infants which included only three infants from whom *U. urealyticum* was recovered within 24 hours of birth found no association with development of CLD (35). The remaining 11 infants were not culturally positive until 48 to 72 hours after birth suggesting that only the three study infants were infected in utero. In another recent study reported by Valencia et al. (36) CLD was found in 26% of *U. urealyticum* infected infants compared to only 4.7% of the noncolonized group. However, these results were not statistically significant possibly because of the small number of patients studied but also possibly because 22% of the patients included did not have cultures performed until between 2 days and 3 months postnatal life.

Isolation of *U. urealyticum* from endotracheal aspirates is not only a risk factor for development of pneumonia but also of precocious dysplastic changes (30). Walsh et al. (38) isolated *U. urealyticum* directly from pleural fluid and

tissue collected by open lung biopsy in four of eight infants cultured who had CLD. We (19) continued to recover ureaplasmas from endotracheal aspirates of infants with CLD for months following initial recovery of the organism from endotracheal aspirates within 12 hours of birth.

Available evidence creates a cohesive argument that *U. urealyticum* infection of the lower respiratory tract is a likely risk factor for, and not only associated with, CLD. Because *U. urealyticum* has only recently been suggested as a cause of pneumonia in newborns, it is not routinely sought by most hospital laboratories. Furthermore, the organism is not susceptible to antibiotics used prophylactically in very low birth-weight infants with evidence of respiratory distress. Consequently, the infection, i.e., pneumonia, goes undetected and untreated. Due to the difficulties in diagnosis, most hospital laboratories do not culture for this organism.

The pathophysiology of CLD in premature infants suggests that *U. urealyticum* produces undetected and untreated pneumonia and results in an increased requirement for oxygen and subsequent development of CLD as a result of oxygen toxicity (33,37) or a synergistic effect between the ureaplasmas and hyperoxia. It has been proposed that hyperoxia-induced lung injury contributes to development of CLD by stimulating the proinflammatory cytokine interleukin (IL)-6 (38). *U. urealyticum* may also contribute to the development of CLD by stimulation of proinflammatory cytokines. Infants from whom ureaplasmas are isolated from endotracheal aspirates within the first 24 hours of life are more likely to have neutrophils in their tracheal aspirates on day 2 than are those not colonized (39). Aspirates from colonized infants are also more likely to have class II cytology than those from uncolonized patients at day 2 of life. This may explain why ureaplasma-infected infants respond to dexamethasone therapy (39). These in vivo findings are consistent with the recent demonstration of *U. urealyticum* induction of IL-6 and IL-8 in human neonatal pulmonary fibroblasts even in the absence of hyperoxia (38). Interestingly, together ureaplasmas and hyperoxia resulted in greater stimulation of IL-6 and IL-8 than either alone. This is consistent with the synergism previously demonstrated in vivo between ureaplasmal infection and hyperoxia (37).

Studies in mice also suggest that increased oxygen requirements of very low birth-weight infants might predispose them to lower respiratory tract infection or, alternatively, that *U. urealyticum* infection potentiates oxygen-induced injury (28,37). Exposure to oxidants is known to enhance respiratory disease and death due to *M. pulmonis* respiratory disease in mice (41).

That *U. urealyticum* is a cause of pneumonia in newborns can no longer be questioned. Data provide strong evidence that *U. urealyticum* can be a primary cause or a contributing cofactor in development of CLD in humans, but the data are not definitive. Cohort studies allow follow-up of exposed persons and thus reduce bias, but the designs of these studies cannot rule out the possibility that a third factor associated with *U. urealyticum* is actually the true cause of CLD. However, a randomized trial of exposure to infection in humans is not ethical or practical. Although a randomized trial of antibiotic treatment could provide critical information related to patient management, it would still not bring us closer to proving causality. Even if treatment is found to be efficacious, conclusions about causation will be limited by the fact that the third factor might also be susceptible to the antibiotic chosen. If it is not found to be efficacious, it may be because ureaplasma infection in utero or soon after birth results in irreversible lung damage. Nevertheless, a treatment trial is urgently needed to determine whether appropriate therapy can reduce the incidence of illness and death associated with CLD. First, studies are needed to determine dose and duration of antibiotic therapy and whether currently available antibiotics will even eliminate the organism.

Mycoplasma pneumoniae and *C. pneumoniae* as Causes of Chronic Asthma

Asthma, a CLD characterized by airway obstruction, inflammation, and bronchial hyperresponsiveness to a variety of stimuli, including infections, is a common illness in both pediatric and adult populations. In the United States alone, approximately 12 million people have asthma, resulting in health-care costs of approximately 4.6 billion dollars annually (42). In children, asthma is the most common reason for hospital admissions and school absenteeism (43). Yet the etiology and pathogenesis of this important disease remains poorly defined. Historically, viruses that commonly infect the respiratory tract have been thought to play a role

in both provoking asthma exacerbations and in altering responses to other environmental agents that might be involved (44).

M. pneumoniae is a common cause of both upper and lower respiratory infection in humans; tracheobronchitis is the most common clinical manifestation (45). Previously thought to cause acute, self-limited disease primarily in persons between 6 and 21 years of age (45), *M. pneumoniae* is now known to be the cause of pneumonia in 20% to 25% of all age groups and to persist in certain persons for weeks to months, resulting in prolonged reduced pulmonary clearance and airway hyperresponsiveness (46,47). Epidemiologic evidence links mycoplasma infection with asthma exacerbation and possibly with the pathogenesis of asthma (47-50).

While *M. pneumoniae* has been associated with exacerbations of asthma, its role in sustaining chronic asthma or in initiating exacerbation is unknown. However, the proven role of mycoplasmas in similar chronic respiratory diseases of numerous animal species, including *M. pulmonis* in rodents, suggests that careful, systematic studies should be undertaken in humans (45).

C. pneumoniae, the most recently described *Chlamydia* species, has been associated with a wide range of respiratory tract illnesses, from pharyngitis to pneumonia with empyema (51). *C. pneumoniae* has been isolated from 15% to 20% of adults and children with community-acquired pneumonia (51). On the basis of serologic results only, *C. pneumoniae* has been associated with acute exacerbations of asthma in adults (52); on the basis of nasopharyngeal cultures, it has been associated with asthma in children (53). In both children and adults, the organism persists for months in the upper respiratory tract of patients with wheezing (54).

If *M. pneumoniae*, or for that matter any infectious agent, is a causal factor in initiating and sustaining asthma in certain persons, the agent should be present and persistent in the lungs of some persons with stable, chronic asthma. We have recently undertaken a study to determine if *M. pneumoniae* can be detected in the lungs of adults with stable, chronic asthma versus asymptomatic controls (55). To facilitate interpretation of results, we also evaluated the presence of other fastidious infectious agents that have previously been implicated in the pathogenesis of asthma, including the seven common respiratory viruses (44) and *C. pneumoniae* (56,57).

M. pneumoniae was detected by PCR in 10 of 18 asthma patients and 1 of 11 controls ($p = 0.02$). All patients were culture, EIA, and serologically negative for *M. pneumoniae*. All PCR and cultures were negative for *C. pneumoniae* and all EIAs for respiratory viruses were negative. Nine persons with asthma and one control exhibited positive serology for *C. pneumoniae* ($p = 0.05$). For *C. pneumoniae*, the lack of correlation between serologic results and culture and PCR was not unexpected. We have seen discordance between culture and serologic results in patients with community-acquired pneumonia (58,59), but in these cases more patients were culture positive than seropositive. Thus, the culture methods we used in the study have previously been shown to be valid.

Our failure to culture *M. pneumoniae* might be explained by its extreme fastidiousness or its low numbers. Culture is the least sensitive of the methods used in this study for detection of *M. pneumoniae*. However, the culture methods we used in this study we also used to evaluate more than 2,000 respiratory specimens during the same period in patients with radiographically confirmed, community-acquired pneumonia. These methods have resulted in recovery of *M. pneumoniae* by culture in up to 17% of patients (G. Cassell, et al., unpub. obs.; 58,59).

Recent studies indicate that some other mycoplasma species of human origin may be able to survive intracellularly in chronic infections of cell cultures (60). Likewise, some strains of *M. hyorhinis*, the etiologic agent of chronic respiratory disease of swine, can become so adapted to growth in the presence of cells that it is no longer cultivable on artificial media (61). If this occurs in vivo, organisms like *M. pneumoniae* could be difficult if not impossible to recover by culture.

In the absence of other known respiratory pathogens in other patient populations, the presence of *M. pneumoniae* can be detected longer by PCR than by either culture or serologic test. Guinea pigs experimentally infected with *M. pneumoniae* become chronically infected as detected by PCR for up to 200 days but are culture negative by 70 days. Also by 70 days, antibody levels become negative (62). Thus, patients with asthma appear chronically infected with *M. pneumoniae*, despite negative culture results, because they are PCR positive. That positive PCR results truly reflect involvement of the lower respiratory tract by *M. pneumoniae* is supported

by the fact that 9 of the 10 *M. pneumoniae*-positive patients were positive in the bronchoalveolar lavage (BAL), bronchial biopsies, or bronchial brush specimens. Furthermore, the organism was detected in the nasopharynx or the throat of only five of the nine asthma-positive patients, thus indicating that detection in the lower tract was not merely due to contamination by organisms from the upper tract during sample collection. More importantly, a significant number of persons with asthma were positive in the lower respiratory tract on repeat sampling (2 to 4 months between samples), thus indicating persistent colonization. By cloning and sequencing the PCR product in BAL from several representative patients, we demonstrated 100% sequence homology with *M. pneumoniae.* Use of multiple primer pairs as well as confirmation of PCR findings in two different laboratories also attests to the validity of the PCR results. Our failure to detect *M. pneumoniae* in specimens from age-matched control patients as well as in specimens from 100 asymptomatic children using the same PCR methods further verifies the specificity of our PCR methods and argues that finding *M. pneumoniae* in persons with chronic asthma does not merely reflect a carrier state.

We have previously noted the lack of antibody response to *M. pneumoniae* in both pediatric and adult populations with community-acquired pneumonia (G. Cassell et al, unpub. obs.; 58,59,63). Study results indicate that a subset of infected persons do not mount an antibody response, perhaps due to genetic differences. Lack of antibody may in fact contribute to the organism's persistence. The immunomodulatory properties of *M. pneumoniae* (12) also could facilitate the organism's persistence.

Recent studies indicate that *M. pneumoniae* respiratory disease is often misdiagnosed and inappropriately treated, which would also contribute to persistence. Admitting physicians chose other pathogens as the most likely agents in 46% of the cases subsequently documented as *M. pneumoniae* infections (64). Even upon correct diagnosis, at least 10% of the patients did not receive appropriate antibiotics during their hospitalization.

In summary, we have demonstrated that persons with chronic asthma, but not healthy persons, exhibit evidence of *M. pneumoniae* colonization of the lower airways. Like several other investigators (56,57), we found more persons with asthma than control subjects had serologic evidence of *C. pneumonia* infection. Further study is needed to determine if these findings are an epiphenomenon or, as we expect, a pathogenic mechanism in asthma. If the latter is correct, greater evaluation of the process involved is needed to further our understanding of the pathogenesis and treatment of asthma.

Role of *C. pneumoniae* in Atherosclerosis

Infection was proposed as a cause of atherosclerosis by Sir William Osler and others at the beginning of the century (65). However, it was not until the 1970s that experimental infection of germ-free chickens with an avian herpesvirus was found to produce arterial disease that resembled human atherosclerosis (66). Associations have since been reported of human coronary heart disease with certain gram-negative bacteria (i.e., *Helicobacter pylori* and *C. pneumoniae)* (67,68), with certain herpesviruses (especially cytomegalovirus) (69), and with clinical markers of chronic dental infection (70). Rather than an exhaustive evaluation of each of these purported associations, it seems reasonable to focus on the respiratory pathogen, *C. pneumoniae,* for which the evidence seems strongest.

C. pneumoniae, like *M. pneumoniae*, is a common cause of community-acquired pneumonia (70,71). *C. pneumoniae* infects more than 50% of people at some point in their lives (51,71). It can often go undiagnosed and improperly treated because again it is fastidious and diagnostic methods are not routinely available. Even in the best reference laboratories, diagnosis can be a challenge (71). It, like *M. pneumoniae,* is also thought to play a role in acute asthma and chronic bronchitis (52) as well as to cause extrapulmonary manifestations (51,71). It, like *M. pneumoniae*, can also result in persistent infection following acute respiratory disease (54).

Eighteen seroepidemiologic studies evaluated the association of *C. pneumoniae* infection and cardiovascular disease (67). Most found at least twofold or larger odds ratios; some reported increasing odds ratios with increasing antibody titers. The general consistency of their findings in a total of 2,700 cases supports the existence of some real association between *C. pneumoniae* and coronary heart disease because the studies were done in different populations, used different criteria for cases, adjusted for potential confounders to differing degrees, and were, therefore, prone

to different biases. While diagnosis by serology has its limitations, *C. pneumoniae* has been demonstrated by a variety of laboratory techniques (including culture, PCR, electron microscopy, and immunocytochemistry) in the atherosclerotic lesions of coronary arteries, carotid arteries, aorta, smaller cerebral vessels, and larger peripheral arteries (72-78). In the more than 13 published studies of *C. pneumoniae* in human pathology samples (67), chlamydiae were present in 257 (52%) of 495 atheromatous lesions but in only 6 (5%) of 118 control samples of arterial tissue, yielding a weighted odds ratio of about 10 (95% confidence interval 5-22). It seems unlikely that sampling biases can entirely account for this extreme difference between case and control tissue.

C. pneumoniae, an obligatory intracellular bacterium capable of causing persistent infection and multiplying in endothelial and smooth muscle cells and macrophages (79), can also be disseminated by macrophages (80). Hence, some have argued that macrophages may ingest *C. pneumoniae* in the lung or elsewhere before migrating to atheromatous lesions, in which case it may only be a bystander. However, in two different rabbit models, atherosclerotic changes develop only after infection with *C. pneumoniae* (81,82). The organism by itself induces the production of cytokines (83) and adhesion molecules (84), and it possesses an endotoxin (85) capable of modulating the host inflammatory response. Thus, the biologic properties of *C. pneumoniae* make it a logical candidate for triggering the chronic inflammation found in atherosclerosis (82).

Finally, some studies have found rising or elevated levels of antibodies to *C. pneumoniae* in some males during the months just preceding a heart attack (86). Recent studies indicate that antibiotics given during or after a first heart attack may decrease the risk of a second cardiac problem (86-88). This finding also raises the possibility that antibiotics may have a role in the treatment of cardiovascular illnesses; that could be especially beneficial in developing countries where traditional treatments like angioplasty are expensive.

Some have proposed additional large-scale antibiotic treatment trials in an attempt to further prove causality. Several major issues need to be resolved first. Ideally, one should treat patients with documented *C. pneumoniae* infection; however, reliable diagnostic methods and treatment protocols are lacking (71). Because most available antibiotics are bacteriostatic, not bacteriocidal, some patients may remain infected up to 11 months after treatment. Even if these issues could be resolved, antibiotic treatment trials will not prove causality, just as is the case with *U. urealyticum* and CLD of prematurity or *M. pneumoniae* and chronic asthma. The nonantimicrobial effects may also influence the outcome of such studies. For example, tetracyclines can inhibit metalloproteinases, which may contribute to acute coronary syndromes (89). Some macrolides have antiinflammatory effects (90-93). Moreover, antibiotics are not selective, thus making it impossible to determine the effects of treatment upon *C. pneumoniae* versus other potential culprits, e.g., *H. pylori,* which is also susceptible to tetracyclines and macrolides. However, if antibiotic treatment could reduce atherosclerotic events, the public health implications could be enormous.

Causal Role of Viruses and Bacteria in Cancer

Early in this century, Peyton Rous (2) established beyond doubt that cancer can be caused by an infectious agent in chickens. Since then, evidence has accumulated that other viruses cause cancer in a number of different animal species (94). A growing body of research suggests that a number of viruses, bacteria, and parasites cause cancer in humans, thus providing new possibilities for treatment and prevention of cancer (94). In 1997, the World Health Organization estimated that up to 84% of cases of some cancers are attributable to viruses, bacteria, and parasites and that more than 1.5 million (15%) new cases each year could be avoided by preventing the infectious disease associated with them (95).

H. pylori, found in the stomachs of a third of all adults in the United States, causes inflammation of the mucous membrane of the stomach (96). In 20% of infected persons, *H. pylori* induces gastric ulcers (96). Peptic ulcer disease, a chronic inflammatory condition of the stomach and duodenum, affects as many as 10% of people in the United States at some time in their lives. In the early 20th century, pathogenesis was believed related to stress and dietary factors. Thus treatment focused on bed rest and bland food. Later, gastric ulcers were believed to be caused by the injurious effects of digestive secretions. Following the identification of the histamine receptor that appeared to be the principal mediator of gastric acid secretion, antagonists of this receptor were used for therapy

for peptic ulcer disease. In 1982, *H. pylori* was first isolated from the human stomach, but it was not until one decade later and after Marshall ingested pure cultures of the organism that causality was accepted by the medical and scientific community (97).

In 1994, the International Agency for Research on Cancer concluded that infection of humans with *H. pylori* is causally associated with the risk of developing adenocarcinoma of the stomach (98), one of the most common malignancies in the world, although relatively uncommon in the United States (24,000 new cases and 14,000 deaths per year). However, also in 1994, a Consensus Panel of the National Institutes of Health (NIH) concluded that available evidence was insufficient to recommend eradication of *H. pylori* for the purpose of preventing gastric cancer (99). The NIH conclusion was based upon the existence of clear examples of disparity in the epidemiology of the two diseases. Gastric cancer is more common in males than in females, whereas the rates of *H. pylori* infection are not different for the two genders. Some populations are reported to have a high rate of *H. pylori* infection but low rates of gastric cancer. Gastric cancer occurs in some persons with no evidence of *H. pylori* infection, and in the United States, fewer than 1% of *H. pylori*–infected persons will ever develop gastric cancer. The strongest evidence that *H. pylori* is associated with gastric cancer comes from three prospective studies that indicate that *H. pylori*–infected persons have a significantly increased rate of gastric cancer (96,98).

Only some retrospective serologic studies have shown an association. These disparities indicate that factors other than *H. pylori* infection are also important in gastric cancer risk. It is possible that only some strains of *H. pylori* are involved in the carcinogenic process. For example, infection with *H. pylori* strains possessing the cagA virulence factor is associated with an increased risk of developing adenocarcinoma of the stomach (100,101).

H. pylori is also associated with two less common forms of cancer, non-Hodgkin lymphoma and mucosa-associated lymphoid tissue lymphomas of the stomach (96). These types of lymphomas in the stomach only arise in the setting of *H. pylori* inflammation. In 70% of *H. pylori*–infected patients with lymphoma, treatment with appropriate antibiotics leads to regression (96). This finding not only suggests a causal role but that treatment of a bacterial infection can actually result in regression of cancer.

Another landmark study, published in June, 1997, shows that a 12-year nationwide vaccination program against hepatitis B virus in Taiwan resulted in a significant reduction in the number of cases of childhood liver cancer (102). The role of chronic infection with hepatitis B virus in the etiology of hepatocellular carcinoma is well established (103,104). Yet this is the first evidence that prevention of a viral infection is also effective against cancer. The implications are profound. Hepatitis B infection causes some 316,000 cases of liver cancer (60% of all liver cancer) a year worldwide (103,104). While hepatitis C causes a further 118,000 cases (22% of all cases) a year (103,104), some cases result from infections with both viruses (104).

The infectious origin of carcinoma of the cervix has long been suspected, because known risk factors for the disease are linked to sexual activity (105). Recent evidence indicates that human papillomavirus (HPV) types 16 and 18 are definitely carcinogenic in humans (94,105). Types 31 and 33 are classified as probably carcinogenic (94,105). In the United States, HPVs, are associated with 82% of the 15,000 cases and 4,600 deaths due to cervical cancer each year. They are also associated with more than a million precancerous lesions of varying severity. The combined direct medical costs due to HPV are approximately 1.3 billion dollars per year in the United States alone. Thus, effective therapy and vaccines would have a major impact.

The pathogenic mechanisms by which infectious agents cause cancer have not been resolved but they appear to be diverse. In cervical cancer, there seems to be a clear role for HPV-encoded genes in tumor cell growth (106). In addition to stimulation of cell proliferation, inactivation of tumor suppressor genes, such as *p53* may be a common pathway leading to malignancy in HPV and hepatitis B virus (106,107). In the case of other viruses and *H. pylori*, active oxygen and nitrogenic species generated by inflammatory cells may cause DNA damage, induce apoptosis, and modulate enzyme activities and gene expression (94,108).

Future Research Opportunities

The basic biology of agents implicated in chronic diseases and cancer, in contrast to many

other infectious agents, is relatively unknown. With rare exception, the means by which pathogens suppress, subvert, or evade host defenses and establish chronic or latent infection have received little attention. Few areas of basic research compared with microbial latency hold greater promise of substantially contributing to our understanding of infectious diseases and the eventual relief of human suffering (109). Given that the diseases discussed are among the most common in the world, even if only some cases are proven to be of infectious origin and effective therapies or vaccines can be developed, the impact on reducing health-care costs would be substantial. Thus, further research to clarify the etiologic agents and pathogenic mechanisms involved in chronic diseases and cancer should be given the highest priority.

To address the potential role of infectious agents in chronic diseases requires a new research paradigm compared to that which most investigators and funding agencies in infectious diseases are accustomed. Such an approach will require high levels of sustained funding of networks of research groups (ideally at least for 10 years). The approach will require collaborative research groups that follow a large number of well-defined patients over long periods. Success will depend on involvement of researchers highly skilled in clinical and epidemiologic investigation supported by laboratory personnel with proven expertise in detection of a wide spectrum of fastidious organisms. No single agent is likely to be the cause of chronic obstructive lung disease, asthma, or cancer; rather a number of infectious agents are likely to have this potential, hence the need for studies of large numbers of patients. Because the infecting agent may only be present in the very early stages of disease followed by an inflammatory response, different stages of disease need to be studied. A critical component of the investigative approach will be the ability to determine the genetic background and immune response of the patients.

The randomized, controlled clinical trial provides a scientific experiment that conforms to the standard model of biomedical research and is undoubtedly the best theoretical approach to evaluating any new therapy (110,111). Antibiotic treatment trials are commonly used to prove an infectious etiology. While clinical trials are at their best in evaluating the efficacy of therapies for acute diseases, clinical trials may not be the best approach for evaluating the efficacy of therapies for chronic diseases, most of which are likely to be complex, multifactorial illnesses in which behavioral and lifestyle factors play an important role. Some of the difficulties associated with this approach have already been discussed.

Well-defined, relevant animal models will be extremely important in elucidating the role of infectious agents in chronic inflammatory diseases. The animal studied should be the most genetically susceptible to the infecting agent and chronic infection. All too often inappropriate conclusions are based on use of a single strain of a single species. The value of using a naturally occurring disease with features that closely parallel those of the human disease cannot be overestimated.

As we attempt to prove the role of infection in chronic inflammatory diseases and cancer, the biggest challenge will be convincing peer review groups who establish research priorities and who facilitate funding decisions that these are not "fishing expeditions." Likewise, the challenge will be to convince journal editors that the findings are not merely coincidental. To make rapid progress we must keep an open mind and accept the likely possibility that fulfillment of Koch's postulates for infectious agents involved in chronic inflammatory diseases and cancer may not be possible.

Dr. Cassell is a recent past president of the American Society for Microbiology, a member of the National Institutes of Health Director's Advisory Committee, and a member of the Advisory Council of the National Institute of Allergy and Infectious Diseases of NIH. She was named to the original Board of Scientific Councilors of the National Center for Infectious Diseases, CDC, and is the immediate past chair of the board.

References

1. Moscicki AB, Palefsky J, Gonzales J, Schoolnik GK. Human papillomavirus infection in sexually active adolescent females: prevalence and risk factors. Pediatr Res 1990;28:507-13.
2. Rous P. Transmission of a malignant new growth by means of a cell-free filtrate. JAMA 1911;56:198.
3. Cassell GH, Cole BC. Mycoplasmas as agents of human disease. N Engl J Med 1981;304:80-9.
4. Lindsey JR, Baker HJ, Overcash RG, Cassell GH, Hunt CE. Murine chronic respiratory disease: significance as a research complication and experimental production with *Mycoplasma pulmonis*. Am J Pathol 1971;64:675-708.
5. Hektoen L. Observations on pulmonary infections in rats. Transactions of the Chicago Pathology Society 1015-1918;10:105-8.
6. Nelson JB. Infectious catarrh of the albino rat. I. Experimental transmission in relation to the role of *Actinobacillus muris*. II. The causal relation of cocobacilliform bodies. J Exp Med 1940;72:645-54, 666-667.

7. Cassell GH, Lindsey JR, Baker HJ. Mycoplasmal and rickettsial diseases. In: Baker HJ, Lindsey JR, Weisbroth SH, editors. The laboratory rat, Vol. I. New York: Academic Press; 1979. p. 243-69.
8. Cassell GH, Lindsey JR, Overcash RG, Baker HJ. Murine mycoplasma respiratory disease. Ann N Y Acad Sci 1973;225:395-412.
9. Davis JK, Parker RF, White H, Dziedzic D, Taylor G, Davidson MK, et al. Strain differences in susceptibility to murine respiratory mycoplasmosis in C57BL/6 and C3H/HeN mice. Infect Immun 1985;50:647-54.
10. Davis JK, Thorp RB, Maddox PA, Brown MB, Cassell GH. Murine respiratory mycoplasmosis in F344 and LEW rats: evolution of lesions and lung lymphoid cell populations. Infect Immun 1982;36:720-9.
11. Cartner SC, Simecka JW, Briles DE, Cassell GH, Lindsey JR. Resistance to mycoplasmal lung disease in mice is a complex genetic trait. Infect Immun 1996;64:5326-31.
12. Simecka JW, Davis JK, Davidson MK, Ross SE, Städtlaender CTK-H, Cassell GH. Mycoplasma diseases of animals. In: Maniloff J, McElhaney R, Finch L, Baseman J, editors. Mycoplasmas: molecular biology and pathogenesis. Washington: American Society of Microbiology; 1992. p. 391-415.
13. Ross SF, Simecka JW, Gambill GP, Davis JK, Cassell GH. *Mycoplasma pulmonis* possesses a novel chemattractant for B lymphocytes. Infect Immun 1992;60:669,674.
14. O'Brodovich HM, Mellins RB. Bronchopulmonary dysplasia. Unresolved neonatal acute lung injury. Am Rev Respr Dis 1985;132:694-709.
15. Saigal S, Rosenbaum P, Stoskopf B, Sinclair JC. Outcome in infants 501-1000 gm birth weight delivered to residents of the McMaster Health Region. J Pediatr 1984;105:969-76.
16. Naeve RL, Dellinger WS, Blanc WA. Fetal and maternal features of antenatal bacterial infections. J Pediatr 1971;79:733-9.
17. Dennehy PH. Respiratory infections in the newborn. Clin Perinatol 1987;14:667-82.
18. Tafari N, Ross S, Naeye RL. Mycoplasma 'T' strains and perinatal death. Lancet 1976;1:108-9.
19. Cassell GH, Waites KB, Crouse DT, Rudd PT, Canupp KC, Stagno S, et al. Association of *Ureaplasma urealyticum* infection of the lower respiratory tract with chronic lung disease and death in very-low-birthweight infants. Lancet 1988;2:240-5.
20. Quinn PA, Gillan JE, Markestad T, St. John MA, Daneman A, Lie KI, et al. Intrauterine infection with *Ureaplasma urealyticum* as a cause of fatal neonatal pneumonia. Pediatr Infect Dis 1985;4:538-43.
21. Gray DJ, Robinson HB, Malone J. Adverse outcome in pregnancy following amniotic fluid isolation of *Ureaplasma urealyticum.* Prenat Diagn 1992;12:111-7.
22. Waites KB, Crouse DT, Philips JB III, Canupp KC, Cassell GH. Ureaplasmal pneumonia and sepsis associated with persistent pulmonary hypertension of the newborn. Pediatrics 1989;83:79-85.
23. Brus F, van Waarde WM, Schoots C, Oetomo SB. Fetal ureaplasmal pneumonia and sepsis in a newborn infant. Eur J Pediatr 1991;150:782-3.
24. Cassell GH, Waites KB, Gibbs RS, Davis JK. The role of *Ureaplasma urealyticum* in amnionitis. Pediatr Infect Dis 1986;5:S247-52.
25. Rudd PT, Carrington D. A prospective study of chlamydial, mycoplasmal and viral infections in a neonatal intensive care unit. Arch Dis Child 1985;59:120-5.
26. Taylor-Robinson D, Furr PM, Liberman MM. The occurrence of genital mycoplasmas in babies with and without respiratory diseases. Acta Paediatrica Scandinavica 1984;73:383-1984.
27. Ollikainen J, Heikkaniemi H, Korppi M, Sarkkinen H, Heinonen K. *Ureaplasma urealyticum* infection associated with acute respiratory insufficiency and death in premature infants. J Pediatr 1993;122:756-60.
28. Rudd PT, Cassell GH, Waites KB, Davis JK, Duffy LB. *Ureaplasma urealyticum* pneumonia: experimental production and demonstration of age-related susceptibility. Infect Immun 1989;57:918-25.
29. Walsh WF, Butler J, Coalson J, Hensley D, Cassell GH, Delemos RA. A primate model of *Ureaplasma urealyticum* infection in the premature infant with hyaline membrane disease. Clin Infect Dis 1993;17:S158-62.
30. Crouse DT, Odrezin GT, Cutter GR, Reese JM, Hamrick WB, Waites KB, et al. Radiographic changes associated with tracheal isolation of *Ureaplasma urealyticum* from neonates. Clin Infect Dis 1993;17:S122-30.
31. Sidiropoulos D, Herrmann U, Morell A. Transplacental passage of intravenous immunoglobulin in the last trimester of pregnancy. J Pediatr 1986;109:505-8.
32. Cassell GH. The pathogenic potential of mycoplasmas: *Mycoplasma pulmonis* as a model. Derrick Edward Award Lecture. Rev Infect Dis 1982;4:S18-34.
33. Cassell GH, Waites KB, Crouse DT. Mycoplasmal infections. In: Remington JS, Klein JO, editors. Infectious diseases of the fetus and newborn infant. Philadelphia (PA): W.B. Saunders Co.; 1994. p. 619-55.
34. Dyke MP, Grauaug A, Kohan R, Ott K, Andrews R. *Ureaplasma urealyticum* in a neonatal intensive care population. J Paediatr Child Health 1993;29:295-7.
35. Saxen H, Hakkarainen K, Pohjavuori M. Chronic lung disease of preterm infants in Finland is not associated with *Ureaplasma urealyticum* colonization. Acta Paediatr 1993;82:198-201.
36. Valencia GB, Banzon F, Cummings M. *Mycoplasma hominis* and *Ureaplasma urealyticum* in neonates with suspected infection. Pediatr Infect Dis J 1993;12:571-3.
37. Crouse DT, Cassell GH, Waites KB, Foster JM, Cassady G. Hyperoxia potentiates *Ureaplasma urealyticum* pneumonia in newborn mice. Infect Immun 1990;58:3487-93.
38. Stancombe BB, Walsh WF, Derdak S, Dixon P, Hensley D. Induction of human neonatal pulmonary fibroblast cytokines by hyperoxia and *Ureaplasma urealyticum.* Clin Infect Dis 1993;17:S154-7.
39. Payne NR, Steinberg S, Ackerman P, Cheenka BA, Sane SM, Anderson KT, et al. New prospective studies of the association of *Ureaplasma urealyticum* colonization and chronic lung disease. Clin Infect Dis 1993;17:S117-21.
40. Cassell GH, Davis RO, Waites KB, Brown MB, Marriott PA, Stagno S, et al. Isolation of *Mycoplasma hominis* and *Ureaplasma urealyticum* from amniotic fluid at 16-20 weeks gestation: potential effect on outcome of pregnancy. Sex Transm Dis 1983;10:294-302.

41. Parker RF, Davis JK, Cassell GH, White H, Dziedzic D, Blalock DK, et al. Short-term exposure to nitrogen dioxide enhances susceptibility to murine respiratory mycoplasmosis and decreases intrapulmonary killing of *Mycoplasma pulmonis*. Am Rev Respir Dis 1989;140:502-12.
42. National Heart, Lung and Blood Institute Data Fact Sheet, Asthma Statistics, May, 1992. Washington: National Institutes of Health; 1992.
43. Rao M, Kravath R, Abadco D, Arden J, Steiner P. Childhood asthma mortality: the Brooklyn experience and a brief review. J Assoc Acad Minor Phys 1991;2:127-30.
44. Sterk PJ. Virus-induced airway hyperresponsiveness in man. Eur Respir J 1993;6:894-902.
45. Cassell GH, Clyde WA, Davis JK. *Mycoplasmal* respiratory infections. In: Razin S, Tully JG, editors. The *Mycoplasmas*. New York: Academic Press; 1985. p. 66-106.
46. Shimuzu T, Mochizuki H, Kato M, Shjigeta M, Morikawa A, Hori T. Immunoglobulin levels, number of eosinophils in the peripheral blood and bronchial hypersensitivity in children with *Mycoplasma pneumoniae* pneumonia. Japanese Journal of Allergology 1991;40:21-7.
47. Sabato AR, Martin AJ, Marmion BP, Kok TW, Cooper DM. *Mycoplasma pneumoniae*: acute illness, antibiotics, and subsequent pulmonary function. Arch Dis Child 1984;59:1034-7.
48. Seggev JS, Lis I, Siman-Tov S, Gutman R, Abu-Samara H, Bouchey H, et al. *Mycoplasma pneumoniae* is a frequent cause of exacerbation of bronchial asthma in adults. Annals of Allergy 1986;57:262-5.
49. Yano T, Ichikawa Y, Komatu S, Arai S, Oizumi K. Association of *Mycoplasma pneumoniae* antigen with initial onset of bronchial asthma. Am J Respir Crit Care Med 1994;149:1348-53.
50. Henderson FW, Clyde Jr WA, Collier AM, Denny FW, Senior RJ, Sheaffer CI, et al. The etiologic and epidemiologic spectrum of bronchiolitis in pediatric practice. J Pediatr 1979;95:183-90.
51. Grayston JT. Infections caused by *Chlamydia pneumoniae* strain TWAR. Clin Infect Dis 1992;15:757-63.
52. Hahn DL, Dodge RW, Golubjatnikov R. Association of *Chlamydia pneumoniae* (TWAR) infection with wheezing, asthmatic bronchitis and adult-onset asthma. JAMA 1991;266:225-30.
53. Emre U, Roblin PM, Gelling M, Dumornay W, Rao M, Hammerschlag MR, et al. The association of *Chlamydia pneumoniae* infection and reactive airway disease in children. Archives of Pediatric Medicine 1994;148:727-32.
54. Hammerschlag MR, Chirgwin K, Roblin PM. Persistent infection with *Chlamydia pneumoniae* following acute respiratory illness. Clin Infect Dis 1992;14:178-222.
55. Kraft M, Cassell GH, Henson JE, Watson H, Williamson J, Marmion BP, et al. Detection of *Mycoplasma pneumoniae* in the airways of adults with chronic asthma. Am J Resp Crit Care Med. In press 1998.
56. Allegra L, Blasi F, Centanni S, Cosentini R, Denti F, Raccanelli R, et al. Acute exacerbations of asthma in adults: role of *Chlamydia pneumoniae* infection. Eur Respir J 1994;7:2165-8.
57. Hahn DL, Golubjatnikov R. Asthma and Chlamydial infection: a case series. J Fam Pract 1994;38:589-95.
58. Block S, Hedrick J, Hammerschlag AR, Craft JC. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in pediatric community-acquired pneumonia: comparative safety and efficacy of clarithromycin vs. erythromycin. Pediatr Infect Dis 1995;14:471-7.
59. Harris JAS, Kolokathis A, Campbell M, Cassell GH, Hammerschlag MR. Safety and efficacy of azithromycin treatment of community acquired pneumonia in children. Pediatr Infect Dis J. In press 1998.
60. Giron JA, Lange M, Baseman JB. Adherence, fibronectin, binding, and induction of cytoskeleton reorganization in cultured human cells by *Mycoplasma penetrans*. Infect Immun 1996;64:197-208.
61. Wise KS, Cassell GH, Acton RT. Selective association of murine T lymphoblastoid cell surface alloantigens with *Mycoplasma hyorhinis*. Proc Natl Acad Sci U S A 1978;75:4479-83.
62. Marmion BP, Williamson J, Worswick PA, Kok TW, Harris RJ. Experience with newer techniques for the laboratory detection of *Mycoplasma pneumoniae* infection: Adelaide, 1978-1991. Clin Infect Dis 1993;17:S90-9.
63. Cassell GH, Drnec J, Waites KB, Pate MS, Duffy LB, Watson HL, et al. Efficacy of clarithromycin against *Mycoplasma pneumoniae*. J Antimicrob Chemother 1991;27:47-59.
64. Gray GC, Duffy LB, Paver RJ, Putnam SD, Reynolds RJ, Cassell GH. *Mycoplasma pneumoniae*: a frequent cause of pneumonia among U.S. marines in southern California. Mil Med 1997;162:524-6.
65. Osler W. Diseases of the arteries. In: Osler W, editor. Modern medicine: its practice and theory. Philadelphia (PA): Lea & Febiger; 1908. p. 429-47.
66. Fabricant CG, Fabricant J, Litrenta MM, Minick CR. Virus-induced atherosclerosis. J Exp Med 1978:335-40.
67. Danesh J, Collins R, Peto R. Chronic infections and coronary heart disease: is there a link? Lancet 1997;350:430-6.
68. Patel P, Mendall MA, Carrington D, Strachan DP, Leatham E, Molineaux N, et al. Association of *Helicobacter pylori* and *Chlamydia pneumoniae* infections with coronary heart disease and cardiovascular risk factors. BMJ 1995;311:711-4.
69. Melnick JL, Adam E, Debakey ME. Cytomegalovirus and atherosclerosis. Eur Heart J 1993;14:30-8.
70. Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. J Periodontol 1996;67:1123-37.
71. File TM, Bartlett JG, Cassell GH, Gaydos CA, Grayston JT, Hammerschlag MR, et al. The importance of *Chlamydia pneumoniae* as a pathogen: the 1996 consensus conference on *Chlamydia pneumoniae* infections. Infec Dis Clin Prac1997;6:S28-31.
72. Kuo C, Shor A, Campbell L, Fukushi H, Patton DL, Grayston JT. Demonstration of *Chlamydia pneumoniae* in atherosclerotic lesions of coronary arteries. J Infect Dis 1993;167:841-9.
73. Kuo CC, Grayston JT, Campbell LA, Goo YA, Wissler RW, Benditt EP. *Chlamydia pneumoniae* (TWAR) in coronary arteries of young adults (15-34 years old). Proc Natl Acad Sci U S A 1995;92:6911-4.
74. Campbell LA, O'Brien ER, Capuccio AL, Kuo C-C, Wang S-P, Stewart D. Detection of *Chlamydia pneumoniae* TWAR in human coronary arterectomy tissues. J Infect Dis 1995;172:585-8.
75. Ong G, Thomas BJ, Mansfield AO, Davidson BR, Taylor-Robinson D. Detection and widespread distribution of *Chlamydia pneumoniae* in the vascular

system and its possible implications. J Clin Pathol 1996;49:102-6.
76. Jackson LA, Lee AC, Cho-Chou Kuo, Rodriquez DI, Lee A, Grayston JT. Isolation of *Chlamydia pneumoniae* from a carotid endarterectomy specimen. J Infect Dis 1997;176:292-5.
77. Blasi F, Denti F, Erba M. Detection of *Chlamydia pneumoniae* but not *Helicobacter pylori* in atherosclerotic plaques of aortic aneurysms. J Clin Microbiol 1996;34:2766-9.
78. Muhlestein JB, Hammond EH, Carlquist JF, Radicke E, Thomson MJ, Karagounis LA, et al. Increased incidence of *Chlamydia* species within the coronary arteries of patients with symptomatic atherosclerotic versus other forms of cardiovascular disease. J Am Coll Cardiol 1996;27:1555-61.
79. Kuo CC, Jackson LA, Campbell LA, Grayston JT. *Chlamydia pneumoniae* (TWAR). Clin Microbiol Rev 1995;8:451-61.
80. Yang ZP, Kuo CC, Grayston JT. Systemic dissemination of *Chlamydia pneumoniae* following intranasal inoculation in mice. J Infect Dis 1995;171:736-8.
81. Fong IW, Chiu B, Viira E, Fong MW, Jang D, Mahony J. Rabbit model for *Chlamydia pneumoniae* infection. J Clin Microbiol 1997;35:48-52.
82. Laitinen K, Laurila A, Pyhala L, Leinonen M, Saikku P. *Chlamydia pneumoniae* infection induces inflammatory changes in the aortas of rabbits. Infect Immun 1997;65:4832-5.
83. Kaukoranta-Tolvanen SS, Teppo AM, Laitinen K, Linnavuori K, Leinonen M. Growth of *Chlamydia pneumoniae* in cultured human peripheral blood mononuclear cells and induction of a cytokine response. Microb Pathog 1996;21:215-21.
84. Molestina R, Miller RD, Summersgill JT, Ramirez. *Chlamydia pneumoniae* stimulates secretion of chemokines and adhesion molecules in human endothelial cells. In: Abstracts of the 96th General Meeting of the American Society for Microbiology 1996. Washington: American Society for Microbiology; 1996. Abstract No. 243.
85. Nurmenen M, Leinonen M, Saikku P, Makela PH. The genus-specific antigen of *Chlamydia*: resemblance to the lipopolysaccharide of enteric bacteria. Science 1988;220:1279-81.
86. Saikku P, Leinonen M, Tenkanen L, Linnanmaki E, Ekman MR, Manninen V, et al. Chronic *Chlamydia pneumoniae* infection as a risk factor for coronary heart disease in the Helsinki heart study. Ann Intern Med 1992;116:273-8.
87. Gupta S, Leatham EW, Carrington D, Mendall MA, Kaski JC, Camm J. Elevated *Chlamydia pneumoniae* antibodies, cardiovascular events, and azithromycin in male survivors of myocardial infarction. Circulation 1997;96:404-7.
88. Gurfinkel E, Bozovich G, Daroca A, Beck E, Mautner B, for the ROXIS Study Group. Randomised trial of roxithromycin in non-Q-wave coronary syndromes: ROXIS pilot study. Lancet 1997;350:404-7.
89. Liddy P, Egan D, Skarlartos S. Roles of infectious agents in atherosclerosis and restenosis. Circulation 1997;96:4095-103.
90. Kadota JL. Non antibiotic effects of antibiotics. J Clin Micro Infect 1996;1:220-2.
91. Labro MT. Intracellular bioactivity of macrolides [suppl]. J Clin Micro Infect 1996:1:24-30.
92. Agen C, Danesi R, Blandizzi C. Macrolide antibiotics as antiinflammatory agents:roxithromycin in an unexpected role. Agents Actions 1993;38:85-90.
93. Kita E, Sawaki M, Mikasa K. Alterations of host response by long term treatment of roxithromycin. J Antimicrob Chemother 1993;32:285-94.
94. Pisani P, Parkin DM, Munoz, Ferlay J. Cancer and infectiou: Estimates of the attributable fraction in 1990. Can Epidemiol Biomarkers Prevent 1997;6:387-400.
95. Infectious diseases and cancer. 1996. In: The World Health Report 1996. Fighting disease fostering development. Geneva: World Health Organization; 1996. p. 59-62.
96. Parsonnet J. *Helicobacter pylori*. Infect Dis Clinics North Amer 1998;12:185-97.
97. Marshall BJ. History of the discovery of *C. pylori*. In Blaser MJ, editor. *Campylobacter pylori* in gastritis and peptic ulcer disease. New York: Igaku-Shoin; 1989. p. 7-23.
98. Moller H, Heseltine E, Vaqinio. Working group report on schistosomes, liver flukes, and *Helicobacter pylori*. Int J Cancer 1995;60:587-9.
99. NIH Consensus Conference. *Helicobacter pylori* in peptic ulcer disease. JAMA 1994;272:65-9.
100. Xiang Z, Censini S, Bayeli PF, Telford JL, Figura N, Rappuoli R, et al. Analysis of expression of CagA and VacA virulence factors in 43 strains of *Helicobacter pylori* reveals that clinical isolates can be divided into two major types and that CagA is not necessary for expression of the vacuolating cytotoxin. Infect Immun 1995;63:94-8.
101. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH. Infection with *Helicobacter pylori* strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 1995;55:2111-5.
102. Chen CJ, Lai MS, Hsu HM, Wu TC, Kong MS, Liang DC, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. N Engl J Med 1997;336:1855-9.
103. Hepatitis viruses. Monographs on the evaluation of carcinogenic risks to humans. Lyon, France: IARC; 1994. IARC Scientific Publ No. 59.
104. Chuang WL, Chang WY, Lu SN, Su WP, Lin ZY, Chen SC, et al. The role of hepatitis B and C viruses in hepatocellular carcinoma in a hepatitis B endemic area. A case-control study. Cancer 1992;69:2052-4.
105. Human papillomaviruses. Monographs on the evaluation of carcinogenic risks to humans. Lyon, France: IARC; 1995. IARC Scientific Publ No. 64.
106. Vousden KH, Farrel PJ. Viruses and human cancer. Br Med Bull 1994;3:580-1.
107. Morris JDH, Eddleston ALWF, Crook T. Viral infection and cancer. Lancet 1995;346:754-8.
108. Ohshima H, Bartsch H. Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. Mutat Res 1994;305:253-64.
109. Mackowiak PA. Microbial latency. Rev Infect Dis 1984;6:649-67.
110. Pincus T. Rheumatoid arthritis: disappointing long-term outcomes despite successful short-term clinical trials. J Clin Epidemiol 1988;41:1037.
111. Feinstein AR. An additional basic science for clinical medicine: II. The limitations of randomized trials. Ann Intern Med 1983;544.

Bioterrorism as a Public Health Threat

D.A. Henderson
The Johns Hopkins University, Baltimore, Maryland, USA

The threat of bioterrorism, long ignored and denied, has heightened over the past few years. Recent events in Iraq, Japan, and Russia cast an ominous shadow. Two candidate agents are of special concern—smallpox and anthrax. The magnitude of the problems and the gravity of the scenarios associated with release of these organisms have been vividly portrayed by two epidemics of smallpox in Europe during the 1970s and by an accidental release of aerosolized anthrax from a Russian bioweapons facility in 1979. Efforts in the United States to deal with possible incidents involving bioweapons in the civilian sector have only recently begun and have made only limited progress. Only with substantial additional resources at the federal, state, and local levels can a credible and meaningful response be mounted. For longer-term solutions, the medical community must educate both the public and policy makers about bioterrorism and build a global consensus condemning its use.

Until recently, biological terrorism had been little discussed or written about. Until recently, I had doubts about publicizing the subject because of concern that it might entice some to undertake dangerous, perhaps catastrophic experiments. However, events of the past 12 to 18 months have made it clear that likely perpetrators already envisage every possible scenario.

Four points of view prevalent among national policy circles and the academic community at various times have served to dismiss biological terrorism as nothing more than a theoretical possibility. l) Biological weapons have so seldom been deployed that precedent would suggest they will not be used. 2) Their use is so morally repugnant that no one would deign to use them. 3) The science of producing enough organisms and dispersing them is so difficult that it is within the reach of only the most sophisticated laboratories. 4) Like the concept of a "nuclear winter," the potential destructiveness of bioweapons is essentially unthinkable and so to be dismissed. Each of these arguments is without validity.

Nations and dissident groups exist that have both the motivation and access to skills to selectively cultivate some of the most dangerous pathogens and to deploy them as agents in acts of terrorism or war. After the Gulf War, Iraq was discovered to have a large biological weapons program. In 1995, Iraq confirmed that it had produced, filled, and deployed bombs, rockets, and aircraft spray tanks containing *Bacillus anthracis* and botulinum toxin (1,2); its work force and technologic infrastructure are still wholly intact. Also in 1995, the Japanese cult, Aum Shinrikyo, released the nerve gas Sarin in the Tokyo subway. The cult also had plans for biological terrorism (3); included in its arsenal were large quantities of nutrient media, botulinum toxin, anthrax cultures, and drone aircraft equipped with spray tanks. Members of this group had traveled to Zaire in 1992 to obtain samples of Ebola virus for weapons development.

Of more recent concern is the status of one of Russia's largest and most sophisticated former bioweapons facilities, called Vector, in Koltsovo, Novosibirsk. Through the early 1990s, this was a 4,000-person, 30-building facility with ample biosafety level 4 laboratory facilities, used for the isolation of both specimens and human cases. Situated on an open plain surrounded by electric fences and protected by an elite guard, the facility housed the smallpox virus as well as work on Ebola, Marburg, and the hemorrhagic fever viruses (e.g., Machupo and Crimean-Congo). A visit in the autumn of 1997 found a half-empty facility protected by a handful of guards who had not been paid for months (P. Jahrling, pers. comm., 1998). No one can say where the scientists have gone, nor is there confidence now that this is

Address for correspondence: D.A. Henderson, The Johns Hopkins University, The Johns Hopkins School of Medicine, 615 North Wolfe Street, Baltimore, MD 21205, USA; fax: 410-955-6898; e-mail: dahzero@aol.com.

the only storage site for smallpox virus outside the Centers for Disease Control and Prevention.

The number of countries engaged in biological weapons experimentation has grown from 4 in the 1960s to 11 in the 1990s (4). Meanwhile, the bombing of the World Trade Center and the Oklahoma City Federal Building have dramatized the serious problems even small dissident groups can cause.

A comprehensive review of the problems posed by biological terrorism and warfare has been published (5). Four observations deserve special note. First, biological terrorism is more likely than ever before and far more threatening than either explosives or chemicals. Second, official actions directed at the threat to the civilian population (less than 2 years in the making) have been only marginally funded and minimally supported (6). Third, preventing or countering bioterrorism will be extremely difficult. Recipes for making biological weapons are now available on the Internet, and even groups with modest finances and basic training in biology and engineering could develop, should they wish, an effective weapon (7) at little cost. Fourth, detection or interdiction of those intending to use biological weapons is next to impossible. Thus, the first evidence of such weapons will almost certainly be cases in hospital emergency rooms. Specialists in infectious diseases thus constitute the front line of defense. The rapidity with which they and emergency room personnel reach a proper diagnosis and the speed with which they apply preventive and therapeutic measures could spell the difference between thousands and perhaps tens of thousands of casualties. Indeed, the survival of physicians and health-care staff caring for the patients may be at stake. However, today few have ever seen so much as a single case of smallpox, plague, or anthrax, or, for that matter, would recall the characteristics of such cases. Few, if any, diagnostic laboratories are prepared to confirm promptly such diagnoses.

Of a long list of potential pathogens, only a handful are reasonably easy to prepare and disperse and can inflict sufficiently severe disease to paralyze a city and perhaps a nation. In April 1994, Anatoliy Vorobyov, a Russian bioweapons expert, presented to a working group of the National Academy of Sciences the conclusions of Russian experts as to the agents most likely to be used (8). Smallpox headed the list followed closely by anthrax and plague. None of these agents has so far effectively been deployed as a biological weapon, and thus no real world events exist to provide likely scenarios. However, we have had several well-documented smallpox importations into Europe over recent decades; two bear recounting.

Smallpox is caused by a virus spread from person to person; infected persons have a characteristic fever and rash. Virus infection invariably results in symptomatic disease. There are no mild, subclinical infections among unvaccinated persons. After an incubation period of 10 to 12 days, the patient has high fever and pain. Then a rash begins with small papules developing into pustules on day 7 to 8 and finally changing to scabs around day 12. Between 25% and 30% of all unvaccinated patients die of the disease. There was, and is, no specific treatment.

Until 1980, essentially all countries conducted vaccination programs of some sort, whether or not they had endemic disease (9). Until 1972, the United States mandated smallpox vaccination for all children at school entry, although the last cases had occurred in 1949, 23 years before. In the United Kingdom, four standby hospitals were to be opened only if smallpox cases were imported, and in Germany, two state-of-the-art isolation hospitals were constructed in the 1960s specifically for the isolation of smallpox cases should they occur.

In 1962, the initial response of U.S. officials to the occurrence of a single case of smallpox illustrated extreme concern. That year, a young Canadian boy returned from Brazil, traveling by air to New York and by train to Toronto by way of Albany and Buffalo (10). Shortly after arrival in Toronto, he developed a rash and was hospitalized. In response to this single case, senior U.S. government officials seriously considered a plan of action that called for the border with Canada to be closed, for mass vaccination campaigns to be conducted in all cities along the route from New York through Albany, Syracuse, Rochester, and Buffalo, and for vaccination of all who had been in Grand Central Station on the day the Canadian boy was there. Sensibly, this plan was soon scrapped for more modest measures, albeit not without considerable debate.

The potential of aerosolized smallpox to spread over a considerable distance and to infect at low doses was vividly demonstrated in an outbreak in Germany in 1970 (11). That year,

a German electrician returning from Pakistan became ill with high fever and diarrhea. On January 11, he was admitted to a local hospital and was isolated in a separate room on the ground floor because it was feared he might have typhoid fever. He had contact with only two nurses over the next 3 days. On January 14 a rash developed, and on January 16 the diagnosis of smallpox was confirmed. He was immediately transported to one of Germany's special isolation hospitals, and more than 100,000 persons were promptly vaccinated. The hospital had been closed to visitors because of an influenza outbreak for several days before the patient was admitted. After the diagnosis of smallpox, other hospital patients and staff were quarantined for 4 weeks and were vaccinated; very ill patients received vaccinia-immune globulin first. However, the smallpox patient had had a cough, a symptom seldom seen with smallpox; coughing can produce a large-volume, small-particle aerosol like what might occur after its use as a terrorist weapon. Subsequently, 19 cases occurred in the hospital, including four in other rooms on the patient's floor, eight on the floor above, and nine on the third floor. Two were contact cases. One of the cases was in a visitor who had spent fewer than 15 minutes in the hospital and had only briefly opened a corridor door, easily 30 feet from the patient's room, to ask directions. Three of the patients were nurses, one of whom died. This outbreak occurred in a well-vaccinated population.

An outbreak in Yugoslavia in February 1972 also illustrates the havoc created even by a small number of cases. Yugoslavia's last case of smallpox had occurred in 1927. Nevertheless, Yugoslavia, like most countries, had continued populationwide vaccination to protect against imported cases. In 1972, a pilgrim returning from Mecca became ill with an undiagnosed febrile disease. Friends and relatives visited from a number of different areas; 2 weeks later, 11 of them became ill with high fever and rash. The patients were not aware of each other's illness, and their physicians (few of whom had ever seen a case of smallpox) failed to make a correct diagnosis.

One of the 11 patients was a 30-year-old teacher who quickly became critically ill with the hemorrhagic form, a form not readily diagnosed even by experts. The teacher was first given penicillin at a local clinic, but as he became increasingly ill, he was transferred to a dermatology ward in a city hospital, then to a similar ward in the capital city, and finally to a critical care unit because he was bleeding profusely and in shock. He died before a definitive diagnosis was made. He was buried 2 days before the first case of smallpox was recognized.

The first cases were correctly diagnosed 4 weeks after the first patient became ill, but by then, 150 persons were already infected; of these, 38 (including two physicians, two nurses, and four other hospital staff) were infected by the young teacher. The cases occurred in widely separated areas of the country. By the time of diagnosis, the 150 secondary cases had already begun to expose yet another generation, and, inevitably, questions arose as to how many other yet undetected cases there might be.

Health authorities launched a nationwide vaccination campaign. Mass vaccination clinics were held, and checkpoints along roads were established to examine vaccination certificates. Twenty million persons were vaccinated. Hotels and residential apartments were taken over, cordoned off by the military, and all known contacts of cases were forced into these centers under military guard. Some 10,000 persons spent 2 weeks or more in isolation. Meanwhile, neighboring countries closed their borders. Nine weeks after the first patient became ill, the outbreak stopped. In all, 175 patients contracted smallpox, and 35 died.

What might happen if smallpox were released today in a U.S. city? First, routine vaccination stopped in the United States in 1972. Some travelers, many military recruits, and a handful of laboratory workers were vaccinated over the following 8 years. Overall, however, it is doubtful that more than 10% to 15% of the population today has residual smallpox immunity. If some modest volume of virus were to be released (perhaps by exploding a light bulb containing virus in a Washington subway), the event would almost certainly go unnoticed until the first cases with rash began to appear 9 or 10 days later. With patients seen by different physicians (who almost certainly had never before seen a smallpox case) in different clinics, several days would probably elapse before the diagnosis of smallpox was confirmed and an alarm was sounded.

Even if only 100 persons were infected and required hospitalization, a group of patients many times larger would become ill with fever

and rash and receive an uncertain diagnosis. Some would be reported from other cities and other states. Where would all of these patients be admitted? In the Washington, D.C., metropolitan area, no more than 100 hospital beds provide adequate isolation. Who would care for the patients? Few hospital staff have any smallpox immunity. Moreover, one or two patients with severe hemorrhagic cases (which typically have very short incubation periods), who would have been hospitalized before smallpox was suspected, would have been cared for by a large, unprotected intensive care team.

What of contacts? In past outbreaks, contacts of confirmed or suspected cases numbered in the thousands, if not tens of thousands. What measures should or could be taken to deal with such numbers? Would patients be isolated as in Yugoslavia, and if so, where? Logistics could be simplified if rapid, easily used laboratory tests could confirm or rule out smallpox among suspected cases. At present, however, such tests are known only to scientists in two government laboratories.

An immediate clamor for mass vaccination (as in the outbreaks in Germany and Yugoslavia) can be predicted. U.S. stocks of smallpox vaccine are nominally listed at 15 million doses, but with packaging, the useful number of doses is perhaps half that number. How widely and quickly should this vaccine be used? Were vaccine to be limited strictly to close contacts of confirmed cases, comparatively few doses would be needed. However, the realities of dealing with even a small epidemic would almost certainly preclude such a cautious, measured vaccination effort. Vaccine reserves would rapidly disappear, and there is, at present, no manufacturing capacity to produce additional vaccine. If an emergency effort were made to produce new stocks of smallpox vaccine, many months to a year or more would be required.

What of anthrax, which has been so enthusiastically embraced by both Iraq and the Aum Shinrikyo? The organism is easy to produce in large quantity. In its dried form, it is extremely stable. The effect of aerosolized anthrax on humans once had to be inferred from animal experiments and the occasional human infection among workers in factories processing sheep and goat hides (12). It was clear that inhalation of anthrax is highly lethal. Just how lethal became evident in the 1979 Sverdlovsk epidemic (13).

In all, 77 cases were identified with certainty; 66 patients died. The actual total number of cases was probably considerably more than 100. The persons affected lived or worked somewhere within a narrow zone extending some 4 km south and east of a military bioweapons facility. An accidental airborne release of anthrax spores occurred during a single day and may well have lasted no more than minutes. Further investigations revealed anthrax deaths among sheep and cows in six different villages up to 50 km southeast of the military compound along the same axis as the human cases.

Of the 58 patients with known dates of disease onset, only 9 had symptoms within a week after exposure; some became ill as late as 6 weeks after exposure. Whether the onset of illness occurred sooner or later, death almost always followed within 1 to 4 days after onset. However, there appeared to be a somewhat higher proportion of survivors after the fourth week. This almost certainly resulted from the widespread application of penicillin prophylaxis and anthrax vaccine, both of which were distributed in mid-April throughout a population of 59,000.

Meselson and his colleagues, who documented this outbreak, calculate that the weight of spores released as an aerosol could have been as little as a few milligrams or as much as "nearly a gram." Iraq acknowledged producing at least 8,000 L of solution with an anthrax spore and cell count of 109/ml (1). The ramifications of even a modest-sized release of anthrax spores in a city are profound. Emergency rooms would begin seeing a few patients with high fever and some difficulty breathing perhaps 3 to 4 days after exposure. By the time the patients were seen, it is almost certain that it would be too late for antibiotic therapy. All patients would die within 24 to 48 hours. No emergency room physicians or infectious disease specialists have ever seen a case of inhalation anthrax; medical laboratories have had virtually no experience in its diagnosis. Thus, at least 3 to 5 days would elapse before a definitive diagnosis would be made.

Once anthrax was diagnosed, one would be faced with the prospect of what to do over the succeeding 6 to 8 weeks. Should vaccine be administered to those who might have been exposed? At present, little vaccine is available, and no plan exists to produce any for civilian use. Should antibiotics be administered prophylactically? If so, which antibiotics, and what should be the criteria for exposure? What quantity would be required to treat an exposed population of

perhaps 500,000 over a 6-week period? Should one be concerned about additional infections resulting from anthrax spores subsequently resuspended and inhaled by others? Should everyone who has been anywhere near the city report to a local physician for treatment at the first occurrence of fever or cough, however mild? Undoubtedly, many would have such symptoms, especially in the winter; how can such symptoms be distinguished from the premonitory symptoms of anthrax that may proceed to death within 24 to 48 hours?

We are ill-prepared to deal with a terrorist attack that employs biological weapons. In countering civilian terrorism, the focus (a modest extension of existing protocols to deal with a hazard materials incident) has been almost wholly on chemical and explosive weapons. A chemical release or a major explosion is far more manageable than the biological challenges posed by smallpox or anthrax. After an explosion or a chemical attack, the worst effects are quickly over, the dimensions of the catastrophe can be defined, the toll of injuries and deaths can be ascertained, and efforts can be directed to stabilization and recovery. Not so following the use of smallpox or anthrax. Day after relentless day, additional cases could be expected, and in new areas.

The specter of biological weapons use is an ugly one, every bit as grim and foreboding as that of a nuclear winter. As was done in response to the nuclear threat, the medical community should educate the public and policy makers about the threat. We need to build on the 1972 Biological and Toxin Weapons Convention to strengthen measures prohibiting the development and production of biological weapons and to ensure compliance with existing agreements. In a broader sense, we need a strong moral consensus condemning biological weapons.

But this is not enough. In the longer term, we need to be as prepared to detect, diagnose, characterize epidemiologically, and respond appropriately to biological weapons use as to the threat of new and reemerging infections. In fact, the needs are convergent. We need at international, state, and local levels a greater capacity for surveillance; a far better network of laboratories and better diagnostic instruments; and a more adequate cadre of trained epidemiologists, clinicians, and researchers.

On the immediate horizon, we cannot delay the development and implementation of strategic plans for coping with civilian bioterrorism. The needed stocking of vaccines and drugs as well as the training and mobilization of health workers, both public and private, at state, city, and local levels will require time. Knowing well what little has been done, I can only say that a mammoth task lies before us.

D.A. Henderson is a distinguished service professor at the Johns Hopkins University, with appointments in the Departments of Health and Epidemiology, School of Hygiene and Public Health. Dr. Henderson directed the World Health Organization's global smallpox eradication campaign (1966-1977) and helped initiate WHO's global program of immunization in 1974. He also served in the federal government as deputy assistant secretary and senior science advisor in the Department of Health and Human Services.

References

1. Ekeus R. Iraq's biological weapons programme: UNSCOM's experience. Memorandum report to the United Nations Security Council; 1996 20 Nov; New York.
2. Zalinskas RA. Iraq's biological weapons: the past as future? JAMA 1997;278:418-24.
3. Daplan E, Marchell A. The cult at the end of the world. New York: Crown Publishing Group; 1996.
4. Roberts B. New challenges and new policy priorities for the 1990s. In: Biologic weapons: weapons of the future. Washington: Center for Strategic and International Studies; 1993.
5. Bioweapons and bioterrorism. JAMA 1997;278:351-70, 389-436.
6. Tucker JB. National health and medical services response to incidents of chemical and biological terrorism. JAMA 1997;285:362-8.
7. Danzig R, Berkowsky PB. Why should we be concerned about biological warfare? JAMA 1997;285:431-2.
8. Vorobyov A. Criterion rating as a measure of probable use of bio agents as biological weapons. In: Papers presented to the Working Group on Biological Weapons Control of the Committee on International Security and Arms Control, National Academy of Sciences; 1994 Apr; Washington.
9. Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi I. Smallpox and its eradication. Geneva: World Health Organization; 1988.
10. Epidemiologic report. Smallpox, Canada. MMWR Morb Mortal Wkly Rep 1962;11:258.
11. Wehrle PF, Posch J, Richter KH, Henderson DA. An airborne outbreak of smallpox in a German hospital and its significance with respect to other recent outbreaks in Europe. Bull World Health Organ 1970;4:669-79.
12. Brachman PS, Friedlander AM. Anthrax. In: Plotkin SA, Mortimer EA, editors. Vaccines. Philadelphia: WB Saunders; 1994.
13. Meselson M, Guillemin V, Hugh-Jones M, Langmuir A, Popova I, Shelokov A, et al. The Sverdlovsk anthrax outbreak of 1979. Science 1994;266:1202-8.

Bioterrorism as a Public Health Threat

Joseph E. McDade* and David Franz‡
*Centers for Disease Control and Prevention, Atlanta, Georgia, USA;
‡U.S. Army Medical Research Institute of Infectious Diseases,
Ft. Detrick, Maryland, USA

In addition to meeting the continuing threat of new and reemerging infectious diseases, public health officials must also prepare for the possible use of infectious agents as weapons by terrorists to further personal or political agendas. These were the conclusions of session panelists Scott Lillibridge, Centers for Disease Control and Prevention (CDC); Michael Skeels, Oregon State Public Health Laboratory; Marcelle Layton, New York City Department of Public Health; David Franz, U.S. Army Medical Research Institute of Infectious Diseases; and Randall Murch, Federal Bureau of Investigation (FBI).

The potential spectrum of bioterrorism ranges from hoaxes and use of non–mass casualty devices and agents by individuals and small groups to state-sponsored terrorism that employs classic biological warfare agents and can produce mass casualties. The agents of anthrax, plague, brucellosis, smallpox, viral encephalidites, and viral hemorrhagic fevers are of particular concern: they are relatively easy and inexpensive to produce, cause death or disabling disease, and can be aerosolized and distributed over large geographic areas. If released under ideal environmental circumstances, these agents can infect hundreds of thousands of persons and cause many deaths. Such scenarios would present serious challenges for patient management and for prophylaxis of exposed persons; environmental contamination could provide a continuing threat to the population (especially those exposed at the beginning of the crisis) and generate panic in the community.

Bioterrorist attacks could be covert or announced and could be caused by virtually any pathogenic microorganism. The case of the Rajneeshee religious cult in The Dalles, Oregon, is an example (1). The cult planned to infect residents with *Salmonella* on election day to influence the results of county elections. To practice for the attack, they contaminated salad bars at 10 restaurants with *S*. Typhimurium on several occasions before the election. A communitywide outbreak of salmonellosis resulted; at least 751 cases were documented in a county that typically reports fewer than five cases per year. Although bioterrorism was considered a possibility when the outbreak was being investigated by public health officials, it was considered unlikely. The source of the outbreak became known only when FBI investigated the cult for other criminal violations. A vial of *S.* Typhimurium identical to the outbreak strain was found in a clinical laboratory on the cult's compound, and members of the cult subsequently admitted to contaminating the salad bars and putting *Salmonella* into a city water supply tank. This incident, among other recent events, underscores the importance of improving preparedness at all levels.

A bioterrorist attack may be difficult to distinguish from a naturally occurring infectious disease outbreak. Investigators must first examine the etiology and epidemiology of an outbreak to identify its source, mode of transmission, and persons at risk. Certain clues may indicate whether an outbreak is the result of purposeful release of microorganisms. Naturally occurring diseases are endemic to certain areas and involve traditional cycles of transmission; some diseases occur seasonally, and sentinel cases are not uncommon. In contrast, a disease outbreak due to bioterrorism could occur in a nonendemic-disease area, at any time of year, without warning, and depending on the etiologic agent and mode of transmission, in large numbers—thousands of cases might occur abruptly. Public health officials must be appropriately sensitized to the possibility of bioterrorism when investigating disease outbreaks. Suspected bioterrorism should be reported promptly to FBI, which is responsible for coordinating interagency investigations of such episodes. FBI scientists are also well trained in forensic methods for criminal investigations

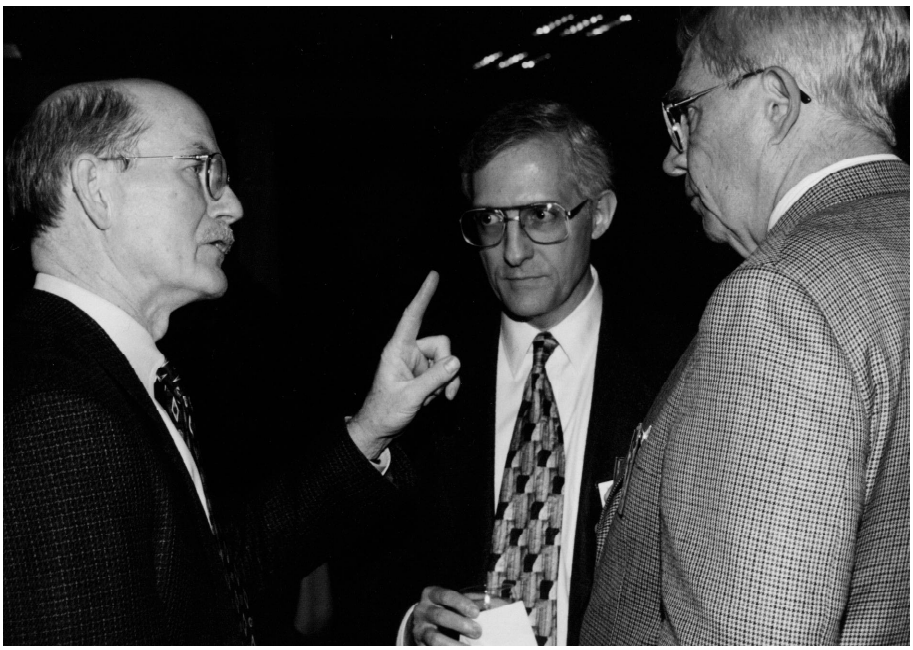
and are prepared to react quickly and effectively.

Maintaining effective disease surveillance is an essential first step in preparedness and is important in helping law enforcement officials to react swiftly. Ensuring adequate epidemiologic and laboratory capacity nationwide are prerequisites to effective surveillance systems. Preparations also must include plans for rapid identification and characterization of agents involved and for emergency distribution of large quantities of medical supplies, especially antibiotics and vaccines. Coordination and communication links also need to be strengthened to minimize response time, especially at first when exposed but asymptomatic persons may still be treated prophylactically. Also, when response time is shortened, the possibility of apprehending perpetrators increases. Education and training in bioterrorism and its potential consequences must become national priorities.

Many agencies and organizations must work collaboratively to ensure national preparedness against bioterrorist attacks. CDC is well positioned to provide leadership in several areas. In partnership with state health departments, the agency maintains infectious disease surveillance systems and provides reference laboratory diagnosis and epidemiologic support, especially during outbreak investigations; disseminates public health recommendations and other information, issues quarantine measures, and provides expert advice on worker health and safety; and is the logical bridge between the public health community and FBI's scientific and response capabilities. Enhancing the public health infrastructure will improve U.S. ability to respond to any infectious disease outbreak and provide added value in the event of a bioterrorist event.

References

1. Torok TJ, Tauxe RV, Wise RP, Livengood JR, Sokolow R, Mauvais S, et al. A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. JAMA 1997;278:389-95.



Joseph McDade (L)
Centers for Disease Control and Prevention, Atlanta, Georgia, USA
Carlos Lopez;
Eli Lilly and Company, Indianapolis, Indiana, USA
D.A. Henderson
The Johns Hopkins University, Baltimore, Maryland, USA

Who Speaks for the Microbes?

Stanley Falkow
Stanford University School of Medicine, Stanford, California, USA

In discussing emerging infectious diseases, the focus is often on the clinical effects of the host-parasite relationship, i.e., the impact on the health and survival of humans and animals, rather than the examination of the biology of the pathogen. It seems fitting to take a moment to reflect on how pathogens "got that way in the first place." Thus, while we discuss emerging infections, it is worthwhile to consider that from the beginning of recorded history—in books or the pictographs of ancient cultures—infectious diseases have been the leading cause of illness and death. Even today, because of infectious diseases most of the world's population does not have the luxury of living long enough to succumb to the chronic diseases of aging.

What were and what remain the reasons that infectious diseases are still the leading cause of death? I believe there are four answers. 1) The presence of human populations was and is large enough to sustain and amplify parasites. We have lived in communities large enough to perpetuate parasites for only about 10,000 years, barely a blink of the eye in the time frame of evolution, which means that most of the well-known infectious diseases adapted to humans are very recent in the evolutionary sense. The black death of the 14th century, just 700 years ago, led to the death of approximately one quarter to one third of the human population of what was then the Western world. We may never understand the full implications of the plague outbreaks of the Middle Ages. The resistance of some caucasian populations to the recent scourge of HIV actually may reflect the genetic consequences of plague survival 20 generations ago. 2) Poverty, with its crowding, unsanitary conditions, and often malnutrition, has led to an increased susceptibility to infection and disease. 3) War, famine, civil unrest, and, indeed, epidemic disease have led to a breakdown in public infrastructure and the increased incidence of infectious diseases. 4) The domestication of animals, beginning about 12,000 years ago, was another important factor. The actual large-scale domestication of animals has slowed and has been replaced by the encroachment of human populations into the domain of animal species all over the globe. It is little wonder that our deliberate destruction of predators and the outgrowth of human populations into virgin land with its attendant destruction of habitat led to the emergence of new diseases such as Lyme disease and murine typhus (spread now by opossums and cat fleas in our slums, instead of by the more classic rat and rat flea vector—"sic transit gloria mundi").

The Enemy Is Us

The cartoon character Pogo, invented by Walt Kelly, once announced to his companions that "the enemy is us." I believe that many of what we refer to as emerging diseases are characterized better as "diseases of human progress." Thus, many major public health crises of the past 2 decades have been infectious in origin. Many, like the outbreaks of Lyme disease and murine typhus, are a natural consequence of human meddling. Similarly, the appearance of infections, like Legionnaires' disease, can be traced to more subtle differences in human behavior and social conventions that have an effect on the microbial world. Thus, the aerosolization of water, now so prominent in the Western world from the widespread use of showers instead of baths to the spraying of produce in large markets to air conditioning, likely has played an important role in the emergence of Legionnaires' disease and also of *Mycobacterium avium* infection in both healthy and immunocompromised persons.

Legionella pneumophila, the Legionnaires' bacillus, is found in nature as an infectious agent of predatory protozoa. Introduction of this organism, often as part of an aerosol of potable water into the alveolus of the lung, results in the microorganism's finding a new niche in the macrophage instead of in its usual host *Acanthamoeba* or *Hartmanella*. More absorbent tampons helped select for a new disease, toxic shock syndrome.

While pathogenic traits of the disease-causing microbes are of consequence, humans

and their technology and social behavior have played a major role in providing pathogenic microbes with new venues for their wares. Food poisoning by *Escherichia coli* O157, *Campylobacter*, and *Salmonella* emerged more from food technology and food distribution networks than from any fundamental change in the virulence properties of the bacteria. In a sense, we have provided these bacteria with a moveable feast.

What Is a Pathogen, Anyway?

Medicine views pathogens as microorganisms capable of causing disease. The emphasis is on disease, not the microorganism. However, from the microbial standpoint, being pathogenic is a strategy for survival and simply one more remarkable example of the extraordinary diversity of the microbial world. Humans are a home to a myriad of other living creatures. From mouth to anus, from head to toes, every millimeter of our cells exposed to the outside world is inhabited by a rich biology. From the mites that may inhabit the eyebrows to the seething cauldron of more than 600 species of bacteria that inhabit the large bowel, we are a veritable garden of microorganisms. Most of these microorganisms are not only innocuous but play a useful, yet unseen, role in our lives. They protect against the few harmful microorganisms that we encounter each day; they provide vitamins and nutrients and help digest food. We have harbored them so long in our evolution that they are even a necessary part of the developmental pathways required for the maturation of intestinal mucosa and the immune system.

Most microbes are commensal; that is, they "eat from the same table." Others are either commensal or transient microbes that are opportunistic; they can cause disease if one (or more) usual defense mechanism, evolved to restrict microorganisms from normally sterile inner organs and tissue, is breached by accident, by intent (as in surgery and, increasingly, in gunshot wounds), or by an underlying metabolic or even infectious disorder. Nevertheless, a small group of microorganisms often causes infection and overt disease in seemingly healthy persons.

Many of the microorganisms, for example, the typhoid bacillus, gonococcus, tubercle bacillus, and treponema of syphilis, are adapted exclusively to humans; others, for example, *Salmonella* Typhimurium, can regularly cause disease in humans, animals, birds, and reptiles. The distinct difference between commensal, opportunistic, and pathogenic microbes is that pathogenic microbes have evolved the genetic ability to breach cellular and anatomic barriers that ordinarily restrict other microorganisms. Thus, pathogens can inherently cause damage to cells to forcefully gain access to a new, unique niche that provides them with less competition from other microorganisms, as well as with a ready new source of nutrients.

For microorganisms that inhabit mammals as an essential component of their survival tactic, success can be measured by their capacity to multiply sufficiently to be maintained or be transmitted to a new susceptible host. This is true for commensal and pathogenic organisms alike. However, if the pathogen gains a new niche free of competition and rich in nutrients, it also faces a more hostile environment designed by evolution to restrict microbial entry and, indeed, to destroy any intruders that enter these protected regions. Thus, pathogens have not only acquired the capacity to breach cellular barriers but also, by necessity, have learned to circumvent, exploit, and subvert our normal cellular mechanisms for their own selfish need to multiply at our expense.

How Did Pathogens Get That Way?

Recent advances in bacterial genetics, molecular biology, and microbial genomics have led to a better understanding of the evolution of bacterial pathogenicity. In genera that have both pathogenic and nonpathogenic organisms, the nonpathogenic bacteria frequently possess one (or more) large genetic insert that contains genes exclusively associated with the pathogenic phenotype. Indeed, in gram-negative enteric bacteria, pathogenic traits are commonly found as large inserts of DNA in the chromosome, as are plasmids dedicated to the pathogenicity of the host microbe. Certain qualities of these DNA inserts suggest that they were acquired by horizontal gene transfer from one microbe to another and that the ultimate origin of these virulence genes was a microbe very different from the organism in which these genes now reside. These "pathogenicity islands" have been the subject of a number of recent articles. However, the evolution of pathogenicity is not the product of a slow, plodding process as much as it is the product of a large single genetic event that had a profound influence on the biology of the

microorganism. Thus, the divergence of *Salmonella* from an ancestor that also gave rise to *E. coli* resulted when the organism received a large pathogenicity island that encoded a contact-dependent secretory system, which gave the host bacterium the ability to cross epithelial barriers. Later on in evolution, some *Salmonellae* received another pathogenicity island that provided the host bacterium with the ability to survive within phagocytic macrophages; finally, other *Salmonellae* that infect only warm-blooded animals eventually inherited a plasmid that appears to permit systemic spread and, perhaps, some degree of host animal preference. These genetic events occurred over millions of years of evolution and were undoubtedly rare, perhaps occurring only once in evolution.

The success of these genetic changes also depended on subsequent selective pressures and genetic fine-tuning by mutation and other genetic mechanisms. Nevertheless, the molecular fossil record in the DNA of contemporary pathogens leads to the inevitable conclusion that microbial evolution is still dynamic and that these periodic genetic upheavals in microbes affecting their pathogenicity can occur at any time. To underestimate the evolutionary potential of microorganisms and their ability to survive, even in the face of enormous pressures to eradicate them and their effects on humankind, would be a mistake.

Infectious agents will emerge so long as there are microorganisms. Humans help the evolutionary process sometimes unwittingly and sometimes by arrogance or ignorance. Antibiotic resistance on a global scale in what seems such a short time comes as no surprise. Does feeding animals antibiotics to promote growth have any effect on human microbes and the health of the human population as a whole?

Rachel Carson's book Silent Spring, which documents the devastating effects of insecticides (e.g., DDT) on the health of a number of living creatures far removed from the insects that were the target, was easily understood. Yet, application of a selective pressure on the microbes of the planet with antibiotics, a pressure that dwarfs the use of DDT in its scope, as well in the number of species that are affected, still remains a subject of debate after 50 years. Is it because we could see the effects of DDT in the pictures of fragile eagle eggs but not in the unseen microscopic world? As Pasteur said, the microbe will endure. Perhaps the fate of the last human is to be consumed by its own microorganisms.

Suggested Bibliography

1. Bäumler AJ. The record of horizontal gene transfer in *Salmonella*. Trends Microbiol 1997;5**:**318-22.
2. Falkow S. The evolution of pathogenicity in *Escherichia*, *Shigella*, and *Salmonella*. In: Neidhardt F, editor. *Escherichia coli* and *Salmonella*: cellular and molecular biology. Washington: American Society for Microbiology; 1995. p. 2723-9.
3. Finlay BB, Cossart P. Exploitation of mammalian host cell functions by bacterial pathogens. Science 1997;276**:**718-25.
4. Galán JE, Bliska JB. Cross-talk between bacterial pathogens and their host cells. Ann Rev Cell Dev Biol 1996;12**:**221-55.
5. Groisman EA, Ochman H. How *Salmonella* became a pathogen. Trends Microbiol 1997;5**:**343-9.
6. Hacker J, Blum-Oehler G, Muhildorfer I, Tachape H. Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. Mol Microbiol 1997;23**:**1089-97.

Stanley Falkow and Lucy Tompkins
Stanford University School of Medicine, Stanford, California, USA

Emerging Diseases—What Now?

George A. O. Alleyne
Pan American Health Organization, Washington, D.C., USA

The Pan American Health Organization (PAHO) was born in 1902 out of concern for the spread of infectious diseases. The outbreak of cholera in Hamburg in 1892 and the epidemics of yellow fever in the Americas led to the decision to establish the International Sanitary Bureau with permanent headquarters in Washington. At the conference that made this historic decision in 1902, participating countries agreed to cooperate with each other and transmit to the bureau "all data of every character relative to the sanitary conditions of their ports and territories and furnish said Bureau every opportunity and aid for a thorough and careful study and investigation of any outbreaks of pestilential disease." All this was to be done to provide the "widest possible protection of the public health of each of the said republics and that commerce between said republics may be facilitated."

To a very large extent, we are still following the bureau's recommendations, only the list of pestilential diseases is shorter by one. Smallpox is no longer with us—and cholera, yellow fever, and bubonic plague are now among the emerging diseases. Cholera is far from disappearing. There were approximately 400,000 cases in the Americas in 1991. This number fell to 18,000 in 1997, but recent reports indicate that as a result of flooding caused by El Niño, the number of cases in Peru has increased dramatically this year. For the first 4 weeks of this year, 2,863 cases were reported compared with 174 for the same period last year and 3,500 for the whole of 1997.

Over the past 5 years, emerging diseases have caused intense concern and activity. The growth in international travel is a major factor. Statistics from the World Tourism Organization show that some 1 million persons per day traveled from their homes by air in 1995. International travel has increased every one of the past 10 years with an average increase of 5.5% per annum. Approximately 1.6 million people cross or recross the U.S.-Mexico border every day by land. Cholera did not spread between the Peruvian towns of Chancay and Chimbote by air travel, but by normal intercity traffic.

The spread of antibiotic resistance is another reason for the emergence of disease; the indiscriminate use of antibiotics is to blame. In the South, antibiotic abuse is facilitated by ready availability without a prescription. In some countries, local pharmacies stock and dispense antibiotics with the same facility as they do cough syrups. In one study of private pharmacies, 42% of the antibiotics were dispensed without prescription (1); in another study, only 23% were given with a physician's prescription (2).

The essential elements of a control strategy for addressing emerging infections are a surveillance system, strengthening the public health infrastructure (including enhancing laboratory capability), stimulation of research, and training of personnel. This strategy is difficult. However, a review of past surveillance activities provides specific lessons.

At the regional level, three disease surveillance systems (for foot-and-mouth disease, poliomyelitis, and measles) have worked and are working. An essential common feature is that surveillance leads to definitive action. For example, detection of cases of poliomyelitis (before the disease was finally eliminated from the Americas) automatically triggered a response. The report of a suspected case now causes resources to be mobilized to establish the validity of the report.

In addition, strong motivation undergirds surveillance. In the case of animal vesicular disease, there is the intense commercial interest behind the maintenance of the system and the possibility of eradication of foot-and-mouth disease. The commercial interest arises because elimination of the disease from the countries of the South represents a possibility of exporting beef worth billions of dollars. Interest rests not only with the national authorities; small communities actually drive the system. An estimated 70% of the cattle are owned by peasants, who each own 10 or fewer. Systematic regular feedback is necessary to maintain interest.

The surveillance systems for these diseases are based on the use of geographic coordinates to

divide the countries into grids that represent the special unit in which the data are collected. Reports are sent by the local veterinary service to the Pan American Foot-and-Mouth Disease Center in Brazil. In recent years, a system has been developed for childhood illnesses that is as sensitive as that which reports animal diseases. The driving force behind the successful development and maintenance of the surveillance system for these childhood illnesses is the possibility of a finite end—eradication and the emotional pride that national health workers and politicians have in reaching this end.

Perhaps the most important aspect of successful surveillance systems is the presence of a credible coordinating international body. No effective international surveillance system can be mounted by a single country, no matter how well it is endowed. External energy, commitment, expertise, and persistence are necessary for such systems to function.

The technology of communication should not become the focus of our efforts. The surveillance and containment systems for smallpox depended on telegrams, telexes, and, I suspect, talking drums. "In India, the largest of the endemic countries, there were no fewer than 8,167 units reporting weekly to 397 district offices, which in turn reported to 31 state program offices and those to the national program office in New Delhi" (3). All this and more was sent to Geneva to be analyzed and reported back faithfully, without the benefit of electronic mail. New information technology is not an indispensable part of the solution.

It is challenging to our sense of superiority as a species to realize that diseases will always emerge. Changes in our social and physical ecology will almost certainly ensure the emergence of new or old diseases, and we are now more vulnerable to these diseases than before. Thus, strategies and policies must be able to be adapted to confront the inevitable new threats; the international community must avoid the peaks and valleys of action that accompany public interest in the exotic.

We have already begun to implement agreed-upon strategies in one particular area. To establish a system for surveillance of antibiotic resistance to enteric pathogens, we identified participating laboratories in 14 countries of the Americas. The next step was to standardize isolation techniques and review methods for measuring antibiotic sensitivity. We are applying an approach similar to the one that proved successful with the Pan American Regional Poliomyelitis Laboratory Network and have adopted "open regionalism"—establishing limited networks that may expand eventually and cooperate among themselves.

PAHO is also creating a functional network of laboratories in the greater Amazon Region to provide data on emerging infections. The participating laboratories' common objective will be the provision of accurate results, prompt sharing of information and research protocols, and a mechanism for rapid transfer of technology. However, the laboratories will need external support to sustain the system.

A strong global system for the application of strategies to control emerging diseases will not occur if the agreement on global action exists only in the sphere of surveillance. There is a fundamental need for other health professionals, in addition to microbiologists, to be convinced of the need for a global approach to some health issues.

The fear of infectious disease has been a powerful stimulus for global action. The successful global system for influenza is due partly to the coordinating efforts of the World Health Organization (WHO) and the work of the key collaborating laboratory centers. Involvement in these efforts keeps laboratories abreast of the latest developments in their special fields.

The need for global health coordination has been very much in the news; the appropriate body to perform that function is WHO. Most nations agree that they must assume responsibility for what are called essential public goods, e.g., immunization, provision of clean water. But some goods are public beyond national considerations, and no single nation can coordinate the availability of these international public goods.

International leadership goes beyond emerging diseases; indeed the success of a global effort to address the threat of these diseases depends largely on the wider perception of responsibilities for global coordination in health. Some believe that the global effort must focus on problems more common in the developing world and that global coordination is a mechanism for channeling resources from the rich to the less fortunate. However, all countries need to appreciate the benefits of global coordination of efforts such as those needed to address emerging diseases. Multilateralism is not antithetical to national interests or bilateral approaches. Success of this multilateral approach will require budgetary support. The annual regular budget of WHO is approximately US$420 million—14% of PAHO's

budget. As Joshua Lederberg said, "Our thinking has been impoverished in terms of budget allocation for dealing with health on an international basis."

Some very successful efforts at global coordination in health have been disease or theme specific, and the "Special Program" approach has given some very good results. However, we should go beyond that and have a global health forum or council in which those agencies and institutions active or becoming increasingly active in health join with WHO in determining how to coordinate the various efforts. I would include in this forum representation from the multilateral financial institutions, the private sector, and nongovernmental organizations. Different spheres of interest and action would complement each other, which should help correct the current ad hoc theme-driven approach that continues to draw criticism.

PAHO has emphasized the benefit of a collective approach, and Panamericanism is one of the major underlying principles of the organization's work. For example, "Health Technology Linking the Americas," a concept that promotes the availability of simple effective technologies throughout the Americas, is a current initiative. Vaccines are one of the technologies emphasized.

In conclusion, we must promote the individual study of the nature and local means of control of emerging diseases. However, we also need a more collective approach at the regional, or even better, the global level—this approach is bound up with the support for global action on other fronts in health. The most powerful instrument we have is multipronged advocacy—advocacy is needed at the political and popular levels for this approach. The public must be engaged on a more regular basis to consider the truism that public health must be a concern of the public. This advocacy has to use some specific examples of those matters that affect the public's health so that emerging diseases are not seen as a threat only on television.

References

1. Brieva J, Danhier A, Villegas G, Yates T, Pérez H. Modalidades del uso de antibióticos en Concepción, Chile. Boletín Oficina Sanitaria Panamericana 1987;103(4):363-72.
2. López R, Kroeger A. Morbilidad y medicamentos en Perú y Bolivia. Universidad Peruana, Cayetano Heredia, Lima, Perú. Acción para la salud, Chimbote, Perú. Ministerio de Salud, La Paz, Bolivia. Universidad de Heidelberg, Alemania, 1990.
3. Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. Smallpox and its eradication. Geneva: World Health Organization; 1988. p. 497.

Plague Diagnostic Workshop[1]

May C. Chu
Centers for Disease Control and Prevention, Fort Collins, Colorado, USA

The Plague Diagnostic Workshop, cosponsored by the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO), was held on March 8 and 12, 1998. Participants represented major laboratories involved in plague diagnostic test implementation and development, the WHO Collaborating Centers for Plague Research and Reference (Almaty, Kazakhstan; Stavropol, Russia; and Fort Collins, Colorado, USA), the WHO Collaborating Center for Yersiniosis (Paris, France), WHO headquarters, and the Pan American Health Organization. Other participants came from Brazil, China, Indonesia, Kazakhstan, Madagascar, Myanmar, Peru, Russia, South Africa, Taiwan, Tanzania, United Kingdom, United States, Venezuela, and Vietnam. From the United States, state and local public health laboratory specialists from California and New Mexico, Naval Medical Research Unit #2, and private industry personnel also participated.

The goals of the workshop were to assess the laboratories' capabilities to perform plague diagnostic tests worldwide; discuss test methods; develop a program for molecular characterization of *Yersinia pestis*, with emphasis on monitoring drug resistance strains; and initiate worldwide electronic links between laboratories. During the first session, representatives reported on their countries' plague activities and presented results on improved and new tests for plague. During the second session, presenters discussed molecular methods used in typing *Y. pestis* and electronic methods for linking laboratories through the Internet. Participants also met during the International Conference on Emerging Infectious Diseases to discuss issues ranging from plague diagnostic criteria to adoption of new test methods.

Recommendations were made to broaden and refine the plague laboratory diagnostic criteria. Three working groups were created to evaluate and develop international standards of *Y. pestis*-specific F1 antigen, F1 antigen-sensitized sheep red blood cells, and specific bacteriophage stock. A fourth working group was charged with evaluating new diagnostic tests. Guidelines and recommendations were made for molecular typing of isolates using plasmid and protein profiling, pulsed-field gel electrophoresis, and ribotyping. The workshop participants also worked toward establishing an electronic bulletin board and soliciting support for another workshop in 2 years to certify the results of the working groups.

[1]Summary of Satellite Session

The U.S.–EU Conference on Extension of the Salm/Enter-net Surveillance System for Human *Salmonella* and *Escherichia coli* O157 Infections[1]

Alexandra Levitt
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

To help extend the European Union's (EU) Enter-net system for the surveillance of *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC)[2] to other countries, a conference was held on March 12, 1998, under the auspices of the U.S.–EU Task Force on Communicable Diseases. The conference was cochaired by James LeDuc (Centers for Disease Control and Prevention [CDC], United States) and Christopher Bartlett (Public Health Laboratory Service [PHLS] Communicable Disease Surveillance Center [CDSC], United Kingdom), who head the Task Force Working Group on Surveillance and Response. Attendees from countries outside EU (South Africa, Hungary, Canada, Japan, Poland, Australia, the Czech Republic, Latvia, and the United States) were invited to describe their countries' procedures for monitoring *Salmonella* and *E. coli* O157:H7.

Enter-net, which has superseded the EU's *Salmonella* surveillance system (Salm-net), is an example of "globalization in action." The network consists of the microbiologist in charge of the member nation's national reference laboratory and the epidemiologist responsible for national surveillance of foodborne diseases. A collaboration of epidemiologists and microbiologists working at the technical level, the network is not a regulatory organization. It includes participants from all 15 EU countries plus Norway and Switzerland, with a combined population of 380 million. Since 1994, Enter/Salm-net has detected 10 international outbreaks resulting from contaminated food or water, including one that involved an Israeli snack food contaminated with *S.* Agona and one due to *S.* Livingstone infection in visitors to Tunisia. Enter-net's objectives are to extend Salm-net monitoring to STEC, including *E. coli* O157:H7, as well as drug-resistant strains of *Salmonella*.

Enter-net participants are working toward a common set of laboratory protocols, including procedures for serotyping, phage typing, and toxin typing. They report disease cases to the international Enter-net database on a regular basis, through the Internet, by using standardized data fields. Every year, the participants from each member country attend a workshop to discuss technical issues and principles of collaboration. Potential conflicts addressed at workshops include ownership of data; confidentiality; outbreak control measures; and liability concerns (e.g., what happens when a food product is implicated by Enter-net as a vehicle of disease transmission). At the next workshop, which will take place in November 1998 in Denmark, Enter-net members will review protocols for collaborative field investigations.

U.S. representatives described U.S. procedures for surveillance of *Salmonella* and STEC, including procedures for antimicrobial resistance monitoring. While Enter-net relies largely on phage typing to define *E. coli* O157:H7 subtypes, pulsed-field gel electrophoresis (PFGE) is the primary *E. coli* O157:H7 subtyping method in the United States. In 1996, CDC initiated PulseNet, a national molecular subtyping network for tracking *E. coli* O157:H7. PulseNet is being expanded to include *Salmonella* and other foodborne pathogens. PulseNet currently includes 26 state and large city health departments and laboratories from the U.S. Department of Agriculture and the Food and Drug Administra-

[1]Summary presented at a satellite meeting, March 12, 1998.
[2]Previously known as verotoxin-producing *Escherichia coli* (VTEC).

tion. An electronic database at CDC will be accessible to all participating PulseNet laboratories and will include DNA patterns of foodborne pathogenic bacteria and epidemiologic information associated with these isolates. Like Enter-net, PulseNet requires that all reporting sites use harmonized laboratory methods and standardized reporting specifications.

Each month, Enter-net's coordinator, based at the Communicable Disease Surveillance Center, applies an automatic cluster-detection algorithm to detect international outbreaks. To make the best use of the algorithm, each country must supply Enter-net with retrospective data from at least 3 years. The United States has an analogous system, the *Salmonella* Outbreak Detection Algorithm (SODA), which analyzes data reported through CDC's Public Health Laboratory Information System (PHLIS). Some U.S. state health departments are beginning to use SODA to perform their own analyses for incident detection.

Over the past few months, Enter-net has begun to define the data that will be collected on isolates of *E. coli* O157:H7; the data will be incorporated in an international database similar to the one used for *Salmonella*. The network has also begun a survey of methods in use for antimicrobial resistance monitoring in its member countries.

Enter-net's goals for 1998 are to conduct an inventory of national laboratory practices related to the diagnosis of STEC and to antimicrobial resistance testing for STEC and *Salmonella*, perform a multicenter study in participating reference laboratories on the detection of drug resistance, upgrade the Enter-net database to include STEC and antimicrobial resistance testing, agree on an outbreak investigation protocol, pilot weekly on-line reporting, and hold a scientific workshop in Denmark in November 1998.

Formal invitations will be sent to non-EU countries that have expressed interest in joining Enter-net. Pilot data exchanges will be initiated in September 1998. If possible, new members will begin routine data exchange by early October and will attend the November workshop in Denmark.

For additional information on Enter-net, contact Ian Fisher (e-mail: ifisher@phls.co.uk), PHLS Communicable Disease Surveillance Centre, 61 Colindale Avenue, London NW9 5EQ, United Kingdom. For PulseNet, contact Bala Swaminathan, National Center for Infectious Diseases, CDC, Mailstop C07, 1600 Clifton Road, N.E., Atlanta, GA 30333, USA.

ASM/CDC/NIH Training in Emerging and Reemerging Infectious Diseases[1]

Karl Western
National Institutes of Health, Bethesda, Maryland, USA

President Clinton's directive on emerging and reemerging infectious diseases calls for the development of domestic and international training programs in this new and expanding field. A training workshop, which coincided with the International Conference on Emerging Infectious Diseases, provided an opportunity to exchange information on current training activities; discuss future plans in clinical, public health, and research training; and, more importantly, generate discussion on unmet needs and improvement of present activities.

NIH Academic Partnerships: Needs and Future Directions

This part of the training workshop was chaired by the National Institute of Allergy and Infectious Diseases (NIAID) Deputy Director, John R. La Montagne. Each participant was asked to address the following five questions: 1) What is emerging infectious disease training? 2) What are its most important priorities and needs? 3) What are your recommendations for curriculum development? 4) What resources are needed to address training and curriculum needs? and 5) How can the American Society for Microbiology (ASM), Centers for Disease Control and Prevention (CDC), National Institutes of Health (NIH), and partners in academia, government, industry, and professional organizations promote and support the training?

Adel Mahmoud, Case Western Reserve University, spoke about the problem of incorporating emerging infections training into medical school curricula. He was followed by David Stephen, Emory University, who spoke on the integration of emerging disease training into infectious disease training; Mary E. Wilson, Harvard School of Medicine, who discussed continuing medical education; Gail Cassell, Eli Lilly and Company, who brought in perspectives from industry and academia; and Robert Webster, St. Jude Children's Research Hospital, who recounted lessons learned from research in Hong Kong during the recent avian influenza outbreak. After questions and answers cochaired by John La Montagne and Joel Breman, Fogarty International Center (FIC), D.A. Henderson, Johns Hopkins University, summed up the discussions and extracted recommendations.

The workshop had the following conclusions. 1) A number of emerging infectious disease training initiatives either under way or under consideration at CDC, the Armed Forces, NIAID, and FIC are modest (given the training needs) and, without exception, underfunded. 2) There is considerable public, private, and Congressional interest in emerging infections, particularly in food safety and vector-borne diseases. 3) Recently, a new element, biological warfare and terrorism, has been added to the equation. 4) Several CDC training initiatives directed at local and state public health authorities are frustrated by lack of resources in the public health trenches. 5) Army and Navy overseas laboratories represent an underappreciated and underutilized resource for training of both U.S. citizens and foreign nationals. 6) NIH training is limited to formal training; it sets ceilings on research training slots and its domestic mission. As a result, most NIH research training is carried out through research awards. The expansion of the NIAID International Collaboration in Infectious Disease Research Program (with increased emphasis on training U.S. scientists) and the announcement of FIC international Actions for Building Capacity are welcome but are still short of what is needed. 7) Industries' contributions, such as Merck's Mectizan and SmithKline Beecham's Albendazole Donation Programs, are welcome. In addition, Lilly's decision, announced this week, to provide CDC with funds for international participants in its training program is an innovative approach to

[1]Summary of satellite session

promote intersectorial cooperation. 8) The recent Hong Kong avian influenza outbreak is a paradigm on how the research, clinical, public health, and industrial communities can cooperate in an emergency situation and prevent a recurrence of an influenza pandemic. Hong Kong may have been a very close call; influenza is the only reemerging infectious disease for which a contingency plan involving all these players exists and is operational.

The workshop recommended the following. 1) Current CDC, NIH, and Department of Defense training programs should receive additional funding and be expanded through increased U.S. government resources and through innovative cooperative efforts with the private sector. 2) U.S. Agency for International Development (USAID), World Health Organization (WHO), and other international organizations should join forces with domestic agencies to provide for training of foreign nationals. 3) Increased communication and coordination between the clinical, public health, and research communities are needed. The veterinary educational model, which looks at populations rather than individual patients, might serve as a model for the medical community. 4) An intersectorial emerging infectious diseases group composed of U.S. members from government agencies (CDC, NIH) and state health departments, universities, industry, schools of public health, professional organizations (e.g., ASM, Infectious Disease Society of America, American Society of Tropical Medicine and Hygiene, and international organizations (USAID, WHO) should be organized to identify training needs. 5) ASM and other professional organizations should work with academic institutions to promote curriculum changes at the professional student, clinical training, and research training levels to increase awareness of and capacity to recognize and treat or prevent emerging infections. 6) continuing medical education courses, audiovisual programs, and interactive educational materials should be developed to address these training needs and should provide opportunities for cooperation with industry and the private sector. 7) Intersectorial efforts should be undertaken to train personnel and support work plans for training and research that will help anticipate and control emerging diseases other than influenza.

Outbreak of Suspected *Clostridium butyricum* Botulism in India

To the Editor: Foodborne botulism, particularly associated with *Clostridium butyricum,* is rare; no cases had been reported in India before this outbreak. A reported case of foodborne botulism represents a public health emergency because of the potential severity of the disease and the possibility of mass exposure to the contaminated product.

In September 1996, the anaerobic section of the All India Institute of Medical Sciences received serum and food samples from the National Institute of Communicable Diseases, Delhi, India, for investigating a possible outbreak of foodborne botulism.

In the early hours of September 18, 1996, 34 of 310 students of a residential school in rural Gujrat complained of abdominal pain, nausea, chest pain, and difficulty in breathing. One of the students, aged 14, died before he could be treated; two others, aged 13, died on their way to the hospital. The remaining 31 students were admitted to a rural hospital; eight were discharged 1 day later after being given symptomatic treatment, while the other 23 were transported by ambulance to an urban emergency department in Ahemdabad, Gujrat. Findings on examination included ptosis, pupillary mydriasis, extraocular palsies, and impairment of conciousness. All students were given symptomatic treatment in the form of stomach lavage and intravenous administration of antibiotics and steroids. Over the subsequent 24 hours, 21 improved clinically and were discharged; however, two (aged 14 and 17 years) had respiratory distress and required mechanical ventilation. Differential diagnosis included botulinum food poisoning, and both patients were administered trivalent (A,B,E) botulinum antitoxin. They responded well to the treatment and were discharged from the hospital 1 month later.

Patients reported that 24 hours before onset of symptoms, they had eaten ladoo (a local sweet), curd, buttermilk, sevu (crisp made of gram flour), and pickle. Food samples were assayed for botulinum toxin and were cultured anaerobically (1). Anaerobic culture of leftover sevu yielded an organism in pure culture whose cultural and biochemical properties were consistent with those of *C. butyricum;* i.e., it was lipase-negative, fermentative, and did not liquefy gelatin (2). Enrichment cultures of the sevu specimens in enriched chopped meat–glucose–starch medium contained toxin after 5 days of anaerobic incubation at 30°C. This was shown by mouse toxicity test in which the enrichment broth of the specimen was injected intraperitoneally into mice; botulinum toxin was detected by observing its lethal effect on mice. This effect was neutralized by specific polyvalent botulinum antitoxin types A, B, E (Biomed, Warsaw, Poland). Cultures of other food items tested negative for toxigenic organisms. Serum specimens (obtained more than 1 week after the onset of illness) from eight patients with mildly symptomatic illness were negative for toxin.

To test the presence of toxin gene in the isolated strain of *C. butyricum*, polymerase chain reaction (PCR) was performed. Degenerate primers BoNT 1 and BoNT 2 were used, which amplify a specific 1.1-kb fragment of neurotoxin gene *C. botulinum* types (A, B, E, F, and G) as well as toxigenic strains of *C. baratti* and *C. butyricum* (3). Five *Escherichia coli* strains containing clones encoding fragments of the *C. botulinum* neurotoxin genes were used as positive controls in the PCR assay (kindly provided by Alison East, Institute of Food Research, United Kingdom). PCR profile used was as follows: 94°C for 2 min, followed by 25 cycles of 92°C for 1 min, 42°C for 1 min, and 62°C for 5 min, then held at 4°C (Alison East, pers. comm.). An amplified product of 1.1 kb was detected from the culture isolate of sevu.

The outbreak described in this report draws attention to the emergence of new foodborne pathogens and to their association with unusual foods. Human botulism is commonly caused by *C. botulinum* neurotoxin type A, B, and E (4). In the present study, we showed that a neurotoxigenic *C. butyricum* was present in the food implicated in a clinically suspected outbreak of botulism in Gujrat, India.

Laboratory studies could not confirm the diagnosis of botulism because clinical materials (such as contents of the gastrointestinal tract, feces) were not submitted for examination for the presence of the botulinum toxin or organisms. It is not surprising that toxin could not be detected in the eight serum samples received by our laboratory. Because of the delay in clinical diagnosis, early serum samples could not be obtained. Toxin is detected in only 13% of serum samples collected more than 2 days after ingestion of botulinum toxin (5). However, the

clinical presentation of the patients, response to trivalent botulinum antitoxin, and isolation of toxigenic *C. butyricum* from one of the consumed food articles strongly suggest that the outbreak was caused by food contaminated with toxigenic *C. butyricum*.

Neurotoxigenic *C. butyricum* was first reported in 1986 in two cases of infant botulism in Rome (6). Recently, neurotoxigenic *C. butyricum* was isolated from the food implicated in an outbreak of clinically diagnosed type E botulism in China (7). In this outbreak, it appears that sevu, because of improper storage, was contaminated with the spores of *C. butyricum*, which subsequently germinated and produced toxin. To the best of our knowledge, this is the first report of neurotoxigenic *C. butyricum* causing foodborne botulism in India.

The changing epidemiology of foodborne disease as highlighted in this report calls for improved surveillance, including the development of new technology for identifying outbreaks.

We thank Alison East, Institute of Food Research, Reading Laboratory, United Kingdom, for supplying *E. coli* clones with BONT gene for PCR; Pradeep Seth, professor and head, Department of Microbiology, All India Institute of Medical Sciences for facilities provided; Biomed Warsaw, Poland, for polyvalent botulinum antitoxin; and the medical and paramedical staff of Civil Hospital, Ahemdabad.

Rama Chaudhry,* Benu Dhawan,* Dinesh Kumar,* Rajesh Bhatia,† J.C Gandhi,‡ R.K. Patel,§ and B.C. Purohit§

All India Institute of Medical Sciences, New Delhi, India; †National Institute of Communicable Diseases, Delhi, India; ‡Health Medical Services and Medical Education (H.S.), Gandhi Nagar, India; and §Civil Hospital, Ahemdabad, Gujrat, India

References

1. Hatheway CL. Botulism. In: Balows A, Hausler WJ, Lennette EH, editors. Laboratory diagnosis of infectious diseases: principles and practice. New York: Springer-Verlag: 1988. p. 111-33.
2. McCroskey LM, Hatheway CL, Fenicia L, Pasolini B, Aureli P. Characterization of an organism that produces type E botulinal toxin but which resembles *Clostridium butyricum* from the feces of an infant with type E botulism. J Clin Microbiol 1986;23:201-2.
3. Campbell KD, Collins MD, East AK. Gene probes for identification of the Botulinal Neurotoxin gene and specific identification of neurotoxin types B.E. and F.J. Clin Microbiol 1993;31:2255-62.
4. Hatheway CL. *Clostridium botulinum* and other clostridia that produce botulinum neurotoxin. In: Hauschild AHW, Dodds KL, editors. *Clostridium botulinum*—ecology and control in foods. New York: Marcel Dekker, Inc.; 1992. p. 3-20.
5. Woodruff BA, Griffin PM, McCroskey LM, Smart JF, Wainwright RB, Bryant RG, et al. Clinical and laboratory comparison of botulism from toxin types A, B, and E in the United States, 1975-1988. J Infect Dis 1992;166:1281-6.
6. Aureli PK, Fenicia L, Pasolini B, Gianfranceschi M, McCroskey LM, Hatheway CL. VII. Two cases of type E infant botulism caused by neurotoxigenic *Clostridium butyricum* in Italy. J Infect Dis 1986;154:207-11.
7. Meng X, Karasawa T, Zou K, Kuang X, Wang X, Lu C. et al. Characterization of a neurotoxigenic *Clostridium butyricum* strain isolated from the food implicated in an outbreak of food-borne type E botulism. J Clin Microbiol 1997;35:2160-2.

Molecular Analysis of *Salmonella paratyphi* A From an Outbreak in New Delhi, India

To the Editor: In the context of emerging infectious diseases, enteric fever caused by *Salmonella paratyphi* A deserves increased attention and vigilance, although its severity is often milder than that of *S. typhi* disease. Outbreaks associated with this organism are exceedingly rare but have recently been reported in India (1) and Thailand. In India, the first reported outbreak of disease associated with *S. paratyphi* A (1) provided an opportunity to study the molecular epidemiology of infection caused by this organism.

A total of 18 human blood isolates of *S. paratyphi* A, 13 from the outbreak in New Delhi, India (from September to October 1996) (1) and 5 sporadic isolates from cases unrelated to the outbreak, were used in this study. A total of 36 culture-positive cases were detected during the 6-week outbreak. All strains were phage type 1 and were sensitive to all antibiotics tested. Isolates were analyzed by ribotyping and pulsed-field gel electrophoresis (PFGE) (2,3). PFGE/ribotype profiles were assigned arbitrary designations and analyzed by defining a similarity (Dice) coefficient, F (3), where F = 1.0 indicates complete pattern identity and F = 0, complete dissimilarity.

The five sporadic isolates of *S. paratyphi* A gave PFGE patterns following *Xba*I (5'-TCTAGA-3') digestion that were unique and distinctly different, with differences of 8 to 12 bands (F = 0.63-0.70). In contrast, the 13 outbreak isolates shared only four closely related PFGE patterns differing only in 1 to 6 bands (F = 0.8-1.0). Among the outbreak strains, two distinct clones were

observed, X1 and X2, which differed by 5 to 6 bands. Furthermore, outbreak isolates X3 and X4 were closely related to X1, differing by four and three DNA fragments, respectively. Similar results were obtained after digestion with a second restriction endonuclease, *Spe*I (5'-ACTAGT-3'; pattern designation S). Although fewer bands were seen compared to PFGE, ribotyping of these isolates using *Spe*I-digested genomic DNA largely confirmed the PFGE results in that the sporadic isolates gave unique profiles and only three closely related ribotype profiles were detected among the outbreak isolates. Two Malaysian isolates of *S. paratyphi* A included for comparison gave patterns very different from the Indian isolates by both PFGE (F = 0.44-0.65) and ribotyping. Also, it was determined that isolates A-117 (X1/S1) and A-123 (X2/S2) belonged to the index cases and that, as the outbreak progressed, other patterns (X3/S3 and X4/S4), which differed from the original patterns by one to four bands, appeared during weeks 2 to 3 of the outbreak. Notably, patterns X1 and X2 reappeared at the end of the outbreak.

Although molecular analysis of *S. typhi* and *S. paratyphi* B by ribotyping (2,4) and PFGE (3) has been reported, to the best of our knowledge the present study is the first performed with *S. paratyphi* A. The data obtained agree with those observed for *S. typhi* (3) in that outbreak isolates are more clonal and limited in diversity, whereas sporadic isolates are more diverse genetically and belong to unrelated clones. According to the criteria of Tenover et al. (5), it seems likely that the present outbreak was associated with two distinct clones/strains of *S. paratyphi* A (X1/S1 and X2/S2) that are related (5) but have distinct PFGE profiles. This observation is perhaps not surprising given the fact that both clones are phage type 1 and that contaminated potable water was incriminated in the outbreak (1). The PFGE results were largely confirmed by ribotyping, although this technique appears to be slightly less sensitive and discriminating in that fewer bands were seen and the differences between outbreak isolates were much less obvious.

We thus conclude that the outbreak in New Delhi, India, was caused by two related but distinct clones of *S. paratyphi* A. There also appears to be substantial genetic diversity among *S. paratyphi* A strains as the Malaysian isolates were very different from those from India. The data also suggested minor genetic changes among the *S. paratyphi* A isolates during the 2-month outbreak. This observation agrees with the high mutation rates noted among pathogenic *Salmonella* spp. (6) and the plasticity of the genome of salmonellae associated with enteric fever (7). How these changes affected the biologic behavior of these isolates will be the subject of further study. Our study reaffirms the usefulness of PFGE and ribotyping in the molecular typing and discrimination of individual *Salmonella* isolates for epidemiologic investigations.

Kwai-Lin Thong,* Satheesh Nair,* Rama Chaudhry,† Pradeep Seth,† Arti Kapil,† Dinesh Kumar,† Hema Kapoor,‡ Savithri Puthucheary,* and Tikki Pang*

*University of Malaya, Kuala Lumpur, Malaysia; †All India Institute of Medical Sciences, New Delhi, India; and ‡Safdarjang Hospital, New Delhi, India

References

1. Kapil A, Sood S, Reddaiah VP, Das B, Seth P. Paratyphoid fever due to *Salmonella enterica* serotype paratyphi A. Emerg Infect Dis 1997;3:407.
2. Pang T, Altwegg M, Martinetti G, Koh CL, Puthucheary SD. Genetic variation among Malaysian isolates of *Salmonella typhi* as detected by ribosomal RNA gene restriction patterns. Microbiol Immunol 1992;36:539-43.
3. Thong KL, Cheong YM, Puthucheary S, Koh CL, Pang T. Epidemiologic analysis of sporadic and outbreak *Salmonella typhi* isolates by pulsed field gel electrophoresis. J Clin Microbiol 1994;32:1135-41.
4. Ezquerra E, Burnens A, Jones C, Stanley J. Genotypic typing and phylogenetic analysis of *Salmonella paratyphi* B and *S. java* with IS200. J Gen Microbiol 1993;139:2409-14.
5. Tenover FC, Arbeit RD, Goering RV, Mickelson PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233-9.
6. Leclerc JE, Li B, Payne WL, Cebula TA. High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. Science 1996;274:1208-11.
7. Liu SL, Sanderson KE. Highly plastic chromosomal organization in *Salmonella typhi*. Proc Natl Acad Sci U S A 1996;93;10303-8.

Unrecognized Ebola Hemorrhagic Fever at Mosango Hospital during the 1995 Epidemic in Kikwit, Democratic Republic of the Congo

To the Editor: We report here the clinical description of a hemorrhagic syndrome observed

in Mosango General Hospital that, retrospectively, was one of the first cases of the Ebola hemorrhagic fever outbreak in the Bandundu province of former Zaire in the spring of 1995 (1).

Case

On April 20, 1995, a 70-year-old nun, working as a nurse in Kikwit General Hospital, was admitted to Mosango General Hospital with a 5-day history of fever, despite antimalarial treatment. The day before hospitalization she had profuse diarrhea, vomiting, high fever, and severe agitation with delirium. On arrival, quiet and apyretic, she complained of headache, loss of appetite, and severe asthenia, but she walked to her room without help. On examination, the only abnormalities recorded were severe dehydration and oral thrush-like lesions, raising a suspicion of candidiasis. Pulse rate was 80/min and blood pressure 120/80. Medical history included an amebiasis liver abscess 15 years ago and chronic coronaritis since 1990.

Electrocardiogram (ECG) abnormalities were consistent with chronic diffuse ischemia. Laboratory investigations showed the following values: few trophozoites on a thick film; erythrocyte sedimentation rate (ESR) 15 mm/h; bleeding time (BT) 7½ min; coagulation time (CT) 9 min; and white blood cells (WBC) $8.4x10^9$/L (73% neutrophils, 23% lymphocytes, 2% eosinophils, 1% basophils, 1% mastocytes). Urinalysis showed proteinuria (++), hyaline cylinders (+++), 50 white cells per field, and hematuria (+). The patient was perfused with 4L/day of glucose and 1.5 g of quinine. She was kept in a private room in the nearby nuns' convent.

Later during the day, high fever (40°C) and severe diarrhea with melena developed; the pulse rate was normal (80/min). Typhoid fever was suspected despite the lack of hepatosplenomegaly; Widal test was not available for confirmation. Treatment was started with intravenous (i.v.) amoxicillin (1g/6h during the first 24 h and then 1g/4h) and i.v. chloramphenicol (2g/24h). Subsequently, coagulation abnormalities developed in addition to the melena; vitamin K and epsilon amino caproic acid were added to i.v. therapy. Watery vomits remained frequent and abundant, and the patient's condition was unresponsive to treatment.

On hospitalization day 2, the clinical picture remained the same, with severe asthenia, anorexia, abundant blackish diarrhea, and watery vomits. An intractable hiccup developed. The fever remained in plateau around 40°C with spikes. Obnubilation occurred during episodes of high fever. Pulse and blood pressure remained stable. ECG showed no modifications. Cutaneous examination detected for the first time a maculopapular rash and petechiae on flanks and limbs, and the patient complained of gastric pain for which the neurologic examination was normal. Urine was abundant and clear.

On hospitalization day 3, high fever continued, with some defervescence during which the patient regained lucidity, although she responded only with monosyllables because of the extreme asthenia and somnolence; diarrhea persisted but without hemorrhage. The patient had less vomiting. Laboratory data showed ESR 35 mm/h; BT 10 min; CT 12 min; WBC $12.6x10^9$/L (70% neutrophils, 24% lymphocytes, 2% eosinophils, 1% basophils, 3% mastocytes). During the night, the patient maintained a high temperature, still with temperature-pulse disparity. The diagnosis of typhoid fever was questioned, and other diagnostic possibilities were reconsidered (shigellosis, mononucleosis); leukocytosis was considered against the possibility of Ebola hemorrhagic fever. Chloramphenicol was switched to rifampicin (1,200 mg/24h).

On April 23, the patient's status was unchanged with fever, asthenia, and diarrhea. Later in the day, her condition deteriorated: petechiae could be seen on the entire body, and for the first time, bruises and bleeding at injection sites were observed and precluded intramuscular injections. The patient had bleeding cracks on the lips and diffuse bleeding in the oral cavity (i.e., gums, tongue). The volume of urine was low, and antibiotic therapy was changed to cephalosporin.

On hospitalization day 5, hemorrhages increased, and fever remained high until the end of the day, when it started to normalize. Urine volume was still low (verified by vesical catheter) despite the i.v. rehydration of 4 L/day. Fresh blood transfusion (300 ml) did not slow the hemorrhaging; disseminated intravascular coagulation was suspected, and heparin treatment was started. The patient became comatose. The laboratory results showed ESR 55mm/h and WBC $30.2x10^9$/L with an unchanged formula. No coagulation was observed on BT and CT. Blood pressure fell (80/50); the clinical status remained unchanged until the patient's death on April 25 at 10:00 a.m.

No special nursing precautions were taken either during the hospitalization or after the death, and the body was transferred to Kikwit to be buried. On April 30, another nun who took care of the index patient during the night of April 23 became ill with fever, headache, and myalgia. Over the next few days, the second patient had a clinical picture identical to that of the index patient, including high fever, severe asthenia, vomiting, hiccups, and diarrhea. On May 5, epistaxis and coagulation abnormalities developed, followed by other clinical signs of the hemorrhagic syndrome. The second patient was transferred to Kikwit General Hospital, where she died 6 days later. A laboratory confirmation of Ebola hemorrhagic fever was made on a blood specimen collected on May 5 and sent to Special Pathogens Branch (Centers for Disease Control and Prevention, Atlanta, GA).

These cases of unrecognized Ebola hemorrhagic fever were part of the hospital outbreak that precipitated and mobilized international community efforts (2). Retrospectively, the clinical symptoms observed were typical of Ebola hemorrhagic fever (3,4) and were described again in subsequent patients during this outbreak (5). In tropical Africa, the presence of hemorrhagic symptoms in the course of a febrile illness should raise the possibility of one of the viral hemorrhagic fever diseases. In viral hemorrhagic fevers, maculopapular rash is constantly observed only in filovirus disease. Typically, the clinical laboratory findings include an early lymphopenia and marked thrombocytopenia. Containment and barrier nursing procedures should be initiated until the diagnosis of viral hemorrhagic fever can be ruled out. The index patient described here was the third patient transferred from Kikwit General Hospital in less than 1 month to die of a hemorrhagic illness after a few days of an unexplained febrile syndrome. Two patients were health-care workers in Kikwit General Hospital. This cluster of hemorrhagic illness and possible human-to-human transmission, particularly among hospital staff, was (and should always be) sufficient to suspect a viral hemorrhagic fever. The laboratory confirmation of this presumptive diagnosis was the clenching factor in the multinational effort in Kikwit.

Marie-Jo Bonnet, Philippe Akamituna, and Anicet Mazaya
Mosango General Hospital, Kikwit, République Démocratique du Congo

References

1. Muyembe T, Kipasa M, the International Scientific and Technical Committee, WHO Collaborating Centre for Haemorrhagic Fevers. Ebola haemorrhagic fever in Kikwit, Zaire. Lancet 1995;345:1448.
2. Khan AS, Kweteminga TF, Heymann DL, LeGuenno B, Nabeth P, Kerstiens B, et al. The reemergence of Ebola hemorrhagic fever, Zaire, 1995. J Infect Dis. In press 1998.
3. Piot P, Sureau P, Breman JG, Heymann D, Kintoki V, Masamba M, et al. Clinical aspects of Ebola virus infection in Yambuku area, Zaire, 1976. In: Pattyn SR, editor. Ebola virus haemorrhagic fever. Amsterdam: Elsevier/North-Holland Biomedical Press; 1977. p. 7-14.
4. Sureau PH. Firsthand clinical observations of hemorrhagic manifestations in Ebola hemorrhagic fever in Kitwit, Democratic Republic of the Congo (former Zaire): clinical observations in 103 patients. Review of Infectious Diseases 1989;11:S790-3.
5. Bwaka MA, Bonnet M-J, Calain P, Colebunders R, De Roo A, Guimard Y, et al. Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo (former Zaire): clinical observations in 103 patients. J Infect Dis. In press 1998.

Classification of Reactive Arthritides

To the Editor: We read with interest J.A. Lindsay's article on sequelae of foodborne disease (1). However, we believe that there are errors in the classification of the reactive arthritides. Lindsay states that ankylosing spondylitis (AS) is a "rheumatoid inflammation of synovial joints and entheses within and distal to the spine." Although not the primary focus of the article, the classification and etiopathogeneses of rheumatoid arthritis (RA) and the seronegative spondyloarthropathies, including AS, should be clarified. The term spondylitis, from the Greek *spondylos*, for vertebra, means inflammation of the vertebrae. The term rheumatoid is generally taken to apply to rheumatoid arthritis, while rheumatic is a more general term applying to all connective tissue diseases.

AS is a chronic, systemic, inflammatory disorder primarily affecting the axial skeleton, with sacroiliac joint involvement as its hallmark. Back pain is the first clinical manifestation in approximately 75% of the patients (2). The backache is usually insidious in onset, dull, and difficult to localize. After several months, it generally becomes bilateral and persistent. The ache is often worse in the morning or after periods of inactivity and improves with move-

ment. The course is highly variable. Involvement of peripheral joints other than hips and shoulders is uncommon.

AS is strongly associated with human leukocyte antigen (HLA) B27, a major histocompatibility complex (MHC) class I allele, and may show familial aggregation. More than 90% of patients with AS have the HLA-B27 allele (3). HLA-B27 is believed to be directly involved in disease pathogenesis. Transgenic rats expressing human HLA-B27 develop a broad spectrum of disease closely resembling human disease. These rats have peripheral and axial arthritis, gastrointestinal inflammation, and diarrhea. Psoriatic-like skin changes and inflammation of the heart and male genitalia are also seen. Histologically, the joint, gut, skin, and heart lesions resemble those seen in HLA-B27-related disease in humans (4).

The inflammatory process in AS involves the synovial and cartilaginous joints, as well as the osseous attachments of tendons and ligaments (entheses). Much of the skeletal pathology of AS can be explained by the changes that take place at the entheses. After an initial inflammatory, erosive process involving the entheses, there is healing in which new bone is formed. The final outcome of this process is an irregular bony prominence with sclerosis of the adjacent cancellous bone (5). This can be contrasted with the pathology of RA, in which there is a greater tendency to affect cartilaginous joints such as the intervertebral discs and symphysis pubis. The process in RA is one of bony erosion rather than new bone formation.

The term ankylosing spondylitis, derived from the Greek for "bent spinal vertebrae," by definition requires exclusion of the other spondyloarthropathies, such as Reiter syndrome and reactive arthritides due to enteric (or urogenital) organisms. Spondylitis may occur in reactive arthritis, psoriatic arthritis, or the arthropathy associated with inflammatory bowel disease, but is less common in these diseases (approximately 50% in reactive arthritis, 20% in enteric arthritis or psoriatic arthritis). All of these diseases can be viewed as seronegative spondyloarthropathies in that, by definition, rheumatoid factor is not present.

RA is a systemic autoimmune disorder of unknown etiology. It is a chronic symmetric arthropathy of peripheral joints, associated with erosive synovitis. Enthesopathy is generally not found. The majority of patients have elevated titers of serum rheumatoid factor, as opposed to the seronegative spondyloarthropathies. Spinal involvement in RA is seen but most often involves the cervical spine. The pathogenesis of the spinal disease is that of synovitis of the odontoid-atlas joints. The major HLA association is with HLA-DR4, an MHC class II allele.

Reactive arthritis is so named because it is felt that the arthritis and other inflammatory manifestations are an immune reaction to a distant infection. There is an association with HLA-B27 but less so than that found in AS (60% to 80%, compared with more than 90% in AS). While bacterial antigens can be found within the joint, the offending infectious process most often subsides before the onset of arthritis, and no living organisms are found in the joint (2). In many cases, no infectious trigger can be identified. Persistence of microbial antigens has been demonstrated and is likely to play a prominent role in the pathogenesis of acute and chronic inflammation. Antigens to several gastrointestinal pathogens have been isolated from the synovial fluid in patients with reactive arthritis. *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter,* and *Borrelia* are the most common pathogens capable of initiating reactive arthritis (2). The arthritis is generally an asymmetric oligoarthritis predominantly affecting the lower extremities and typically develops 6 to 14 days after a bout of diarrhea. However, onset can occur up to 3 months later. Diarrhea can also be absent, and there is no relationship between the severity of the arthritis and the severity of the diarrhea.

Reiter syndrome is in fact a reactive arthritis. In 1916, Hans Reiter described a triad of arthritis, urethritis, and conjunctivitis in a soldier with dysentery. However, the disease was actually first described by Sir Benjamin Brodie in the early 1800s (6). The complete triad is actually seen in only a minority of patients. Arthritis develops 1 to 3 weeks after the diarrhea or urethritis. It is generally asymmetric, involving large joints, especially in the lower extremities. The term Reiter syndrome actually refers only to the triad of arthritis, urethritis, and conjunctivitis. Reiter syndrome is both clinically and historically more accurately termed reactive arthritis. Nevertheless, the term reactive arthritis does not reflect the systemic nature of the disease.

In summary, while both reactive arthritis and ankylosing spondylitis are seronegative

spondyloarthropathies, they are separate entities. Both are distinct from rheumatoid arthritis.

Darren R. Blumberg and Victor S. Sloan
Robert Wood Johnson Medical School, New Brunswick, New Jersey, USA

References

1. Lindsay JA. Chronic sequelae of foodborne disease. Emerg Infect Dis 1997;3:443-52.
2. Veys EM, Mielants H. Enteropathic arthropathies. In: Klippel JH, Dieppe PA, editors. Rheumatology. St. Louis: 1994; 3.35.
3. Khan MA. Seronegative spondyloarthropathies. In: Schumache HR, editor. Primer on rheumatic diseases. Atlanta (GA): Arthritis Foundation; 1993.
4. Hammer RE, Maika SD, Richardson JA, Tang J-P, Taurog JD. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human a2m: an animal model of HLA-B27-associated human disorders. Cell 1990;63:1099-112.
5. El-Khoury GY, Kathol MH, Brandser EA. Seronegative spondyloarthropathies. Radiol Clin North Am 1996;34:343-57.
6. Toivanen A. Reactive arthritis. In: Klippel JH, Dieppe PA, editors. Rheumatology. St. Louis: 1994: 4.9.

Reply to Drs. Blumberg and Sloan

To the Editor: I concur with your comments. After reviewing the literature related to foodborne disease, it appears that the original classification of reactive arthritides has been in error for some time. I certainly appreciate the correction.

James A. Lindsay
University of Florida, Gainesville, Florida, USA

Cost of Blood Screening

To the Editor: In reference to G.A. Schmunis' article on the risk for transfusion-transmitted infections in Central and South America (1), I would like to comment on the cost of blood screening. In a screening program, the objective is to have safe blood units, not to assess the prevalence of different infections among potential or actual donors. Thus, while acknowledging all infections present in a given donor or potential donor is not required, detecting at least one of the infections that would make a donor noneligible is. If samples from every potential donor are subjected (by default) to all the tests, information on every infection present is provided, and the cost of screening this donor is the sum of the cost of every test applied; in this case, both the information and the cost are greater than necessary.

Information on the prevalence of bloodborne infections among the general population or, preferably, among potential donors (particularly where professional donors are frequent) along with information on the costs of the tests to be used can form the basis of a stepwise screening scheme. Tests for infections with the highest prevalence would be applied first. For example, in many areas of Peru, using the Venereal Disease Research Laboratory (VDRL) test (for screening *Treponema pallidum* infection) first would reduce the number of samples to be subjected to other more expensive and often less available tests (e.g., HIV enzyme-linked immunosorbent assay [ELISA] or hepatitis C virus [HCV] ELISA); in others areas, a test for hepatitis B virus antigen (HB_sAg) should be used before HIV ELISA. The reduction in cost provided by stepwise screening will depend on the prevalences of the more frequent infections and the frequency of concurrent infections.

The questionnaires applied to candidate donors should be validated, and the benefit of using them should be assessed. In most settings, candidate donors are either ignorant of their status as carriers of bloodborne infection or ready to deny it; therefore, the questionnaire is of little use. In some cases candidate donors are turned down because of "hepatitis history" when in fact they have not had bloodborne hepatitis.

Finally, screening tests seem to be quite more expensive than reported in Table 4 of the Schmunis article. In Lima, at a ministry of health facility, some prices are as follows: HIV ELISA US$12.50, VDRL US$6.40, HB_sAg US$13.90.

O. Jaime Chang
Instituto Nacional de Salud, Ministerio de Salud, Lima, Peru

Reference

1. Schmunis GA, Zicker F, Pinheiro F, Brandling-Bennett D. Risk for transfusion-transmitted infectious diseases in Central and South America. Emerg Infect Dis 1998;1:5-11.

Emerging Infections. R.M. Krause, Editor. Academic Press, New York, 1998, 513 pages.

Emerging Infections is the first volume in a new series entitled Biomedical Research Reports, edited by John Gallin and Anthony Fauci. The volume contains 17 chapters, all outstanding and for the most part both timely and comprehensive, written by experts in the field. After an intellectually stimulating introductory chapter by Richard Krause, we are treated to an analysis of epidemics by one of the supreme authorities, Roy Anderson of Oxford University. Included here is a valuable discussion on the transmission of microbial infections in populations as well as the development of drug resistance.

Chapters on emerging bacterial diseases include a superb one on Persisting Problems in Tuberculosis, by McKinney, Jacobs, and Bloom, which is right up to date yet includes fascinating literary quotes, from Charles Dickens to Sir Arthur Conan Doyle. The possible role of mobile genetic elements in the emergence of new strains of cholera is briefly discussed by Rubin, Waldor, and Mekalanos. *Escherichia coli* O157:H7 and its evolution as an emerging infectious disease are considered by Whittam, McGraw, and Reid. A useful overview of group A streptococcal diseases, combined with an overview of staphylococcal toxic shock syndrome, is given by Musser and Krause and followed by a scholarly account of Lyme disease by Allen Steere. Finally, Davies and Webb devote nearly 40 pages to a discussion of the emergence of antibiotic resistance in bacteria.

There are six chapters on viral diseases, beginning with Robert Webster on influenza, the classic pandemic disease threat. Webster's review provides a remarkably current description (up to mid-1997) of what we know about influenza pandemics and their origins, including a discussion of the first H5N1 influenza case in a human in Hong Kong. The emergence of dengue and the complexities of dengue hemorrhagic fever and dengue shock syndrome are reviewed by Holmes, Bartley, and Garnett of Oxford University, with a strong emphasis on the epidemiologic aspects. This discussion is followed by an authoritative review of the AIDS epidemic by Quinn and Fauci, who include sobering predictions of future epidemics in Asia and Africa.

A short chapter on hantavirus by Nathanson and Nichol is followed by a searching account of Ebola virus emergences, including fascinating speculations on their possible origin, by Murphy and Peters. The final chapter, related to virus diseases, by Tabachnick, considers arthropod-borne pathogens and is dedicated to George Craig, a leader in the field of vector biology.

Two chapters are devoted to emerging parasitic diseases. Adel Mahmoud reviews *Giardia, Cryptosporidium, Isospora*, and *Cyclospora* organisms, whose role in human diseases has only recently been recognized. Karen Day of Oxford University discusses malarial infection and disease and the factors that have led to the current world in which the effects of malaria in many regions are the same or worse than at the turn of the century.

Finally, a chapter on transmissible spongiform encephalopathies by Hope brings us up to 1996 when new variant Creutzfeldt-Jakob disease (bovine spongiform encephalopathy agent in humans) was first recognized.

Emerging Infections sets a high standard for future volumes in this series. Nicely produced, it is recommended reading for everyone with an interest in infectious diseases and in strategies for research, understanding, and control of the complex factors that lead to infectious disease emergence and reemergence.

Brian W. J. Mahy
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

CDC To Release Updated Emerging Infectious Disease Plan

Preventing Emerging Infectious Diseases: A Strategy for the 21st Century outlines new measures toward achieving emerging infectious disease prevention and control. The updated plan signals the second phase of the campaign launched in 1994 with the publication of Addressing Emerging Infectious Disease Threats: A Prevention Strategy for the United States, a collaborative effort of the Centers for Disease Control and Prevention under the leadership of the National Center for Infectious Diseases and institutions and agencies throughout the United States and abroad.

The objectives and activities in the updated plan are organized under the same four goals described in the 1994 publication: surveillance and response, applied research, infrastructure and training, and prevention and control. Nine specific priority program areas are outlined: antimicrobial resistance; foodborne and waterborne diseases; vector-borne and zoonotic diseases; diseases transmitted through blood transfusions or blood products; chronic diseases caused by infectious agents; vaccine development and use; diseases of people with impaired host defenses; diseases of pregnant women and newborns; and diseases of travelers, immigrants, and refugees.

Achieving the goals outlined in the updated plan will continue to require sustained and coordinated efforts of agencies and organizations, state and local health departments (surveillance of infectious diseases), academic centers and other federal agencies (research), health-care providers and health-care networks (guideline development and dissemination), international organizations (outbreak responses overseas), and other partners.

The executive summary of Preventing Emerging Infectious Diseases: A Strategy for the 21st Century will be released as a special issue of the Morbidity and Mortality Weekly Report on September 10, 1998. An electronic version of the full document as well as information on how to order a print copy will be available shortly afterwards at http://www.cdc.gov/ncidod/ncid.htm.

First Congress of the European Society for Emerging Infections, September 13-16, 1998, Budapest, Hungary

Founded in 1997 by human and veterinary infectious disease specialists, the European Society for Emerging Infections (ESEI) forms a European network for the study of new or emerging infectious diseases. This interdisciplinary forum was a necessity because most emerging infections are zoonoses or are linked with animal care or with animal product handling. ESEI is holding its first International Congress in the Atrium Hyatt Conference Centre, Budapest, Hungary, September 13–16, 1998. The opening lecture, "Emerging infections—an overview," will be given by Prof. Luc Montagnier. The meeting will consist of invited lectures, two free paper sessions, a roundtable discussion, and daily poster presentations. Conference topics include risk factors for emergence of pathogens, tick-borne diseases, hantavirus infections, transmissible spongiform encephalopathies, Borna disease, lyssavirus infections, and foodborne diseases. A banquet cruise on the Danube will end the Congress on Wednesday evening, September 16, 1998.

Abstracts should address one of the above topics and be submitted before the deadline of May 31, 1998. For more information, please contact ESEI President Prof. M. Granström, Microbiology, Karolinska Hospital, S-171 76 Stockholm, Sweden; fax: 46-8-30-80-99; e-mail: marta@mb.ks.se or the local organizer Dr. A. Lakos, Centre for Tick-borne Diseases, Visegradi 14, H-1132 Budapest, Hungary, fax: 36-1-349-49-26, e-mail: alakos@helka.iif.hu.

Foodborne Illness: A Disease for All Seasons, October 27 and 28, 1998, Newark, Delaware

Sponsored by the Public Health Laboratories of Delaware, Maryland, New Jersey, and Pennsylvania and the National Laboratory Training Network, Eastern Office, this seminar will provide up-to-date information on changes in epidemiology in foodborne diseases, emerging infectious organisms, proper food and clinical specimen collection and testing, and strategies to

decrease foodborne illness. Speakers will represent the Centers for Disease Control and Prevention, the Food and Drug Administration, Minnesota Department of Agriculture, Minnesota Department of Public Health, and the University of Maryland.

For more information, contact Christine Ford, National Laboratory Training Network, Eastern Office, Delaware Public Health Laboratory; tel.: 302-653-2841; fax: 302-653-2844; e-mail: ford115w@cdc.gov.

December 1998 International Conference on Antiretroviral Therapy, St. Thomas, West Indies

The International Medical Press will sponsor the International Conference on the Discovery and Clinical Development of Antiretroviral Therapies from December 13-17, 1998. In addition to plenary talks from invited speakers, the conference will feature oral presentations based on selected abstracts and scientific poster sessions. Topics include drug design and discovery, chemistry and preclinical development, pharmacology, virology and drug resistance, and clinical development (phase I/II/III and novel combination therapies).

Registration is limited, and preference will be given to those delegates who submit an abstract. For further information, contact the International Medical Press; tel: 404-233-6446; fax 404-233-2827; e-mail: ICDCD@intmedpress.com; or Website: http//:www.intmedpress.com/ICDCD.

Erratum

Vol. 4, No. 2

In the article, "Accommodating Error Analysis in Comparison and Clustering of Molecular Fingerprints, by H. Salamon, M.R. Segal, A. Ponce de Leon, and P.M. Small, in Table 1 on page 162, mean kilobases for H37Rv band 12 should be 0.936.

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 and French translations of some articles can be accessed through the journal's homepage at www.cdc.gov/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).

Instructions to Authors

Manuscript Preparation

Follow "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Ann Int Med 1997:126[1]36-47) (http://www.acponline.org/journals/resource/unifreqr.htm).

Begin each of the following sections on a new page and in this order: title page, abstract, text, acknowledgments, references, tables, figure legends, and figures.

Title page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Also provide address for correspondence (include fax number and e-mail address).

Abstract and key words. Avoid citing references in the abstract. Include up to 10 key words; use terms listed in the Medical Subject Headings from Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Type only on one side of the paper and number all pages, beginning with the title page. Indent paragraphs 5 spaces; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use Courier font size 10 and ragged right margins. Italicize (rather than underline) scientific names when needed.

Electronic formats. For word processing, use WordPerfect or MS Word. Send graphics in either (TIFF), or .EPS (Encapsulated Postscript) formats. The preferred font for graphics files is Helvetica. Convert Macintosh files into one of the suggested formats. Submit slides or photographs in glossy, camera-ready photographic prints.

References. Follow the Uniform Requirements style. Place reference numbers in parentheses, not in superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title in full. List the first six authors followed by "et al."

Tables and figures. Create tables within the word processing program's table feature (not columns and tabs within the word processing program). For figures, use color as needed; send files, slides, photographs, or prints. Figures, symbols, lettering, and numbering should be clear and large enough to remain legible when reduced. Place figure keys within the figure.

Units of measurement. Consult Uniform Requirements.

Abbreviations. Use abbreviations sparingly. Spell out a term the first time it is used.

Manuscript Submission

Include a cover letter verifying that the final manuscript has been seen and approved by all authors.

Submit three copies of the original manuscript with three sets of original figures and an electronic copy (on diskette or by e-mail) to the Editor, Emerging Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd., MS C-12, Atlanta, GA 30333, USA; e-mail eideditor@cdc.gov.

Types of Articles

Perspectives: Articles in this 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 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: This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging 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: These brief articles are updates on infectious disease trends and research. The articles include descriptions of new methods for detecting, characterizing, or subtyping new 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) need not be divided into sections. Provide a short abstract (50 words); references, not to exceed 10; figures or illustrations, not to exceed two; and a brief biographical sketch.

Letters: This section includes letters that give preliminary data or comment on published articles. Letters (500 to 1,000 words of text) should not be divided into sections, nor should they contain figures or tables. References (not more than 10) may be included.