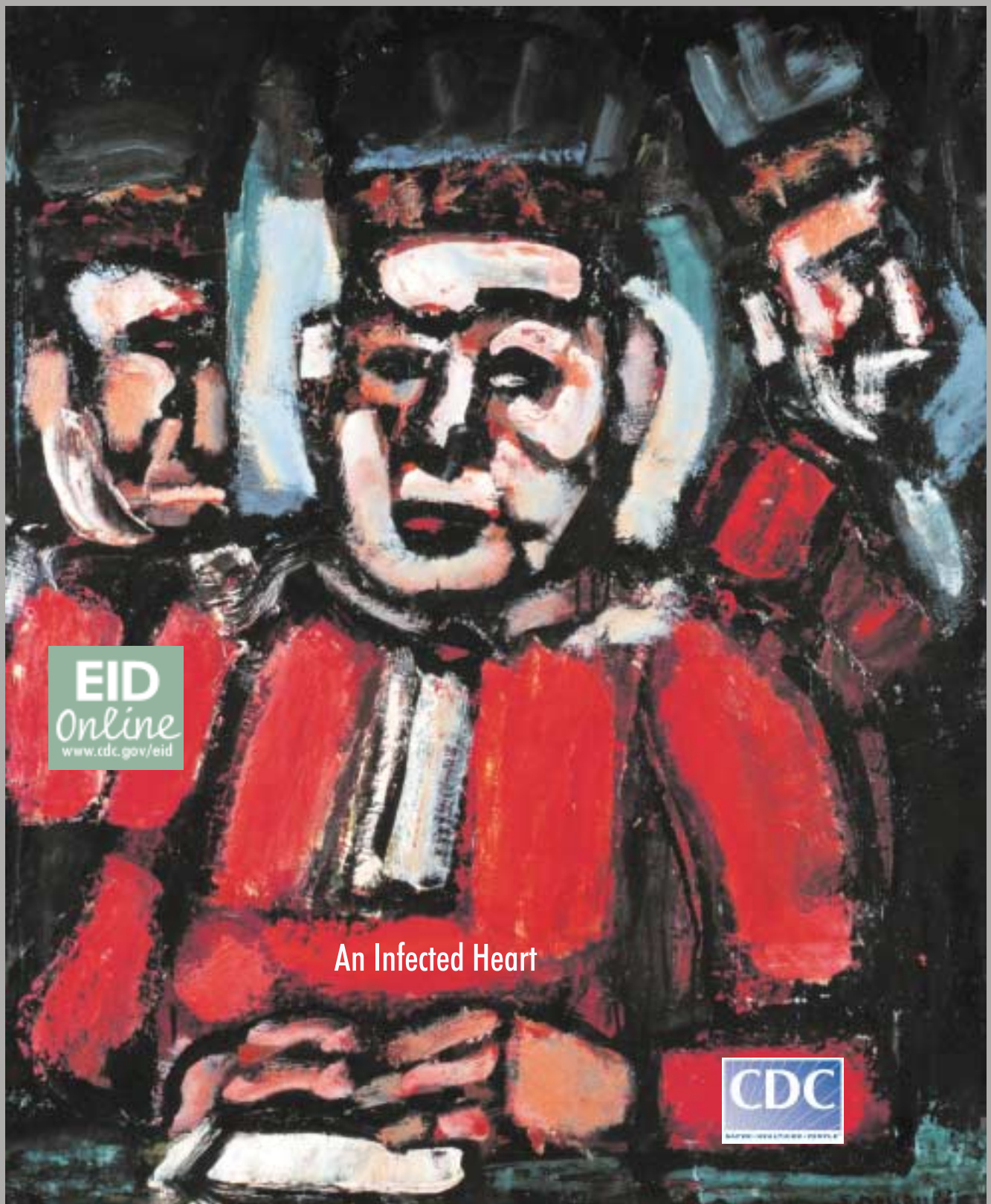
EMERGING INFECTIOUS DISEASES

A Peer-Reviewed Journal Tracking and Analyzing Disease Trends Vol.7, No.3, Supplement 2001



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Emerging Infectious Diseases

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Cover: Les Trois Juges (ca. 1936) by Georges Rouault.

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About the International Conference on Emerging Infectious Diseases 2000

D. Peter Drotman,* Harold W. Jaffe,* Charles A. Schable,* Lori Feinman†
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Why would more than 2,000 epidemiologic, clinical, laboratory, veterinary, and other public health professionals from more than 70 nations gather in a very hot July in Atlanta, Georgia to discuss problems that vex the world? The International Conference on Emerging Infectious Diseases (ICEID) 2000 was the occasion, and they attended the conference because they are committed to working and learning together to make the world a safer and healthier place. The mission of preventing and responding to epidemics and epizootics represents a worthy challenge and tends to draw an eclectic partnership, which is well and good, for that is exactly what is necessary to accomplish this goal. More than 50 public and private, international and federal, academic and professional, charitable and corporate, and other organizations joined the partnership.

Atlanta was once again the site for the conference, which built upon its predecessor in 1998 (1). Plans are well under way for the third ICEID in March 2002 in Atlanta. Built around 12 plenary and 18 panel sessions, ICEID 2000 included more than 100 oral presentations, 300 poster presentations, four meet-the-professor/expert sessions, special sessions on bioterrorism and newsmedia coverage of health stories, an opening session that featured speakers James Hughes, from the Centers for Disease Control and Prevention, Senator Bill Frist, David Heymann, from the World Health Organization, Enriqueta Bond, from the Burroughs Wellcome Fund, and George Lundberg, editor-in-chief of Medscape, along with a closing session on West Nile virus encephalitis. Clearly, the amount of information provided was more than most participants could absorb. To assist them, as well as their colleagues around the world who could not attend, ICEID 2000 has been made available on the Internet. Audio and visual access is available for most of the plenary sessions, many of the panel sessions, and the opening and closing general sessions. Many PowerPoint slide presentations, graciously donated by the presenters, are also posted. (http://www.cdc.gov/iceid/)

Coincidentally, the same week that ICEID 2000 took place, the British Medical Journal published an article in its ongoing series on medical careers on communicable disease control, calling it "arguably the most successful specialty of all" (2). ICEID attendees and readers of Emerging Infectious Diseases can certainly relate to the list of pros and cons Dr. Sarah Woodhouse listed in the article:

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Advantages

- Dynamic nature of work. Communicable diseases are constantly adapting and evolving.
- Diversity. Disease control is both reactive and proactive work.
- Multidisciplinary. Health professionals have increasing opportunities to work in teams with a variety of other professionals.
- Flexible. The on-call commitment rarely interferes with normal activities.
- Opportunities for career development. Regional epidemiology work, research, and teaching are just a few examples of professions relating to communicable diseases.

Disadvantages

- Communicable disease control function is still underresourced in many areas.
- The role of health-care workers in disease prevention is often poorly understood by medical and other colleagues.
- Communicable disease control offers limited opportunities for lucrative private work.

Many persons in the public health community can clearly see how these issues influence the local and global effots to prevent infectious disease emergence and reemergence. We may, in part, be victims of our own recent and past successes. If the price of liberty is eternal vigilance, similarly the price of a world free of plagues is eternal surveillance and appropriate response. Perhaps the most important lessons from ICEID 2000 are that there is no reason for any of us to relax our efforts and that the need for ICEID will continue for a long time to come.

Acknowledgments

The organizers of the International Conference on Emerging Infectious Diseases 2000 thank Carol Snarey, Kelly Holton, and Margaret Songe for their assistance in editing the conference presentations.

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Emerging Infectious Diseases: A CDC Perspective

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

Dr. Jeffrey Koplan, Director of the Centers for Disease Control and Prevention (CDC), has set four goals for the agency to accomplish. Each is directly related to issues being discussed at the 2000 ICEID conference. The first goal is to strengthen the science base for public health action. The second goal is to collaborate with healthcare partners for disease prevention; it is essential that individuals in clinical and academic medicine work closely with colleagues in public health to address these issues. The third goal is to promote healthy living for people at every stage of life. Finally, and very importantly to participants in this conference, the fourth goal for the agency is to work with partners to improve global health.

An article in Morbidity and Mortality Weekly Report in 1999 contains a summary of progress made in infectious disease control in the United States during the 20th century when the number of deaths resulting from infectious diseases decreased dramatically (1). However, the dramatic spike in the number of deaths from 1918 to 1919 resulting from the first of three influenza pandemics, is clearly evident (Figure 1). In addition, the number of deaths caused by infectious diseases increased between 1980 and 1995. Because of the excellent progress made against infectious diseases during much of the 20th century, many people felt that the problem of infectious diseases had been sufficiently addressed. Nearly 40 years ago, Sir MacFarlane Burnett wrote, "One can think of the middle of the twentieth 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" (2). This quotation reveals the complacency that has existed since and goes a long way toward explaining why we have gotten behind both nationally and globally in terms of capacity required to deal with the problems of infectious diseases.

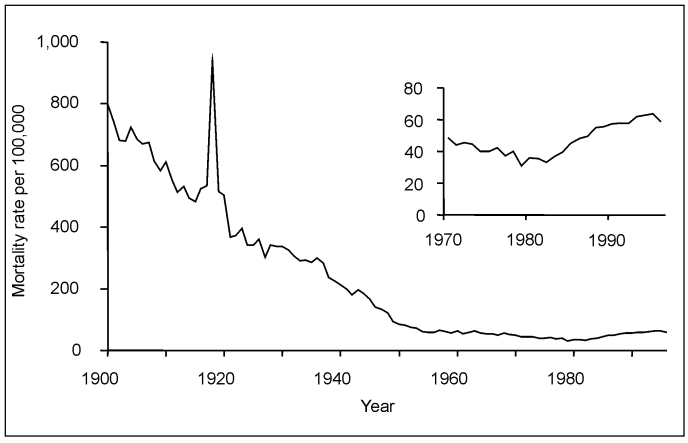

Figure 1. Trends in Infectious Diseases Mortality, 1900-1996. Deaths resulting from infectious diseases decreased markedly in the United States during most of the 20th century. However, between 1980 and 1992, the death rate from infectious diseases increased 58%. The sharp increase in infectious disease deaths in 1918 and 1919 was caused by an influenza pandemic, which killed more than 20 million people.

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The current problems we face as a result were highlighted in a very important 1992 Institute of Medicine (IOM) report, Emerging Infections: Microbial Threats to Health in the United States (3). This seminal work represents the effort of an expert committee cochaired by Dr. Joshua Lederberg and Dr. Robert Shope. This committee defined emerging infections as "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."

The committee also identified six major factors that contribute to disease emergence and reemergence: 1) changes in human demographics and behavior, 2) advances in technology and changes in industry practices, 3) economic development and changes in land-use patterns, 4) dramatic increases in volume and speed of international travel and commerce—movement not only of people but of animals, foodstuffs, and other commodities, 5) microbial adaptation and change (a factor that makes infectious diseases unique and particularly challenging), and 6) breakdown of public health capacity required for infectious diseases at the local, state, national, and global levels. In most cases, more than one of these factors are applicable to the emergence or reemergence of an individual disease or syndrome.

The IOM report contains 15 recommendations, many of which we felt were directed specifically to CDC. We responded to that report by developing a CDC Emerging Infections Plan, issued in 1994 (4), and an updated version, published in 1998 (5), that outlines a strategy for CDC to work with many partners throughout the country and around the world to address these issues. The plan contains four goals. The first emphasizes the need to strengthen infectious disease surveillance and response; this approach is necessary to ensure timely detection and control of diseases and their agents. Second, many research issues raised by these challenges need to be addressed. Third, the public health system is in urgent need of repair so that it can deal with these issues; the CDC strategy emphasizes the training needs associated with human resource development, an important goal of this conference. The final, ultimate goal stresses the need to strengthen prevention and control programs locally, nationally, and globally.

This conference has several dominant themes. The first is antimicrobial resistance. The IOM has maintained a strong

interest in emerging infectious disease control by recently issuing a report by an ongoing forum on antimicrobial resistance (6). Foodborne disease and food safety is another prominent theme. A number of presentations include data from a national surveillance network, the National Molecular Subtyping Network for Foodborne Disease Surveillance (PulseNet, Figure 2), which represents the vision for modern infectious disease surveillance (7).

Electronic linkages of individuals at local, state, and national levels who are utilizing modern molecular epidemiologic techniques in public health laboratories are absolutely essential to ensure the rapid identification of emerging foodborne diseases. This approach needs to be expanded beyond foodborne disease and linked with healthcare facilities and clinical laboratories to integrate our infectious disease surveillance systems. PulseNet represents a partnership between CDC, the Food and Drug Administration, the U.S. Department of Agriculture, the Association of Public Health Laboratories, and many individual state public health laboratories throughout the country.

This conference also emphasizes the global nature and scope of infectious diseases. Another recent IOM report acknowledges this point and concludes, "Distinctions between domestic and international health problems are losing their usefulness and often are misleading" (8). Before 1999, West Nile virus had never been found in the Western Hemisphere, though it was a well-recognized cause of disease in Africa, Europe, and the Middle East. Recent experience reinforces the need to address not only surveillance of and capacity to respond to vectorborne diseases, but also the importance of research on infectious diseases that exist in other parts of the world.

In the 8 years since the IOM Emerging Infections report was published, CDC has collaborated with Dr. David Heymann and his colleagues at the World Health Organization (WHO), along with many other individuals in many countries around the world, to deal with a number of infectious disease outbreaks. Lessons from this experience consistently emphasize the importance of infectious disease surveillance, the ability to rapidly conduct an epidemiologic investigation, and the need for trained staff and modern laboratory facilities to diagnose these diseases accurately and rapidly. Many outbreaks have reminded us of the disruption of travel and commerce that can occur when local outbreaks have global implications.

The West Nile encephalitis outbreak reinforces these lessons: that all of us need to keep an open mind about possible causes of a particular infectious disease outbreak; that clinicians and public health workers need to collaborate closely; and that people involved in human medicine and human public health need to interact on a more regular basis with colleagues in veterinary medicine and veterinary public health. State public health veterinarians have an important role in this regard. The experience with West Nile encephalitis also highlights the necessity of developing public

Figure 2. The National Molecular Subtyping Network for Foodborne Disease Surveillance. Area Lab Service and Support Zones.

health laboratory capacity and of continuing to invest in training of young people in disciplines such as entomology and wildlife biology, and it also reveals a number of critical communication issues that such outbreaks raise.

We have been very pleased during the past few years to work with the Association of Public Health Laboratories to increase CDC's role in training public health laboratory scientists. We have done this, in part, through an Emerging Infectious Diseases Laboratory Fellowship Program that initially had a domestic focus, but, with the support of Eli Lilly and Company and the CDC Foundation, now includes an international track to bring scientists from other countries to work with us at CDC or with colleagues in state public health department laboratories to acquire critical public health laboratory skills.

The West Nile virus outbreak also provides a vivid reminder that we need to consider the possibility that a complex infectious disease outbreak may result from bioterrorism. Preparing for this possibility will strengthen the national and global ability to address emerging and reemerging infections. In an address at the National Academy of Sciences in 1999, President Clinton said, "These cutting edge efforts (focused on bioterrorism preparedness) will address not only the threat of weapons of mass destruction but also the equally serious danger of emerging infectious diseases" (9). The future is hard to predict, but we can be pretty certain that we are going to continue to be challenged by the problem of antimicrobial resistance. We will eventually experience another influenza pandemic, and urban yellow fever threatens to reemerge in Latin America. Recent experience suggests that we will continue to need to deal with regional, national, and global outbreaks of foodborne disease. We are going to continue to be surprised by the range of chronic diseases that have infectious causes. Finally, we know that we are going to have to be prepared to confront the unexpected.

The intelligence community has acknowledged that infectious diseases represent a threat to national security (10). Leaders of the Group of Eight Industrialized Nations have made a commitment to substantially reduce the global burden of HIV infection, tuberculosis, and malaria by 2010 (11). This conference provides a timely opportunity for CDC and its many partners to examine lessons learned and review our commitment to rebuild national and global public health systems in order to address these three diseases as well as the numerous challenges posed by other emerging infectious diseases.

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Emerging Viral Diseases of Southeast Asia and the Western Pacific

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Over the past 6 years, a number of zoonotic and vectorborne viral diseases have emerged in Southeast Asia and the Western Pacific. Vectorborne disease agents discussed in this article include Japanese encephalitis, Barmah Forest, Ross River, and Chikungunya viruses. However, most emerging viruses have been zoonotic, with fruit bats, including flying fox species as the probable wildlife hosts, and these will be discussed as well. The first of these disease agents to emerge was Hendra virus, formerly called equine morbillivirus. This was followed by outbreaks caused by a rabies-related virus, Australian bat lyssavirus, and a virus associated with porcine stillbirths and malformations, Menangle virus. Nipah virus caused an outbreak of fatal pneumonia in pigs and encephalitis in humans in the Malay Peninsula. Most recently, Tioman virus has been isolated from flying foxes, but it has not yet been associated with animal or human disease. Of nonzoonotic viruses, the most important regionally have been enterovirus 71 and HIV.

With a few exceptions, most interest and attention regarding emerging viral diseases in Southeast Asia and the Western Pacific have been directed at zoonotic and vector-borne diseases. However, other viral diseases have also been prominent and will be mentioned briefly. Enterovirus 71, for example, one of the common causes of hand, foot, and mouth disease, has caused a number of regional epidemics that have included cases of encephalitis. It is also not possible to discuss emerging viruses without briefly referring to HIV infection and AIDS in the region.

Several reviews have described the emergence of viruses in one or more countries in the region (1-8). Building on these earlier papers, this article will provide an up-to-date summary of the major viruses and their recent outbreaks (Figure).

Vector-borne Viral Disease Agents

Dengue viruses and Japanese encephalitis (JE) viruses are the major vector-borne disease agents in the Asia-Pacific region, with Ross River, Chikungunya, and Barmah Forest viruses important in relatively restricted geographic areas. Dengue viruses cause frequent epidemics throughout the region and are endemic in a number of countries, including Indonesia, Papua New Guinea, Malaysia, Thailand, Cambodia, and Vietnam. Epidemic activity in northeastern Australia and the Pacific island nations is the result of reintroductions by viremic travelers (8). This report will focus

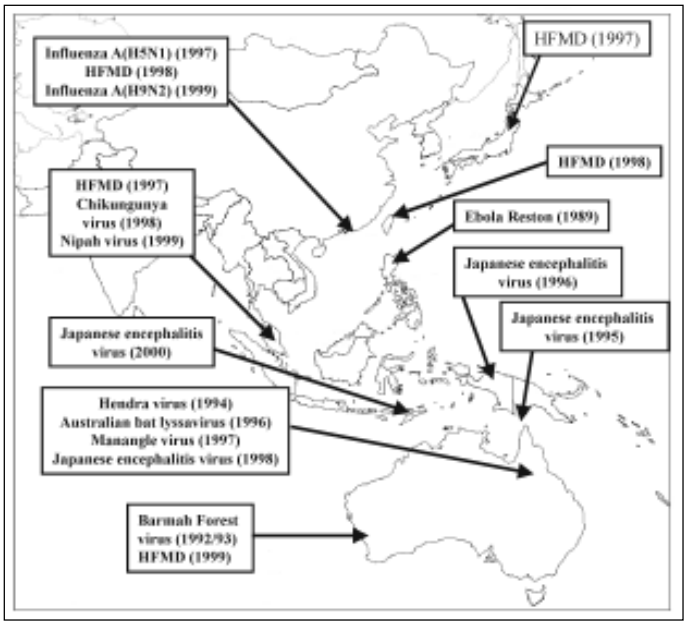

Figure. Outbreaks of viral diseases in Southeast Asia and the Western Pacific over the past decade.

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on JE virus, with short descriptions of the Ross River, Chikungunya, and Barmah Forest viruses.

JE Virus

JE virus is endemic throughout much of Southeast Asia (9), but, with the exception of serologic evidence of activity on Lombok Island and a single isolate from Flores Island (10), it was not known to occur in the Australasian zoogeographic region. However, it emerged in the Torres Strait of northern Australia in 1995 to cause three cases of encephalitis on Badu Island, two of which were fatal (11,12). Ten isolates of JE virus were obtained, two from human serum specimens drawn from subclinical infections and eight from *Culex annulirostris* mosquitoes. Subsequent seroepidemiologic studies showed that JE virus had existed in the Daru area of the Western Province of Papua New Guinea since at least 1989 and that it was spreading rapidly in several provinces. Virus activity has been observed in the northern Torres Strait every year since 1995, except 1999. In 1998, another case of encephalitis occurred on Badu Island (13), and widespread seroconversions were found in sentinel pigs. A total of 43 virus isolates were obtained from *Cx. annulirostris* and one from *Aedes vigilax* mosquitoes on Badu Island.

Virus activity also spread southward into northern mainland Australia; the first clinical case was seen in a fisherman who contracted the infection at the mouth of the Mitchell River in southwestern Cape York. Serologic evidence of infection in pigs indicated that transmission cycles had occurred in two local communities, but no other JE infections were noted in community residents (13). JE virus activity was also observed in northern Cape York in various communities near Bamaga; seroconversions had occurred in sentinel pigs, and JE virus was isolated from three of the pigs. Once again, no evidence of subclinical human infections was found in communities near Bamaga. No isolates of JE virus were obtained from pools of *Cx. annulirostris* collected at various sites in Cape York, even though the number of mosquitoes processed was equal to the number of those from Badu Island (14). The epidemic activity of JE virus on Badu Island in 1995 had been driven by the very close proximity of domestic pigs, mosquito breeding sites, and human habitation (11), and this almost certainly was true again in 1998.

After the 1998 case, a new communal piggery was constructed about 3 km from the community. In 2000, further JE virus activity was found on Badu Island with sentinel pig seroconversions, three of which yielded virus isolates; one virus isolate was obtained from *Cx. gelidus* mosquitoes, but no human cases occurred. The absence of human cases may have been due to the widespread use of JE vaccine, which contained inactivated virus, in the central and northern Torres Strait islands and may also have been associated with the move of domestic pigs away from backyards to the communal piggery. The isolation of virus from *Cx. gelidus*, a major vector of JE virus in Southeast Asia, was particularly important because this species of mosquito had not previously been recognized in Australia (it had earlier been identified as the closely related *Cx. vicinus*) (15). Recently, however, *Cx. gelidus* has become established at a number of sites across northern Australia (P. Whelan, S.A. Ritchie, unpub. data). This reinforced concern about the potential for the virus to spread across Australia where suitable vectors and vertebrate hosts are plentiful (16,17).

The role of marsupials as possible vertebrate hosts remains to be determined. However, some preliminary bloodmeal studies indicate that the most prevalent mosquito vector, *Cx. annulirostris*, prefers to feed on wallabies and other marsupials rather than feral pigs (A. Van Den Hurk, pers. comm.). Under experimental conditions, wallabies do not appear to be viable hosts because of the low level of viremia elicited by JE infection (P. Daniels, unpub. data).

JE virus occurs widely in the Western Province of Papua New Guinea as evidenced by virus isolations taken from *Cx. sitiens* group mosquitoes; a number of clinical cases of encephalitis, and seropositive humans and pigs over a wide area (17-19). Serologic evidence also suggests that JE virus has spread from the Western Province into the Southern Highlands and Gulf Provinces (17) and that it has emerged in the West Sepik Province in the north and been responsible for outbreaks of encephalitis on Normanby Island and at Alatau in Milne Bay Province in the eastern part of the country. A probable human case of JE infection has also been reported from Irian Jaya (20), and antibodies to JE have been found in human serosurveys there (21). The rapidity with which JE virus has spread through Papua New Guinea places some nearby Pacific nations, such as the Solomon Islands and Vanuatu, at risk.

Molecular phylogenetic studies have clearly demonstrated that the JE virus that spread into the Torres Strait in 1995 originated in Papua New Guinea. Indeed, all JE virus isolates from the 1995 incursion, as well as those from Cape York and the Torres Strait in 1998, were almost identical to each other and to three isolates from Papua New Guinea and were most closely related to JE strains from Malaysia, southern Thailand, and Indonesia (unpub. data) (13,15,17,19,22). In addition, these viruses shared an 11-base deletion in the 3' untranslated regions (UTR) immediately downstream from the termination codon of the virus's single open-reading frame (23). The virus isolates obtained from pig sera and from *Cx. gelidus* mosquitoes collected on Badu Island in 2000, however, showed considerable variation from previous isolates. Although they retained the 11-base deletion in the 3′ UTR, their nucleotide sequences differed markedly in the prM gene, E gene, and the NS5-3UTR regions of the genome. The Badu 2000 isolates appear to be phylogenetically more closely related to viruses from Cambodia, northern Thailand, and Korea (A.T. Pyke, D.T. Williams, D.J. Nisbet, A.F. van den Hurk, C.T. Taylor, C.A. Johansen, unpub. data).

The direction and mechanism of the spread of JE virus from the oriental zoogeographic zone to the Australasian zoogeographic zone remain unknown (10,17). The most likely mechanism, however, is the gradual spread in mosquito-bird and mosquito-pig transmission cycles across the eastern Indonesian archipelago from Bali in the west to Irian Jaya in the east. In support of this theory, antibodies to JE virus were found in sera taken from pigs at various sites, including Timor and Jayapura (24), and the very recent serologic diagnosis of clinical cases of JE in Timor (L. Hueston, unpub. data). Computer simulation suggests that low pressure systems west of the Torres Strait/Cape York area produce strong northerly winds that could carry infected mosquitoes from the

New Guinea mainland to the Torres Strait and Cape York Peninsula (S.A. Ritchie, W. Rochester, unpub. data).

Barmah Forest Virus

Barmah Forest (BF) virus is an alphavirus that is enzootic to Australia. It circulates among mosquitoes and terrestrial animals, especially certain marsupial species (25,26) and causes an epidemic polyarthritis-like disease known as Barmah Forest virus disease (27,28). Human infection with BF virus has been recognized since 1986 and its incidence has increased since then, at least partly as a result of greater clinical awareness and availability of diagnostic reagents. From its endemic foci in northern and eastern Australia, the virus has spread into other geographic areas during the past decade, causing epidemics in north, northwest, and southwest Australia and recently in southeast Australia (28-30), establishing a low level endemic pattern with a relatively stable number of reported cases since BF disease became reportable in Australia in 1995 (Table). However, human disease due to BF virus in southern Australia or Tasmania has not yet been confirmed.

Ross River Virus

Ross River (RR) virus causes an epidemic polyarthritis (27,28), and, to avoid confusing it with other viruses that cause similar symptoms, the disease is now referred to as Ross River virus disease. It is found in all Australian states and territories, as well as Papua New Guinea. Serologic evidence indicates that it also occurs in the Solomon Islands. The concept that RR virus is an emerging disease is somewhat difficult to justify, however. Although the number of reported cases of RR virus disease has increased slightly during the past decade (Table), this increase can be largely attributed to a combination of improved diagnostic reagents, greater awareness by clinicians, a trend towards new housing developments in coastal regions adjacent to salt marsh wetlands, and changing demographics as people migrate northward to warmer climates in retirement. However, RR virus clearly has the potential to spread, as demonstrated by the very extensive outbreak in Pacific Island nations in 1979-80 (25).

Chikungunya Virus

Chikungunya virus was relatively common in southern and southeastern Asia in the 1960s. After causing outbreaks in India, Sri Lanka, Burma, and Thailand, it all but disappeared in India, Sri Lanka, Burma (31), and Bangkok (32). However, localized outbreaks and sporadic cases continued in Burma, Thailand, and the Philippines in the 1980s. In addition, the virus spread into Indonesia for the first time from 1982 to 1985, with outbreaks in South Sumatra, Java, and West Kalimantan (1982); southern, eastern, and central Kalimantan (1983); southern Sulawesi (1993); eastern Timor and eastern Nusatengarra (1984); Mollucas Islands (1985); North Sulawesi (1985); and Irian Jaya (1985 to 1986) (33). Outbreaks occurred in Thailand in 1995 (34) and Malaysia in 1998 to 1999 (S.K. Lam, K.B. Chua, D.W. Smith, unpub. data). The latter was the first outbreak to be recorded in Malaysia, although in the 1960s, antibody to Chikungunya virus was relatively common in the people of Malay Peninsula and Sarawak. The outbreak involved 51 confirmed cases in a densely populated, urban area near Kuala Lumpur. The major symptoms were fever (2 to 5 days), transient maculopapular rash on the trunk and limbs (2 to 3 days), and severe back pain. About 80% of patients had some form of joint symptoms, either arthralgia or arthritis, involving the small joints of hands and feet.

Emerging Zoonotic Viruses

A number of viruses have emerged from fruit bats (flying foxes), particularly members of the genus *Pteropus*, over the past 6 years. These viruses include Hendra and Nipah, two members of a new genus within the Paramyxoviridae; Menangle and Tioman viruses, two new members of the *Rubulavirus* genus in the family Paramyxoviridae; and Australian bat lyssavirus, a member of the *Lyssavirus* genus in the family Rhabdoviridae, closely related to classic rabies virus.

Hendra Virus

In September 1994, a sudden outbreak of an acute respiratory syndrome occurred among thoroughbred horses in a training complex in Brisbane, Australia; 13 horses and their trainer died. The causal agent, a previously undescribed member of the family Paramyxoviridae, was initially named equine morbillivirus (35), but was renamed Hendra virus (after the Brisbane suburb where the outbreak occurred). A second (apparently unrelated) outbreak resulted in the death of two horses and their owner near Mackay, nearly 1000 km north of Brisbane(36-38). The outbreak preceded the events at Hendra and was retrospectively identified in 1995. Most recently, a single fatal equine case occurred near Cairns in North Queensland in January 1999 (39,40).

To evaluate the theory that Hendra virus existed in a wildlife reservoir, serologic surveillance of wildlife species was undertaken, and, in April 1996, anti-Hendra virus antibodies were identified in a black flying fox (*Pteropus alecto)*. Within weeks, evidence of infection was found in the other three species of Australian flying foxes; gray-headed flying fox (*P. poliocephalus*), little red flying fox (*P. scapulatus*), and spectacled flying fox (*P. conspicillatus*) (41). In 1996, a Hendralike virus was isolated from the reproductive tract of a seemingly healthy, pregnant gray-headed flying fox. A range of tests showed the bat isolate to be indistinguishable from

Table. Annual case notifications of Ross River (RRV) and Barmah Forest (BFV) virus infections, 1991–1999

	1991	1992	1993	1994	1995	1996	1997	1998	1999
RRV	3,352	5,630	5,428	3,960	2,602	7,823	6,686	3,094	4,407
BFV	N/A	N/A	N/A	N/A	736	837	704	558	628

N/A: not available (BFV was not made a notifiable disease until 1995).
Communicable Diseases Network—Australia, New Zealand—National Notifiable Diseases Surveillance System, pers. comm.

the Hendra virus isolated from horses (42). However, no evidence of illness exists in flying foxes infected naturally (K. Halpin, unpub. data) or infected experimentally (43,44) that can be attributed to infection with Hendra virus, supporting epidemiologic evidence (H.E. Field, unpub. data) that flying foxes are the probable hosts of Hendra virus.

Hendra virus does not appear to be very contagious, and there has been no evidence of infection in humans even in those who have had close contact with injured bats (45). Transmission from flying foxes to horses has not been demonstrated; however, studies done on different species infected experimentally and flying foxes and horses infected naturally have indicated possible modes of transmission. Virus has been isolated from the kidney, urine, and (less so) oral cavity of horses and from the kidney and urine of cats experimentally infected with Hendra virus. Horses have been experimentally infected by the naso-oral route, and cat-to-cat transmission and suspected cat-to-horse transmission have been reported (43,46).

Biologically and genetically, Hendra virus differs significantly from other members of the Paramyxoviridae family. They show morphologic differences, seen in two distinct lengths of surface projections (47), and genetic differences, demonstrated by the genome size. The genome is longer (18,234 nucleotides) than those of members of the *Respirovirus* and *Morbillivirus* genera because it has longer intergenic noncoding sequences and a larger L protein gene (48). These differences, together with limited homology to other members of Paramyxoviridae, indicate that Hendra virus should be classified as the first member of a new genus in this family; the name *Henipavirus* has been suggested (48).

Nipah Virus

A major outbreak of disease in pigs and humans in the Malay Peninsula from September 1998 to April 1999 resulted in 265 infected persons, 105 of whom died (49), and the eventual destruction of about 1.1 million pigs. The disease in pigs was highly contagious and symptoms included acute fever, respiratory problems, and neurologic signs in infected pigs of all ages. The predominant clinical syndrome in humans was encephalitic rather than respiratory, with clinical signs including fever, headache, myalgia, drowsiness, and disorientation, sometimes proceeding to a coma within 48 hours (50,51). Most infected persons had a history of direct contact with live pigs, and most were pig farmers. Epidemiologic evidence suggested that the disease had been spread primarily by pigs that were transported between farms or to other regions. The primary mode of transmission on pig farms was believed to be through the respiratory route, and this was subsequently confirmed with experiments (52). Investigations have revealed that the virus has caused disease in pigs in Peninsular Malaysia since late 1996. Eleven cases of encephalitis and pneumonia resulting from Nipah virus infection also occurred in Singapore during the outbreak in Malaysia; one abattoir worker who worked on pigs imported from Malaysia died (53).

Preliminary research on the new virus, subsequently named Nipah virus, revealed that it had ultrastructural, antigenic, serologic, and molecular characteristics similar to Hendra virus (49). Molecular studies confirmed that Nipah virus was closely related to Hendra virus, with specific genes sharing 70% to 88% nucleotide homologies and 67% to 92% amino acid homologies, and with identical intergenic regions and nearly identical gene start-and-stop sequences (54). Thus, these two viruses are members of a new proposed genus within the family Paramyxoviridae (48).

Surveillance of wildlife species for evidence of the origin of Nipah virus was an integral part of the outbreak investigation (55). Knowing the similarities between Nipah virus and Hendra virus, attention was focused on surveillance of bats. In common with most countries in Southeast Asia, Peninsular Malaysia has a great diversity of bat species: at least 13 species of fruit bat (suborder Megachiroptera), including two flying fox species, and at least 60 species of insectivorous bats (suborder Microchiroptera) (56).

Antibodies that neutralize Nipah virus were found in 21 bats from five species (four species of fruit bat, including two flying fox species and one insectivorous species) (J. M. Yob, H.E. Field, unpub. data). Cross-neutralization of Nipah antigen by antibodies to Hendra virus was excluded as the cause of reactivity. Attempts to detect the virus in sera using both culture and amplification of RNA in reverse transcriptase-polymerase chain reaction were unsuccessful. However, Nipah virus has recently been isolated from the urine of flying foxes (K.B. Chua, S.K. Lam, unpub. data).

Menangle and Tioman Viruses

A previously undescribed virus, Menangle virus, was isolated from stillborn piglets with deformities at a large commercial piggery in New South Wales (57). The virus was responsible for a reduced farrowing rate and for causing the stillbirths with deformities. The affected stillborn piglets frequently showed severe degeneration of the brain and spinal cord, arthrogryposis, brachygnathia, and, occasionally, fibrinous body cavity effusions and pulmonary hypoplasia. Virus was isolated from lung, brain, and heart tissues of infected piglets and shown to be morphologically similar to viruses in the family Paramyxoviridae. No disease was seen in postnatal animals of any age, but a high proportion of serum specimens (>90%) collected from animals of all ages contained high titers of antibodies that neutralized the virus. Phylogenetic studies with nucleotide sequences generated from cDNA of Menangle virus showed that the virus was a member of the *Rubulavirus* genus within the family Paramyxoviridae and unrelated to any other virus known to infect pigs. Convalescent-phase serum samples from two persons who worked on pigs were found to have high titers of antibodies that neutralized the new virus. Both workers had an influenzalike illness with a rash during the pig outbreak, and extensive serologic testing showed no evidence of any alternative cause. Therefore, the illness was likely caused by the Menangle virus (58).

Notably, a large breeding colony of gray-headed and little red flying foxes roosted within 200 m of the affected piggery. In a preliminary study, 42 of 125 serum samples collected from the bats had antibodies that neutralized the new virus. In addition, antibodies were found in sera collected in 1996, before the outbreak, and from a colony of flying foxes 33 km from the piggery (57). Thus, flying foxes were likely the primary hosts of the virus that caused the outbreak. All other sera collected from a variety of wild and domestic animals in the vicinity of the affected piggery tested seronegative for the virus.

The search for the natural host of Nipah virus led to the discovery of another new member of the Paramyxoviridae

family, Tioman virus, which was isolated from the urine of flying foxes (*P. hypomelanus*) and found on Tioman island off the eastern coast of the Malay Peninsula (K.B. Chua, unpub. data). Electron microscopic analysis of virus-infected cells revealed spherical and pleomorphic enveloped virus particles (100 nm to 350 nm) compatible in structure with those of viruses in the family Paramyxoviridae. Tioman virus failed to react with antibodies against a number of known Paramyxoviridae members but did cross-react in immunofluorescence tests with antisera to Menangle virus. However, antiserum to Menangle virus failed to neutralize Tioman virus. To characterize the molecular structure of Tioman virus, a cDNA subtraction strategy that isolated virus-specific cDNA from virus-infected cells was employed. Complete gene sequences for the nucleocapsid protein (N) and phosphoprotein (P/V) have been determined and recombinant N protein produced in baculovirus. The recombinant Tioman virus N and V proteins reacted with porcine antisera to Menangle virus in Western blots, confirming the serologic cross-reactivity observed during initial virus characterization. Phylogenetic analysis indicated that Tioman and Menangle viruses are closely related members of the *Rubulavirus* genus. Sequences of the nucleocapsid protein gene of the two viruses are approximately 70% identical at the nucleotide level and approximately 85% identical at the amino acid level (K.B. Chua, L.F. Wang, unpub. data). The potential of Tioman virus to cause disease in animals and humans is unknown. Its relationship with Menangle virus highlights the need to determine not only if it will replicate and cause disease in pigs, but also if pigs can act as amplifying hosts and transmit the disease to other species, as appears to be the case with Menangle virus.

Australian Bat Lyssavirus

Australian bat lyssavirus (ABLV) was first discovered in a black flying-fox bat (*P. alecto*) in Ballina in northern New South Wales that was displaying neurologic signs (59). ABLV has since been discovered in all four species of flying-fox bats (black, gray-headed, little red, and spectacled) throughout their geographic range (H.E. Field, unpub. data) and in an insectivorous bat (yellow-bellied, sheath-tail, *Saccolaimus flaviventris* species) in Queensland. The virus was antigenically similar to classic rabies virus and therefore a member of lyssavirus serotype 1, but its genetic sequence was distinguishable and was therefore ascribed a new genotype number—genotype 7 (60). Further research has shown that two closely related, but genetically distinguishable, strains of ABL occur in Australia, one in flying fox bats and the other in insectivorous bats. Researchers at the Centers for Disease Control and Prevention have found that rabies vaccine may elicit a protective immune response to ABLV (61,62), and vaccination is now offered to all those at risk of exposure. The first human case of ABLV infection occurred in 1996; a 39-year-old animal handler, who had been scratched and possibly bitten 5 weeks earlier by a yellow-bellied sheath-tailed bat, died of encephalitis (63). The second case, also manifested by fatal encephalitis, occurred in a 27-year-old woman who had been bitten by a flying fox more than 2 years previously (64). In both instances, the clinical signs were consistent with classic rabies infection. The incidence of ABLV infection in bats is unknown. In one study, about 6% of sick, injured, or orphaned bats were antibody-positive for ABLV (65). However, antibodies have also been found in apparently healthy bats (P.W. Daniels, R. Lunt, H.E. Field, unpub. data), but the role that these bats play in the ecology of ABLV, while of concern, remains to be elucidated. Virus isolations from bats, however, have generally come from animals exhibiting behavioral or neurologic signs. Most infected bats appear depressed, although some exhibit aggressive behavior (66). Histopathologic examinations of infected bats have been carried out, and in most bats the lesions found were nonsuppurative meningoencephalitic and ganglioneuritic in nature, similar to that seen in rabies, except that the number of Negri bodies was variable (65). Immunoperoxidase tests showed lyssaviral antigen was variable in intensity and distribution. Reactions did not always occur in the salivary glands, even if virus was present in the brain (67).

The finding of ABLV in Australian frugivorous and insectivorous bats has had major public health implications: people at risk for exposure must be vaccinated, and those with suspected infection must undergo expensive postexposure prophylaxis (65). Indeed, flying foxes are common in urban areas of eastern and northern Australia; many towns and cities are home to colonies of many hundreds.

Other Viral Diseases

Enterovirus 71 Infection

Enterovirus 71 (EV71) infection manifests most frequently as a mild childhood illness known as hand, foot, and mouth disease (HFMD) and is clinically indistinguishable from HFMD caused by coxsackievirus type A16 (CA16). However, EV71 has a propensity to cause severe neurologic disease during acute infection (68,69), a feature not observed in CA16 infections. Children under 4 years of age are particularly susceptible to the most severe forms of EV71-associated neurologic disease, including meningitis, brainstem or cerebellar encephalitis (or both), and poliomyelitis-like paralysis. The neurologic complications of EV71 infection may occasionally cause permanent paralysis or death.

Since 1997, several large epidemics of EV71 infection have been reported in East and Southeast Asia and Australia. The first epidemic occurred in 1997 in Sarawak (70), followed by smaller outbreaks in Singapore, Japan (71) and the Malay Peninsula (72). These outbreaks were associated with numerous cases of HFMD in young children and were accompanied by neurologic complications such as aseptic meningitis, poliomyelitislike paralysis, and cerebellar ataxia in a small number of cases. However, a syndrome of rapidly fatal neurogenic pulmonary edema and hemorrhage was also observed during these outbreaks (73,74). Thirty-four deaths occurred in Sarawak as a result of this disease (70); four deaths were reported in Kuala Lumpur (72) and three in Japan. In 1998, the largest recorded epidemic of EV71-associated HFMD occurred in Taiwan (75,76), involving the whole island, with approximately 130,000 cases of HFMD reported. There were 405 cases of severe neurologic disease and 78 cases of fatal neurogenic pulmonary edema (75). A small outbreak was also reported in Hong Kong at the same time.

The most recent large outbreak of EV71 infection occurred in Perth, Australia, in 1999 (77). Numerous cases of HFMD were reported over a 6-month period (March to August), and 29 cases of severe neurologic disease were

diagnosed. The spectrum of neurologic disease seen in Perth included aseptic meningitis, acute cerebellar ataxia, and acute flaccid paralysis; however, no cases of fatal neurogenic pulmonary edema were observed.

Before the large outbreaks of EV71 infection in the Asian-Australasian region, only one case of brainstem encephalitis and neurogenic pulmonary edema due to enterovirus 71 infection had been described (78). Several postmortem studies on those who died of neurogenic pulmonary edema have been published (70,72,79,80). In each case, disease appears to be confined to the brainstem, with histologic evidence of acute inflammatory encephalitis and isolation of EV71 or identification of EV71 antigen within neurons. These studies strongly suggest that pulmonary edema and hemorrhage are of neurogenic origin and secondary to brainstem encephalitis. These findings are supported by neuroradiologic evidence of brainstem pathology in many people who died of fulminant pulmonary edema (71,79,81).

Despite radiologic and histologic evidence of brainstem encephalitis in people who died of neurogenic pulmonary edema and immunohistochemical evidence of the direct involvement of EV71 in brainstem encephalitis, the cause of death in children who contracted the disease during the 1997 outbreak in Sarawak remains controversial. Although many children died as a result of rapidly progressive pulmonary edema (70) similar to that observed elsewhere, a clinical diagnosis of acute myocarditis was made in many cases. In addition, both EV71 and a novel group B adenovirus were isolated from specimens from sterile sites (including brain and heart) and nonsterile sites taken both before and after patients' deaths. The authors suggest that this adenovirus might have played a causative role in these fatal cases either as the primary pathogen or by interacting with EV71. Unfortunately, the data currently available in published literature do not allow a rigorous assessment of the role of adenovirus in this syndrome. Review of additional published postmortem studies done in Sarawak will be necessary to clarify this issue.

Several reports on the molecular epidemiology of recent EV71 activity in Asia have been published (82-85). Unfortunately, the data in these reports cannot be compared directly as different parts of the viral genome were analyzed in these studies. However, all three studies indicate that at least four genetic lineages of EV71 have circulated in Asia since 1997. In addition, there does not appear to be a single neurovirulent genotype associated with severe and fatal cases because three distinct genotypes have been isolated from people who died as a result of infection with EV71 in Sarawak, Peninsular Malaysia and Taiwan. The EV71 outbreak in Western Australia was caused by two distinct genetic lineages of the organism, determined by using VP1 gene-sequencing (P.C. McMinn, unpub. data). The predominant genotype, which was associated with HFMD and some cases of aseptic meningitis, was most closely related to the genotype of viruses isolated in Sarawak during 1997 (>98% nucleotide homology), suggesting a direct link between the two epidemics. The second, minor genotype, which was associated with severe neurologic disease (acute flaccid paralysis, cerebellar ataxia), was most closely related to the genotypes of EV71 strains isolated in Victoria (eastern Australia) in 1995 (>96% nucleotide homology).

Thus, EV71 activity has increased markedly in the Asia-Pacific region during the past 4 years. In addition, a new clinical manifestation of EV71 infection, a rapidly fatal syndrome of neurogenic pulmonary edema associated with brainstem encephalitis, has been identified. Molecular genetic studies of EV71 isolates have indicated that several distinct viral genotypes circulated in Sarawak, Peninsular Malaysia, Japan, Taiwan, and Western Australia between 1997 and 2000, but, unfortunately, it has not yet been possible to show an association between a particular viral genotype and the development of fatal brainstem encephalitis.

HIV Infection and AIDS

Although discussion of HIV infection and AIDS in Southeast Asia and the Western Pacific region is beyond the scope of this short review, a few comments need to be made about increasing incidence as a component of disease emergence. Two countries in the region, Cambodia and Papua New Guinea, are of particular concern because of the high and increasing incidence of HIV infection, primarily through heterosexual transmission (86). Indeed, Cambodia has the most serious HIV epidemic situation in the region, with the highest HIV infection rate in Asia—3.3% of the most sexually active population (ages 15 to 49). In Papua New Guinea, the prevalence rate is believed to be about 0.6% in the most sexually active population and growing alarmingly, but this figure refers only to the capital city, Port Moresby. Little is known of the prevalance elsewhere, although the second largest city, Lae, probably has a prevalance similar to that of Port Moresby. There is also evidence of increasing HIV infection among people who live along major highways from Lae, especially the highway to Goroka and the Highlands. In addition, because of the very high mobility of the Papua New Guinea population, people in many remote communities have contracted AIDS or HIV infection (M.P. Alpers, pers. comm.). There is also a high incidence of HIV infection among those who inject drugs and increasing heterosexual transmission of HIV in China and Vietnam.

Most emerging diseases in the Asia-Pacific region are due to either novel zoonotic viruses or to an increased incidence or geographic spread of known viruses. The importance of the emergence of novel zoonotic diseases from wildlife cannot be overemphasized. Currently, very few countries anywhere have active wildlife diseases surveillance, but it is hoped that such surveillance activities will eventually increase.

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Epidemiology, Evolution, and Future of the HIV/AIDS Pandemic

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We used mathematical models to address several questions concerning the epidemiologic and evolutionary future of HIV/AIDS in human populations. Our analysis suggests that 1) when HIV first enters a human population, and for many subsequent years, the epidemic is driven by early transmissions, possibly occurring before donors have seroconverted to HIV-positive status; 2) new HIV infections in a subpopulation (risk group) may decline or level off due to the saturation of the susceptible hosts rather than to evolution of the virus or to the efficacy of intervention, education, and public health measures; 3) evolution in humans for resistance to HIV infection or for the infection to engender a lower death rate will require thousands of years and will be achieved only after vast numbers of persons die of AIDS; 4) evolution is unlikely to increase the virulence of HIV; and 5) if HIV chemotherapy reduces the transmissibility of the virus, treating individual patients can reduce the frequency of HIV infections and AIDS deaths in the general population.

Of all the infectious diseases first recognized in the 20th century, AIDS has had not only the most profound effect on human illness and death, it ended the developed world's complacency about infectious diseases. Caused by HIV, AIDS is, as far as we know, always fatal, even with effective therapy. Within the past 50 to 100 years, HIV went from being maintained primarily, if not exclusively, in sooty mangabeys (HIV-2) and chimpanzees (HIV-1) (1-3) to being the etiologic agent of a worldwide pandemic. AIDS was not recognized as a specific disease until 1980, and HIV was not identified as the etiologic agent until 1983. Nevertheless, an estimated 16 million persons have died from AIDS worldwide with 50 million currently infected with HIV.

HIV exhibits considerable evolutionary potential and, with drug-resistant bacteria, may have done more to enhance widespread understanding of the importance of population and evolutionary biology to human health and medicine than any other example this past century. Although HIV was initially susceptible to a variety of drugs, resistance mutations have enabled the virus to skirt every drug in the biotech arsenal. In part because of this capacity for rapid evolution, developing an effective vaccine will be difficult.

In the study reported here, we used mathematical models to consider the epidemiologic and evolutionary future of the HIV/AIDS pandemic. We addressed four questions: 1) What factors contribute to the spread and limiting the spread of HIV/AIDS in human populations? 2) How long will it be before resistance to HIV infections and/or their pathology evolves in the human population? 3) Will evolution in the HIV-infected population favor an increase or decrease in the virulence of the virus? 4) What are the epidemiologic consequences of life-prolonging treatment on the incidence of HIV-infected persons and AIDS patients?

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Age of Infection (AoI) Model

To consider the epidemiologic and evolutionary future of HIV/AIDS, we developed a mathematical model for the population dynamics of HIV/AIDS. Our model is based on those typically employed by demographers and actuaries (see reference 4 for our previous publication of it in a mathematical context). Changes in the numbers of persons infected are treated as a birth and death process; the "births" are new infections, and "death" is removal of infected hosts from the population. The course of an infection in an individual is characterized by (i) how many new infections it generates at each time interval (week) since that host was first infected (the equivalent of the birth rate), and (ii) the weekly likelihood of the removal of a host from the population (the death rate). By "age," we mean the "age of infection" (AoI)—the time in weeks since that host was first infected. Within this framework, an HIV infection has a life cycle different from that of most viral and other microparasitic infections, because the onset of the disease, AIDS, occurs long after the person is infected with HIV and the microparasite has started to proliferate. Although the passage through time and progression to disease is continuous, for tradition as well as convenience it is useful to characterize an HIV infection as having four distinct stages, which are described and given parameters in Table 1.

In the numerical (computer) simulation used here, infected persons pass through the HIV/AIDS gauntlet on a weekly basis. Each week throughout stage i of the infection they cause R_i/L_i new infections (R = new infections; L = duration in weeks); these newly infected hosts then enter the gauntlet. (Note that the rate of transmission during a stage thus depends both on R_i and L_i, not just on R_i.) Each infected host continues to progress through the different stages and transmit the virus until the final week of the third stage, L_3, when the infected host dies. For more details

Table 1. Stages in the age of infection (AoI) model used in this investigation

Stage	Characteristics	Duration (weeks)	New infections[a]	Death rate
0	Establishment: virus enters and colonizes host	L_0	0	0
1	Primary infection: virus proliferates to high densities; the host seroconverts, which causes an abrupt decline in the titer of circulating virus	L_1	R_1	0
2	Asymptomatic: circulating virus remains at low levels, and disease symptoms are absent	L_2	R_2	0
3	AIDS	L_3	R_3	100% at end

[a]Total number of new infections produced by persons in this stage.

about the model see (4). Copies of the FORTRAN 77 program used for the numerical results presented here can be obtained from Bruce Levin.

HIV Dynamics

When an infectious disease is first introduced into a population, it has the greatest opportunity to spread because all hosts are susceptible. Thus, if we introduce a single infected person into a wholly susceptible population, the maximum opportunity exists for producing secondary infections, the number of which is traditionally designated as R_0 (1). Although R_0 is a measure of the potential of a disease to spread in a population, it not a measure of the rate at which that disease will spread. One way to measure the rate at which a disease spreads in a wholly susceptible host population is that used by demographers to represent the geometric expansion rate of populations, the intrinsic rate of increase, r . N persons at time 0 become Ne^{rt} persons at time t, where e is the base of the natural logarithm (if N were an amount of money, r would be the compound interest rate; in this case, r is the intrinsic rate of increase in the number of infected persons). With age structure in the model, a certain "settling out" period occurs in which the ratios of numbers of infections at different stages oscillate. As these oscillations decay, r approaches its steady state value, which can be calculated from the rate of change in any of the age categories of the AoI distribution.

The long duration of infection is important in understanding the intrinsic rate of increase of HIV and its dissemination through a population. During the epidemic phase of the disease, when there are many susceptible hosts and the number of new infections is increasing geometrically, the contribution of transmissions occurring at later stages of the infection to the spread of the virus is severely discounted (4, 5). Thus, new infections transmitted by recently infected persons, in stage 1, contribute much more to the spread of HIV than infections from persons in stage 3 (12 years later).

To illustrate this principle, let us use the AoI distribution employed in our original study (4) and assume that all transmission of the virus is confined to just one of the four stages. A rate of increase of HIV of 0.50 per year (HIV infections doubling every 1.4 years) would require (a) 1.1 secondary infections if transmission occurred solely in stage 1 (between weeks 6 and 12 after the host is infected in our example); (b) 5.2 secondary infections if transmission occurred solely during the asymptomatic period (an average of 10 years in our example); and (c) 72 secondary infections if transmission occurred solely during the period after the onset of AIDS (at an AoI between 10 and 12 years in our examples. In perhaps more familiar terms, we can assume a direct analogy between these results and the concept of compound interest; models for the spread of disease in a population are formally analogous to economic models for the growth of money in an account. An interest rate of 1% per day, compounded daily, yields an annual rate of 3,800%. In a similar manner, new infections produced during stage 1 compound themselves many times within the 10-year period during the advance to AIDS. One implication of this result is that when HIV first enters a naïve population, if transmission occurs within the first month of infection, this early transmission will drive the epidemic. Using a different model and a closer tie to real data, Jacquez, Koopman and colleagues made a similar argument that early transmission is important in driving the epidemic (6, 7). This conclusion has a number of implications, the most immediately practical of which is that public health and education procedures to control the epidemic will fail if they are based on using serologic test results to identify infected persons (6). Infected persons may well have transmitted the virus before they seroconverted.

Factors Limiting the Rate of Spread of HIV/AIDS

What limits the rate at which HIV spreads through a population? Although at least 50 million persons are infected with HIV, the human population (more than 6 billion persons) consists almost entirely of uninfected persons, and the global rate of increase in new HIV infections does not appear to have abated. However, unlike the case with influenza and measles, considerable geographic and cultural variation exists in the epidemiology of HIV/AIDS. In effect, the HIV pandemic has been largely restricted to subpopulations—risk groups within which the likelihood of infection is substantially greater than that in the population at large, e.g., gay men, injection drug users, and sex workers, their patrons, and their spouses (or other sex partners).

It seems reasonable as well as hopeful to expect that the rate of increase in new HIV infections will decline in a number of different populations. What processes can account for these declines in the incidence of new HIV infections and reductions in the rate of spread of this virus? Do they reflect the efficacy of public health measures and education programs leading to more prudent sexual and needle use behavior? Has chemotherapy reduced the transmissibility of the virus? Is evolution making these viruses less transmissible or humans less susceptible to HIV infections or both? Although it would be difficult to reject the possibility of these different factors contributing to reductions in the rate at which new HIV infections are increasing, it may well be that the dominant reason for observed declines in the rate of spread of this retrovirus lies in the progression (and confinement) of the epidemic in particular subpopulations (risk groups). The

reductions in the spread of HIV in these subpopulations could be due to the saturation of the pool of susceptible hosts in these groups rather than to successful intervention or behavioral changes (see 7).

To illustrate the effect of the saturation of susceptible hosts in risk groups, we can consider a single AoI distribution in which the duration of the four different stages (L_0-L_3) are, respectively,4, 6, 520, and 104 weeks. During each of the latter three stages, in a wholly susceptible population, each infected person produces one secondary infection, $R_{01} = R_{02} = R_{03} = 1$.

As the infection spreads, fewer susceptible persons exist, and the number of secondary infections caused by each infected individual will be somewhat less than the maximum rate. We assume that the realized rates of transmission of HIV during each stage of the infection (R_1, R_2, and R_3) decline at a rate proportional to their respective maximum rates and the fraction of the population that is susceptible to the infection. For example, at an given time, t, $R_1(t) = R_{01}S(t)/N$, where S(t) is the number of susceptible hosts at time t and N is the total number of persons in that population, which is held constant. Since we are assuming that AIDS is the only cause of death, to maintain N, a susceptible host replaces each person that dies of AIDS.

Figure 1A shows how the densities of susceptible hosts, HIV-positive persons without AIDS, and persons with AIDS change over the course of time in a population with an initial number of 10^4 susceptible hosts and two HIV-positive persons at the earliest age of the infection (week 1). The virus rapidly spreads through the host population who exhibit no sign of AIDS for the first 10 years. By the time the first AIDS cases are recognized, more than half of the original population of 10,000 hosts are infected with the virus. Because of the relative dearth of susceptible hosts, the rate of spread of HIV to new hosts has already declined. Eventually, equilibrium is achieved and the infection maintains a steady state. In this endemic phase, the densities of susceptible hosts, HIV-positive hosts not manifesting the symptoms of AIDS, and AIDS patients level off. With these parameters, this endemic phase is reached in about 30 years.

A historical interpretation of this result is that by the time HIV infection was recognized as a specific disease in the gay male populations of San Francisco, Los Angeles, and New York in the early 1980s, a substantial proportion of persons in those subpopulations, were already infected with the virus (6). Moreover, by that time, HIV/AIDS may have already been approaching its endemic phase in these risk groups. The rate at which endemic phase is approached as well as the frequency of HIV-positive persons and AIDS patients within a subpopulation depends on the absolute rate of transmission. This is illustrated in Figure 1B. The parameters used for generating this figure are identical to those in Figure 1A, except for the maximum rates of increase, which have been reduced by a factor of two, $R_{01} = R_{02} = R_{03} = 0.50$.

As a consequence of this lower rate of transmission, the endemic phase is not reached for more than 100 years, and the proportion of the population that is HIV-positive and has AIDS is markedly reduced.

The simple explanation of these results is that an epidemic cannot continue forever because the number of uninfected hosts eventually declines, which stops the expansion of infections. At equilibrium, the fraction of infected versus uninfected hosts depends on various

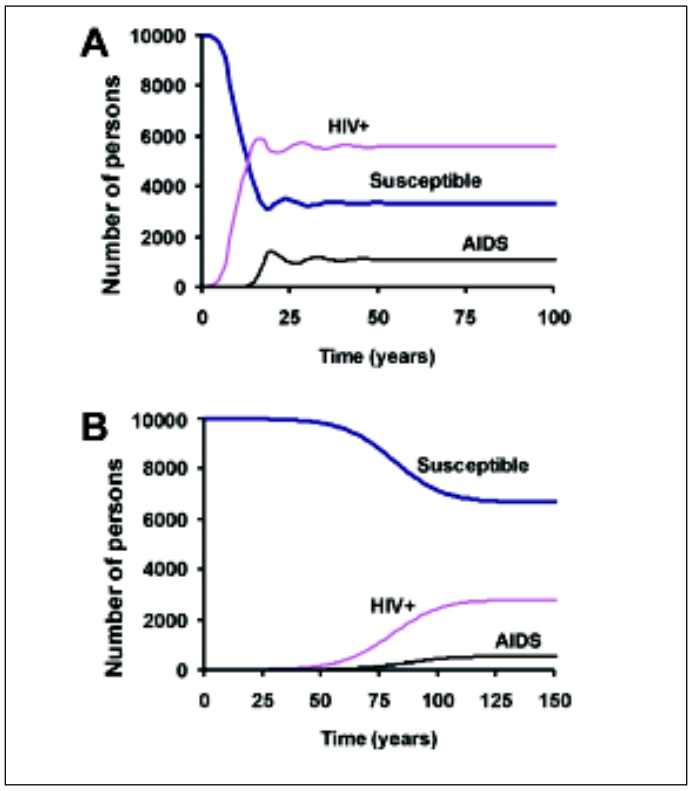

Figure 1. The spread of HIV/AIDS in a steady population of 10,000. In this figure, HIV-positive includes all persons infected with this virus, but not manifesting the symptoms of AIDS. In this simulation, the four stages of the infection, 0, 1, 2, and 3 are, respectively, 4, 6, 520, and 104 weeks ($L_0 = 4$, $L_1 = 6$, $L_2 = 520$, and L3 = 104). We assume that HIV is not transmitted during the first 4 weeks, stage 0. A) In a wholly susceptible host population during each stage 1, 2, and 3, infected host will be responsible for one secondary infection, $R_{01} = 0$, $R_{01} = R_{02} = R_{03} = 1.0$. B) In a wholly susceptible host population during stages 1, 2, and 3, each infected host will be responsible for one secondary infection, $R_{01} = 0$, $R_{01} = R_{02} = R_{03} = 0.50$.

parameters that are subsumed in the R_{0i}s of our model. Using condoms, reducing the numbers of sexual partners, providing sterile needles for injection drug users, and any other factor that reduces the likelihood of transmission of the virus would further reduce the fraction of the subpopulation infected with HIV. Also affecting the rate of spread of the disease would be the rate at which susceptible hosts enter a risk group. We hope this rate can be reduced by education.

Evolution

Evolution in the human population could ultimately reduce the likelihood of becoming infected with a microparasite or of acquiring the disease if infected. Such changes in the host population could also impact the epidemiology and evolution of that microparasite. In this section, we describe simple models for human evolution in response to HIV and evolution of HIV's virulence in HIV-infected persons. We argue that it will take thousands of years before evolution in the human population substantially increases the fraction of persons resistant to HIV/AIDS. Evolution in the HIV-infected population at large, on the other hand, can proceed at an extremely high rate. On epidemiologic grounds, it is unlikely that evolution in this

virus will make it more virulent (reduce the time between infection and the onset of AIDS) and may, in fact, favor reductions in virulence.

Host Evolution

As long as an infectious disease causes some persons to die before or during reproductive years or to otherwise reduce the number of children they produce, natural selection will favor persons who are less susceptible to the infection and its deleterious effects. In the case of HIV, some evidence exists for inherited variation in the likelihood of HIV infection and in the rate of progression to AIDS among HIV-infected persons (8, 9). On the other hand, even under optimal conditions for rapid evolution—disease resistance is complete and determined by the genotype at a single locus—if the resistance gene is initially rare, it will take millennia before a substantial fraction of the population is of the resistant genotype.

This slowness of human evolution is illustrated in Table 2, in which we calculate the number of years required for a gene that confers a 10% advantage on a favored gene to reach a frequency of 0.50 for different initial frequencies of that gene and different modes of inheritance. For this calculation, we use the standard population genetics model for selection in a diploid population (10) and assume an average generation time of 20 years. In the case of infectious disease, the intensity of selection depends on the incidence of the disease as well as its effect on the fitness of infected hosts, so a 10% advantage for resistance could represent 20% of the population infected and a 50% loss of fecundity per infection, and so on. Thus, even if the genetic conditions for selection for resistance to AIDS were optimal, and the fertility of infected persons was substantially reduced, the intensity of selection for the resistant genotype would be no greater than the frequency of the infection in the population. Although in some populations the frequency of HIV infections is tragically and appallingly high, in the human population at large that frequency remains substantially less than 1%. Moreover, HIV-infected persons do produce viable uninfected children. The implications of this are straightforward if not optimistic: we cannot count on evolution in our population to save us from the AIDS epidemic, at least not in our lifetimes or that of many generations to come. On the other hand, in some areas, sub-Saharan Africa in particular, the incidence of the disease in the heterosexual population is so high that the intensity of selection for resistance would be considerably greater than 1%. If genes for resistance to HIV were present in the African population, resistance may in fact become common in sub-Saharan Africa more rapidly than in the human population at large. In any event, many persons will die of AIDS during the evolution process (11).

Table 2. Years before the frequency of a gene that confers a 10% advantage[a] to reach 0.50

Initial frequency	Mode of inheritance		
	No dominance	Dominant	Recessive
0.01	1,838	1,054	41,038
0.001	2,763	1,964	401,963
0.0001	3,684	2,884	4,002,884

[a]We are assuming that favored genotype has a 10% advantage over the other genotypes; in the no dominance case, the relative fitness of the heterozygote is intermediate between that of the two homozygotes. With a 1% selective advantage, it would take 10 times as long for the gene to reach a frequency of 50%.

HIV Evolution of Virulence

Although human evolution is slow by our standards, HIV evolution will likely be rapid. Indeed, this retrovirus has already demonstrated its capacity for rapid evolution on several fronts, for example, the development of drug resistance and the ability to avoid the immune system. There is every reason to expect that HIV could evolve to a form with a different level of virulence in human hosts. Not so clear, however, is whether natural selection will favor changes in the virulence of this retrovirus or, if so, in what the direction that change would be. To predict the direction of natural selection on the virulence of HIV, we have to know the relationship between the virulence of this virus and its capacity for infectious transmission. Will HIV variants that engender a higher rate of progression to AIDS also be more transmissible and thus have an advantage over HIV variants that engender a lower rate of progression? Although a positive relationship between the transmissibility of HIV and its virulence has been proposed (12), no evidence supports this interpretation. Indeed, theoretical studies of the mechanisms of HIV virulence and experimental studies with simian retrovirus SIV_{SM}, which is almost identical to HIV-2, have found no evidence of a relationship between progression to AIDS and viral load or of a positive relationship between the transmissibility and virulence of HIV (13-19; M. Feinberg and S. Staprans, pers. comm.). Models of the epidemiology of HIV/AIDS can be used to elucidate how natural selection will operate on the virulence of HIV under different assumptions about the rate of progression to AIDS and the transmissibility of the virus.

Towards this end, we used our AoI model to explore how natural selection will operate in populations of humans infected with HIV who have different rates of progression to AIDS as measured by the length of the asymptomatic period. We made the simple and plausible assumption that transmission rates are constant within each stage of the infection and across different viruses, but that the total number of transmissions over the course of the infection varies only with the length of the stage of infection, L_i. This assumption constitutes a relationship between the virulence of this virus and its capacity for infectious transmission in a direction opposite from that assumed in (12), in that an earlier onset of AIDS is associated with fewer total transmissions from an infection.

Contrary to what may be anticipated from equilibrium considerations, with this model, a strain with a lower net yield of secondary infections can, under some conditions, have a selective advantage over a more productive strain. More specifically, if virulence is associated with a greater rate of transmission early in the infection, during the epidemic phase of the disease it could be favored, even if the overall transmission rate is reduced due to the earlier death of the infected host. This, too, is a manifestation of the advantages of early and discounting late transmission. On the other hand, as the disease approaches the endemic phase, the total amount of transmission over the term of the infection becomes increasingly important. During that stage, more virulent strains will be at a disadvantage unless they also have a higher overall rate of transmission.

To illustrate these points about the relationship between the epidemiology of the disease and the direction of selection for and against virulence, we used the AoI model to consider two distributions based on different lengths of time in the asymptomatic phase. The AoI distribution for the more

virulent strain is characterized by parameters denoted with an asterisk (*) and that of the less virulent strain is the same as in Figure 1 (the asymptomatic period lasts 10 years). For the more virulent strain, the asymptomatic phase is 5 years ($L_2 = 10$, $L_2^* = 5$). The weekly rate of transmission within each stage is the same for both variants, but the more virulant variant experiences a shorter infection life span and thus produces proportionally fewer secondary infections, than the less virulent strain ($R_{02} = 1.0$, $R_{02}^* = 0.5$).

Figure 2A plots the changes in the total density of susceptible persons, HIV-positive persons, and persons with AIDS and the relative frequency of the more virulent virus. The frequency of the more virulent strain increases initially due to its early progression to AIDS and the consequent higher weekly rate of transmission during that stage. As the epidemic wanes and the endemic phase approaches, the frequency of this more virulent strain declines because it produces fewer secondary infections over the lifetime of the infection. Thus in this case, selection temporarily favors an increase in the virulence of HIV, but over the long term, reductions in the virulence of HIV will be favored.

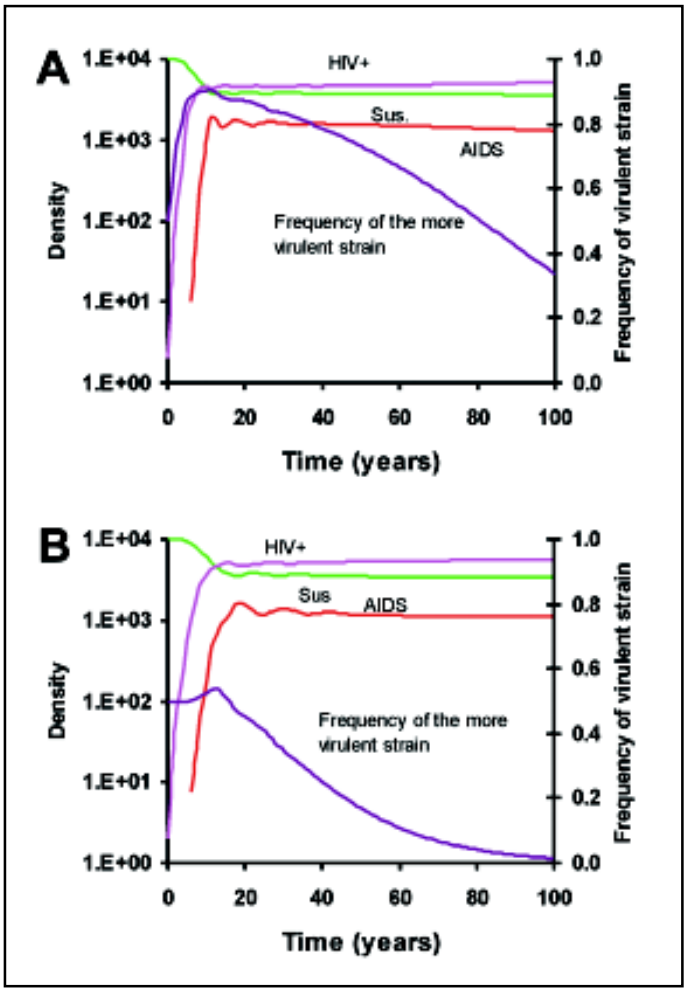

Figure 2. Evolution of HIV virulence in a steady state host population of 10,000. There are two HIV strains in these simulations. For one, the asymptomatic periods is 520 weeks and, in the more virulent population (*), it is 260 weeks. The duration of stages 0,1, and 3 is identical for both populations and the same as those in Figure 1. A) The weekly rates of transmission of the virus are identical in both populations, so that during the asymptomatic period in a wholly susceptible host population, the more virulent strain of HIV is responsible for 0.5 rather than 1 secondary infection, $R_{02}^* = 0.5$, while $R_{01} = 1.0$. The remaining R_0s of these two populations are identical to those in Figure 1. B) Early transmission in the more virulent strain is greater than that in the less virulent, $R_{01}^* = 1.05$, while $R_{01} = 1.0$ secondary infections. The weekly rates of transmission of are same for rest of the stages, $R_{00}^* = R_{00} = 0$, $R_{02}^* = 0.5$, $R_{02} = 1$, and $R_{03}^* = R_{03} = 1$.

If, for physiologic reasons, a faster progression to AIDS (stage 3) is associated with a higher absolute rate of transmission during earlier stages, during the epidemic phase of the disease, the rate of increase of the more virulent strain would be greater. Also greater would be the frequency before onset of the endemic phase and the intensity of selection against virulence (compare Figures 2A and 2B). Stated another way, even if there were a direct association between transmission and virulence during the early stage of the infection, virulence would eventually be selected against as the virus became endemic, provided that the total number of transmissions is lower for the more virulent strain.

Nonetheless, one should interpret these results cautiously because the evidence that no relationship exists between the virulence of HIV and its transmissibility remains largely circumstantial, albeit more compelling than that for a positive relationship. Until the results of studies addressing this issue become unequivocal, we cannot rule on the plausibility of the different scenarios for evolution of increasing or decreasing virulence of this retrovirus.

Epidemiologic Consequences of Treatment

It may be some time before we have vaccines that are effective in preventing HIV infections. On the other hand, multidrug chemotherapy substantially prolongs the life of HIV-infected persons. For those who can afford this relatively expensive therapy or otherwise have access to these drugs, multidrug chemotherapy has literally been a lifesaver. From an epidemiologic perspective, however, is there a downside to this therapy?

On first consideration, it seems obvious that if treated HIV-infected persons survive longer and continue to transmit the virus at the same rates as they would have without chemotherapy, the virus will spread more rapidly than it would in the absence of treatment. This "perverse" effect of therapy was in fact explained nearly 10 years ago in a theoretical study by Anderson, Gupta and May (20). That research was based on a compartment model that was more specific about the mode of transmission than is our AoI model, but it did not take into account either the AoI distribution or reductions in transmission rates due to the limitation of susceptible hosts. They concluded "that in communities where the transmission rate of HIV is low, but sufficient for long-term persistence (R_0 not much greater than unity), treatment that lengthens the infectious period is likely to be able to increase the overall transmission rate to more than counterbalance the greater longevity of infected persons who are treated." Anderson and his collaborators also concluded that when transmission rates are already high, community-wide treatment would benefit both the individual and the community.

We used the AoI model to explore this question of the effect of treatment on the epidemiology of AIDS. If we assume that treatment extends the survival time of AIDS patients and has no

effect on the rate transmission, then our results are the same as those of Anderson and colleagues (20). Treatment can increase the rate at which persons become HIV-positive and later acquire AIDS. On the other hand, there is every reason to expect that anti-HIV chemotherapy will markedly reduce the density of HIV in serum and strong evidence that transmission rates are directly proportional to the density of HIV in serum. Indeed, the results of an impressive recent study of HIV transmission by Quinn and colleagues (21) suggest that transmission will not occur at all when the viral titers are <1,500 copies/ml. With successful multidrug HIV therapy, viral titers of that level and lower can be expected and sustained for some time during the course of treatment.

Thus, the question of concern now is—what are the effects of reduced transmission of HIV from treated patients on the epidemiology of HIV/AIDS? To address this question, we used our AoI model to explore the effects of chemotherapy on the fraction of HIV-positive persons (non-AIDS) and persons with AIDS in treated and untreated groups during the epidemic and endemic phases of the disease. We considered a situation in which the overall rate of transmission in untreated hosts is relatively low, $R_{00} = 0$, $R_{01} = R_{02} = R_{03} = 0.5$ when the negative epidemiologic consequences of treatment are anticipated to be most profound (20). We assumed that treatment would extend the time before a person manifests the symptoms of an HIV infection, AIDS, by a factor of three, from 2 to 6 years. Here, parameters for a treated host will be denoted with *; $L_3 = 104$ weeks, $L_3^* = 312$ weeks. In one case, we assumed that treatment has no effect on the total number of viruses transmitted by a person with AIDS, but that it reduces the weekly rate of transmission by a factor of three. That is, in the course of the threefold increase in survival time, treated persons would be responsible for as many secondary infections as untreated AIDS patients ($R_{03} = R_{03}^* = 0.5$). In the second case,we assumed that treatment reduces the overall transmission by persons with AIDS by a factor of two ($R_{03} = 0.5$, $R_{03}^* = 0.25$).

To illustrate the effect of treatment in these situations, we compare what happens to the incidence of HIV and AIDS in a population in which AIDS patients are treated with a corresponding population in which they are not. If treatment has no effect on the overall rate of transmission, extending the life of AIDS patients will have virtually no effect on the fraction of the population infected with HIV (Figure 3A). While the infection is in the endemic phase, treatment increases the fraction of the population with AIDS by a factor of three, primarily by increasing the lifespan of AIDS patients by that amount. If, as seems reasonable to expect, chemotherapy actually reduces the overall transmission by persons with AIDS (Figure 3B), its epidemiologic effects will be positive. The incidence of HIV infections will be markedly reduced, and not until later in the endemic phase will the proportion of the population with AIDS increase, and that will be due largely to extending the lifespan of AIDS patients.

Conclusions, Caveats, and Recommendations

The results of this theoretical study and others have generated the following hypotheses, predictions, and speculations about the epidemiologic and evolutionary future of HIV/AIDS. 1) The AIDS epidemic has been driven primarily by transmission of the virus early in the course of infection. 2) Declines and leveling off in the incidence of new HIV infections in subpopulations (risk groups) could be largely due to a dearth of susceptible hosts in (or entering) the subpopulation rather than to the efficacy of public heath measures, education, and chemotherapy or to the evolution of the virus. 3) Although AIDS-mediated selection in the human population will eventually increase the overall level of resistance to HIV infection or reduce the rate (and maybe even the likelihood) of progression to AIDS, it will take millennia before human evolution alone will significantly increase our resistance to HIV/AIDS. 4) Epidemiologic considerations provide no reason to anticipate that evolution will increase the virulence of HIV. 5) In populations in which HIV is relatively rare, treatment that simultaneously extends the lifespan of persons with HIV, and also reduces the rate of transmission of the virus, can lead to substantial declines in the number of HIV-infected persons in the general population.

We have evaluated the possible consequences of different properties of HIV transmission and evolution. However, despite all that has been learned about HIV/AIDS, existing knowledge about the biology and epidemiology of this retrovirus is still too rudimentary to employ empirical estimates of these parameters. Thus, it is not yet possible to make robust, quantitative predictions about (and explanations for) the epidemic and endemic behavior of HIV or the evolution of its virulence. Towards these desired ends, however, we believe that the AoI model

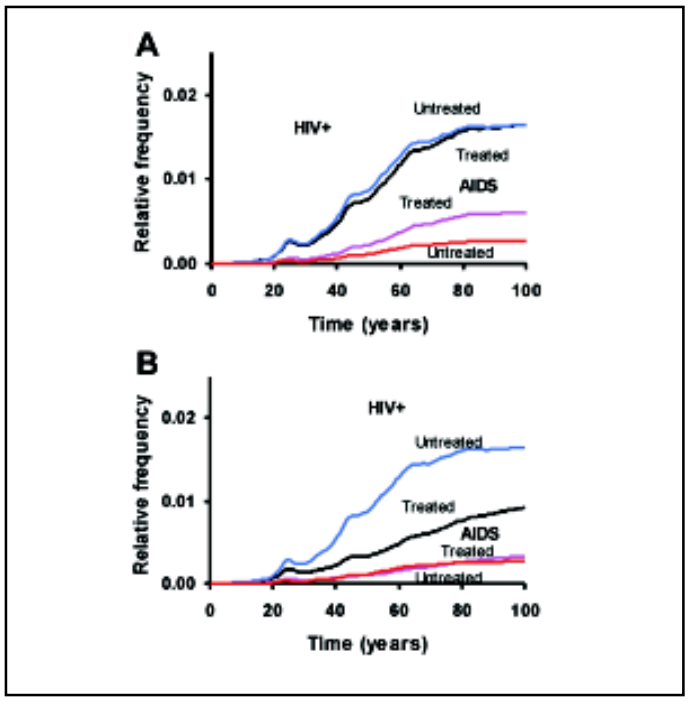

Figure 3. The effect of treatment on the relative frequency of HIV-positive persons (non-AIDS), and persons with AIDS in a steady state population of 10,000. In the untreated population, the duration of AIDS is 2 years, 104 weeks, before the patient dies, whereas in the treated population (*), it is 6 years, 312 weeks. The length of the other stages are identical to those in Figure 1. A) No effect of treatment on the overall transmission of HIV by AIDS patients, but a 1/3 reduction in the weekly rate of transmission. $R_{00} = R_{00}^* = 0$, $R_{01} = R_{01}^* = R_{02} = R_{02}^* = 0.5$, $R_{03} = 0.5$, $R_{03}^* = 0.5$. B) Treatment reduces the overall rate of transmission by AIDS patients by a factor of two. $R_{00} = R_{00}^* = 0$, $R_{01} = R_{01}^* = R_{02} = R_{02}^* = 0.5$, $R_{03} = 0.25$, $R_{03}^* = 0.25$

considered here and other mathematical models of the epidemiology of HIV serve the important role of revealing which properties of infections with this retrovirus and transmission are critical to understanding how it spreads, how to control that spread, and what to look for to predict the direction of evolution of its virulence.

Even without precise estimates of the values of these parameters, theoretical studies of the epidemiology of HIV make a number of unequivocal predictions. One is that early transmission will dominate the spread of HIV in naive populations. Another is that in populations in which HIV is relatively rare, treatment that does not reduce transmission rates can exacerbate the epidemic, and treatment that does reduce transmission can benefit the population as well as the patient. A broader, more definitive, and more quantitatively precise set of predictions about the epidemiologic and evolutionary future of HIV/AIDS will require data addressing the following questions. What are the rates of transmission of HIV during different stages of the infection? What effect does multidrug therapy have on the rate at which this virus is transmitted? Also critical to predicting the future HIV/AIDS is an objective and quantitative assessment of the demographic, behavioral, medical, and other reasons for changes in the incidence of HIV infections in different subpopulations. Are the declines in the rate of new HIV infections due to the efficacy of public health and education measures or, as suggested here, are they due to the saturation of the susceptible hosts in that risk group? Finally, to formally address the question of how HIV evolution will affect virulence of this retrovirus, we must know how much of the variation in the rate of progression to AIDS can be attributed to variation in the HIV-infected population.

Such data are not easy to obtain. Indeed, the potential importance of early HIV transmission (before seroconversion) was identified nearly a decade ago, yet little data have been collected on the magnitude of early transmission or on the amount of transmission occurring during other stages of the infection. From the narrow perspective of funding and careers, embarking on a research program directed at the acquisition of such data may be unwise. Gathering those kinds of data certainly lacks the romance and appeal of vaccine and drug development or the yield of generating more data on sequence variation. Nevertheless, without these transmission data, predictions about the epidemiologic and evolutionary future of HIV/AIDS will have to be relegated entirely (and, we believe, unsatisfactorily) to mathematical modeling.

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Antibacterial Household Products: Cause for Concern

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The recent entry of products containing antibacterial agents into healthy households has escalated from a few dozen products in the mid-1990s to more than 700 today. Antibacterial products were developed and have been successfully used to prevent transmission of disease-causing microorganisms among patients, particularly in hospitals. They are now being added to products used in healthy households, even though an added health benefit has not been demonstrated. Scientists are concerned that the antibacterial agents will select bacteria resistant to them and cross-resistant to antibiotics. Moreover, if they alter a person's microflora, they may negatively affect the normal maturation of the T helper cell response of the immune system to commensal flora antigens; this change could lead to a greater chance of allergies in children. As with antibiotics, prudent use of these products is urged. Their designated purpose is to protect vulnerable patients.

Antibiotics are critical to the treatment of bacterial infections. However, after years of overuse and misuse of these drugs, bacteria have developed antibiotic resistance, which has become a global health crisis (1, 2). The relatively recent increase of surface antibacterial agents or biocides into healthy households may contribute to the resistance problem. The antibacterial substances added to diverse household cleaning products are similar to antibiotics in many ways. When used correctly, they inhibit bacterial growth. However, their purpose is not to cure disease but to prevent transmission of disease-causing microorganisms to noninfected persons. Like antibiotics, these products can select resistant strains and, therefore, overuse in the home can be expected to propagate resistant microbial variants (3-6). Moreover, these agents, like antibiotics, are not cure-alls but have a designated purpose. Whereas antibiotics are designed to treat bacterial (not viral) infections, antibacterial products protect vulnerable patients from infectious disease-causing organisms. Neither are demonstrably useful in the healthy household.

Proliferation of Antibacterial Products

Seven years ago, only a few dozen products containing antibacterial agents were being marketed for the home. Now more than 700 are available. The public is being bombarded with ads for cleansers, soaps, toothbrushes, dishwashing detergents, and hand lotions, all containing antibacterial agents. Likewise, we hear about "superbugs" and deadly viruses. Germs have become the buzzword for a danger people want to eliminate from their surroundings. In response to these messages, people are buying antibacterial products because they think these products offer health protection for them and their families. Among the newer products in the antibacterial craze are antibacterial window cleaner and antibacterial chopsticks. Antibacterial agents are now in plastic food storage containers in England. In Italy, antibacterial products are touted in public laundries. In the Boston area, you can purchase a mattress completely impregnated with an antibacterial agent. Whole bathrooms and bedrooms can be outfitted with products containing triclosan (a common antibacterial agent), including pillows, sheets, towels, and slippers.

Development of Resistance

Bacteria are not about to succumb to this deluge, however. Through mutation, some of their progeny emerge with resistance to the antibacterial agent aimed at it, and possibly to other antimicrobial agents as well (4). Laboratory-derived mutants of *Pseudomonas stutzeri* with resistance to the cationic biocide chlorhexidine were also cross-resistant to antibiotics (nalidixic acid, erythromycin, and ampicillin) (7). In a recent study, 7% of *Listeria monocytogenes* strains isolated from the environment and food products showed resistance to quaternary ammonium compounds (8).

Laura McMurry in my laboratory group conducted experiments to determine whether triclosan had a particular cellular site for its antibacterial activity. She used a classic genetic technique, the isolation of resistant mutants of *Escherichia coli*, to identify its possible target. Surprisingly, finding the cellular site proved easy. In fact, mutants appeared with low, medium, and high-level resistance (3). They all had a mutation in one gene, the *fabI* gene (3) (Table 1). This finding indicated that triclosan had a target for the enoyl reductase essential in fatty acid biosynthesis. In the presence of triclosan, or a known FabI inhibitor (diazoborine), fatty acid biosynthesis was inhibited, whereas the antibiotics chloramphenicol or ciprofloxacin with other targets had little effect on fatty acid

Table 1. Selection of *Escherichia coli* with triclosan resistance (3)

E. coli	MIC (µg/ml)	Change (fold)	Mutated gene
AG100	0.05	1.0	——
AG100-1	0.20	4.0	*fabI* (F203L)
AG100-2	1.90	40.0	*fabI* (M159T)
AG100-3	25.00	500.0	*fabI* (G93V)

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biosynthesis (Table 2). In comparison with the wild-type *E. coli*, the mutant required up to 100 times more triclosan to show even minimal inhibition of fatty acid biosynthesis (3).

Table 2. Effect of various drugs on fatty acid/lipid synthesis in intact cells

Strain	Drug	µg/ml	% inhibition
AG100	Triclosan	0.24	92
(wild-type)	Diazaborine	8.0	93
	Chloramphenicol	13.0	19
	Ciprofloxacin	0.045	2
AGT11	Triclosan	0.24	2
(G93V)	Triclosan	1.4	7
	Triclosan	8.6	37
	Triclosan	25.9	75

One might argue that the high concentration of triclosan usually found in soap, e.g. 2,500 µg/ml, is enough to kill even resistant strains. We examined this question by testing triclosan activity in a commercial soap. To achieve a 90% death rate, wild-type *E. coli* required exposure to 150 µg/mL of triclosan in soap for 2 hours at 37ºC. Two to four times that amount was required by the mutant. By itself, triclosan was more active, killing *E. coli* at 6 µg/ml, and there was an even greater difference between the amounts required to kill wild-type and mutant *E. coli*. The soap seemed to decrease triclosan's effectiveness (Table 3). The mutant *E. coli* strains are truly resistant and would survive in triclosan-treated soaps diluted with as little as 3 parts water. Most importantly, the time, temperature, and amount needed to kill the bacteria greatly exceeded the average 5-second hand

Table 3. Effect of triclosan in soap on *Escherichia coli*[a]

Strain	In nutrient broth	In nutrient broth with commercial soap
AG100[b]	6	150
AGT11[c]	>32	300-600

[a] Amount (µg/ml) needed to kill 90% of *E. coli* (2 h at 37°C).
[b] Wild-type
[c] Mutant

washing performed by most people.

The finding of a mutation in the *fabI* gene led to a study of its homologue, *inhA*, the gene for one of the proposed targets of isoniazid, an anti-tuberculosis drug. Whether selected in triclosan or isoniazid, mutants of *Mycobacterium smegmatis* showed cross-resistance to both drugs via a mutation in the *inhA* gene (Table 4) (9). Moreover, triclosan-resistant *E. coli* mutants also showed resistance to an experimental antibiotic, diazoborine (3). Other drugs currently under development may target *fabI*; these potentially new antibiotics may also be affected by triclosan resistance.

The data clearly suggest that antibacterial agents will have an impact on the environmental flora and on resistance emergence. For instance, use of triclosan could select bacteria which have intrinsic resistance to the chemical. Some gram-positive bacteria such as *Enterococcus faecalis* and *Streptococcus pneumoniae*, which do not have *fabI*, have a related enoyl reductase gene, *fabK* (10). The *fabK* gene in those organisms is naturally resistant to triclosan, so

Table 4. Mutations in InhA confer triclosan resistance in *Mycobacterium smegmatis* (9)

Strain	Selected on[a]	InhA mutation	Relative MIC (in 7H9 medium) TRC	INH
mc^{2}155 (wt)	—	None	1.0	1.0
MT 1	TRC	M161V	6.3	8.5
MT 9	TRC	M103T	6.3	1.2
MT 17	TRC	A124V	5.8	2.0
mc^{2}651	INH	S94A	6.3	22.0
mc^{2}155 (p::inhA)	—	None	6.3	>64

[a]TRC, triclosan; INH, isoniazid

triclosan usage can potentially enhance their growth at the expense of susceptible strains. At the American Society for Microbiology meetings in May 2000, a number of papers described the isolation of bacteria resistant to triclosan or to other antibacterial agents (11-13).

The other known mechanism by which bacteria resist these drugs is by pumping them out of the cell by an efflux mechanism. The key genes in *E. coli* involved in this form of resistance are a regulatory gene, *marA*,and an efflux gene complex, *acrAB* (14). *MarA* is a component of a multiple antibiotic resistance locus, *marRAB*. When *marA* is activated, the cell becomes resistant to antibiotics, oxidative stress agents, organic solvents, and antibacterial agents (14). Over 60 different genes are affected when *marA* is overexpressed in *E. coli*, indicating a very large regulon (15). Strains that overproduce the marA or soxS protein (which is a *marA* homologue) upregulate the AcrAB multidrug efflux pump which pumps out pine oils, organic solvents, triclosan, quaternary ammonium compounds, chloroxanol, and chlorhexidine (4). Triclosan is also a substrate for multidrug efflux pumps in *Pseudomonas aeruginosa* (16).

Efflux pumps can affect antibiotic efficacy in a number of ways. A triclosan-resistant mutant of *E. coli* does not lyse easily in the presence of triclosan, making the strain difficult to kill. Triclosan lyses the wild-type cell (AG100) at about 8 µg/ml, but the mutant AGT11 requires at least four times that amount (=32 µg/ml) (Table 5). When the *acrAB* gene locus is deleted from the wild-type cell, lysis occurs at a lower concentration, i.e., 3 to 4 µg/ml. More importantly, with removal of the AcrAB pump, the mutant bacteria and the wild-type cells were killed by the same amount of triclosan, i.e., 3 to 4 µg/ml, despite residual *fabI* resistance in the mutant to the growth inhibitory action of triclosan (Table 5). Therefore, the normal expression of a multidrug efflux pump in *E. coli* is critical to the activity of triclosan.

Table 5. Effect of triclosan in liquid culture on growth and lysis of *Escherichia coli* strains with and without AcrAB efflux pump (4)

Strain	Characteristics	Concentration (µg/ml) that Inhibited growth 50%	(90%)	Caused lysis
AG100	Wild-type	0.15	(0.60)	8
AG100A	AG100 *acrAB::kan*	0.02	(0.05)	3-4
AGT11	AG100 *fabI* (G93V)	13.0	(>32)	>32
AGT11K	AGT11 *acrAB::kan*	1.3	(2.10)	3-4

Mar mutants generally express low levels of antibiotic resistance and are precursors to mutants with high-level antibiotic resistance (14). We have identified clinical strains of *E. coli* that are resistant to triclosan because they are also Mar mutants (4). From these and other data, selection for Mar mutants can potentially occur by antibiotics or by antibacterial agents.

Consequences of Resistance

Community-acquired methicillin-resistant *Staphylococcus aureus* (cMRSA) has become an increasing problem worldwide. These community-derived strains show an antibiotic susceptibility profile that is markedly different from hospital-acquired MRSA. cMRSA strains are chiefly resistant to the beta-lactam antibiotics (penicillins and cepholosporins). Interesting laboratory findings suggest a link between this resistance in cMRSA and the use of antibacterial products. Investigators in Japan selected MRSA mutants with a twofold higher minimal inhibitory concentration for benzalkonium chloride (5 to 10 µg/ml) (17). Resistance to methicillin and to a number of cephalosporins and penicillins dramatically increased with this mutation (Table 6), but susceptibility to other antibiotics was essentially unchanged. The laboratory mutants, in fact, mirror the phenotype of the MRSA that has emerged in the community. Is there a connection? The findings warrant further study.

Table 6. Antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* and benzalkonium chloride-resistant derivatives[a]

	MRSA[b]		
Antibiotic	Parent	BZ-R-1	BZ-R-2
Oxacillin	16.0	512	512
Cloxacillin	0.5	256	512
Moxalactam	64.0	256	1024
Flomoxef	8.0	128	128
Cefmetazole	8.0	128	64
Cephalothin	64.0	128	128
Ampicillin	16.0	32	32
Chloramphenicol	4.0	4	4
Ofloxacin	8.0	32	32
Tetracycline	128.0	128	128
Benzalkonium chloride	5.0	10	10

[a]Adapted from (17).
[b]MIC (µg/ml) (BZ-R = parent resistant to benzalkonium chloride)

Antibacterial agents and antibiotics share the same resistance problem. Resistance will certainly increase as the drug persists, especially at low levels (e.g., residues) for long periods of time. Of course, that concern is irrelevant with substances that do not leave residues (e.g., alcohols, bleaches, and peroxides). No current data demonstrate any health benefits from having antibacterial-containing cleansers in a healthy household. However, use of these products may change the environmental microbial flora.

Unfortunately, the antibacterial indulgence is coincident with the trend toward shorter hospital stays. An estimated 5% of hospital patients in Massachusetts go home for continued care, often with intravenous parenteral drugs. For these vulnerable patients, the use of antibacterial products protects them from disease caused by commensal as well as pathogenic bacteria. A cause for concern now is that homes, which are becoming end-of-therapy quarters for patients, may be becoming havens for "hospital-like" bacteria as well.

The Antibacterial Products-Allergy Link

Besides resistance, the antibacterial craze has another potential consequence. Reports are mounting about a possible association between infections in early childhood and decreased incidence of allergies (18). In expanding this "hygiene hypothesis," some researchers have found a correlation between *too much* hygiene and *increased* allergy (18-21). This hypothesis stems from studies that revealed an increased frequency of allergies, cases of asthma, and eczema in persons who have been raised in an environment overly protective against microorganisms. In one rural community, children who grew up on farms had fewer allergies than did their counterparts who did not live on farms (19). Graham Rook, University College, London, has likened the immune system to the brain. You have to exercise it, that is, expose it to the right antigenic information so that it matures correctly. Excessive hygiene, therefore, may interfere with the normal maturation of the immune system by eliminating the stimulation by commensal microflora (20).

For normal maturation, the immune system must be stimulated to achieve the right balance between the T-helper 1 (TH-1) cells providing cellular immunity and the TH-2 cells promoting antibody production. When investigators examined people with allergies and eczema, they noted an imbalance between TH-2 and TH-1 activities as compared with the mechanisms in control groups. In those with allergies, antibody production predominated over cell-mediated responses. Other studies showed a correlation between the presence of an immune response to organisms contracted by the oral-fecal route and decreased likelihood of atopy (21). In those persons who demonstrated a prior exposure to one, two, or all three of the organisms tested (*Toxoplasma gondii*, *Helicobacter pylori*, hepatitis A virus), the odds ratio for allergy became substantially lower than that seen in the control group (21). This correlation was not found for prior contact with organisms causing infections by other routes (e.g., mumps, measles, varicella). The authors concluded that "hygiene and a westernized, semi-sterile diet may facilitate atopy by influencing the overall pattern of commensals and pathogens that stimulate the gut-associated lymphoid tissue …" (21). Of note, children vaccinated with bacillus Calmette-Guérin appeared to be protected as well against atopy (22), and this finding was also related to stimulation of the TH-1 response. The combined data led one group to conclude that an "antigenically rich (dirty) environment may be essential for normal immune maturation preventing atopic disease" (23).

Antibiotics may also be implicated in the hygiene hypothesis. Because they eliminate common bacteria, antibiotics may cause the same consequence as too much hygiene. Some infants begin to get antibiotics as soon as a few days after birth. They mature in an antibiotic-laden environment. What antigens do they confront daily? What kind of immune response are they developing?

We must think not just in terms of resistance but also in terms of the changes in the microbial ecology of our infants and our homes. We exist in the bacterial world, not bacteria in ours. Unfortunately, we believe that we can rid ourselves of

bacteria when, in fact, we cannot. Instead, we should "make peace" with them. Although we need to control pathogens when they cause disease, we do not have to engage in a full-fledged "war" against the microbial world. Improved antibiotic use, including shorter treatments and removal of improper usage, will encourage the return of antibiotic-susceptible, commensal flora and return the environment to what it was before the antibiotic/antibacterial onslaught.

A new approach focusing on commensals has been initiated through an agreement between the Alliance for Prudent Use of Antibiotics (APUA) (www.apua.org) and the University of Illinois, funded by the National Institute of Allergy and Infectious Disease. This initiative, entitled ROAR (Reservoirs of Antibiotic Resistance; http://www.roar.apua.org) focuses on monitoring and managing the commensal bacteria that harbor pools of resistance genes that can be passed on to pathogens. Through education, APUA strives to foster control of pathogens without decimation of the non-pathogens. In this goal, prudent use applies to both antibiotics and antibacterial products.

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Food Safety and Irradiation: Protecting the Public from Foodborne Infections

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Early in the 20th century, when food safety was a major concern to the public, two technologies, milk pasteurization and retort canning, were developed, promoted, and virtually canonized as prevention measures against foodborne diseases. Fear of contracting typhoid fever from watered milk and outbreaks of botulism from commercially canned products are now part of the distant past, controlled by these food industry processes in many countries. Nonetheless, at the beginning of the 21st century, foodborne disease remains a major threat to public health, as new pathogens and products have emerged (1). Many of these threats can be controlled by applying new technologies, when we as a society are willing to use them.

In the United States, foodborne infections cause an estimated 76 million cases of illness and 325,000 hospitalizations annually—more than 1 in 1,000 are hospitalized each year (2). The economic burden is substantial, estimated at up to $6.7 billion annually in patient-related costs for treatment of bacterial infections alone (3). Five pathogens account for much of the most severe illness: *Salmonella*, *Escherichia coli* O157 and other Shiga toxin–producing *E. coli*, *Campylobacter, Listeria*, and *Toxoplasma* cause an estimated 3.5 million infections, 33,000 hospitalizations, and 1,600 deaths each year (2).

In the early 1990s, large and devastating foodborne outbreaks of *E. coli* O157:H7 infections heightened public concern about foodborne diseases (4). Efforts to improve food safety were intensified in industry and regulatory agencies and supported by the National Food Safety Initiative (5). As a result of these efforts, the process control strategy of the Hazard Analysis–Critical Control Point (HACCP) is becoming the norm to use for producing many foods. In slaughter inspection it is replacing manual and visual carcass-by-carcass inspections. An expanded focus on regulating sanitation and hygiene with good manufacturing and agricultural practices means that food would be produced under cleaner conditions. In restaurants and home kitchens, new attempts have been made to educate food preparers in the basic principles of food safety, though paid sick leave for foodhandlers is still the exception, and handwashing is intermittent. These developments may collectively help explain a decline in the reported incidence of *Salmonella* and *Campylobacter* infections that was observed in active surveillance by FoodNet between 1996 and 2000 (6). However, we are still far from the public health goals established for 2010. These goals include reducing the national incidence of infections with *Salmonella*, *E. coli* O157, *Campylobacter*, and *Listeria* to 50% of their 1997 incidence (7). Reaching those goals means preventing 50% of foodborne diseases now occurring. This will require new approaches for prevention.

Traditional Methods: Sanitation and Pasteurization

In general, effective vaccines are not available to protect against pathogens that cause foodborne diseases, either for immunizing humans or for animals that serve as hosts and may be eaten by humans. Educating foodhandlers, consumers, and food producers in basic food safety is important but is not sufficient by itself. Protecting consumers from the most severe diseases has been achieved by increasing the safety of food along the chain of production, from farm to table (Figure 1). For many foodborne infections, control has been most successful when mechanisms of transmission are understood well enough to prevent contamination from occurring before consumers purchase food. This has meant rethinking food production processes and sometimes introducing new safety steps to reduce levels of microbial contamination. The degree of safety built into the process varies, depending on the risk and the technologies available to address the risk.

For all foods, using basic principles of sanitation and food hygiene preserves wholesomeness and shelf life. For foods susceptible to contamination with particularly deadly

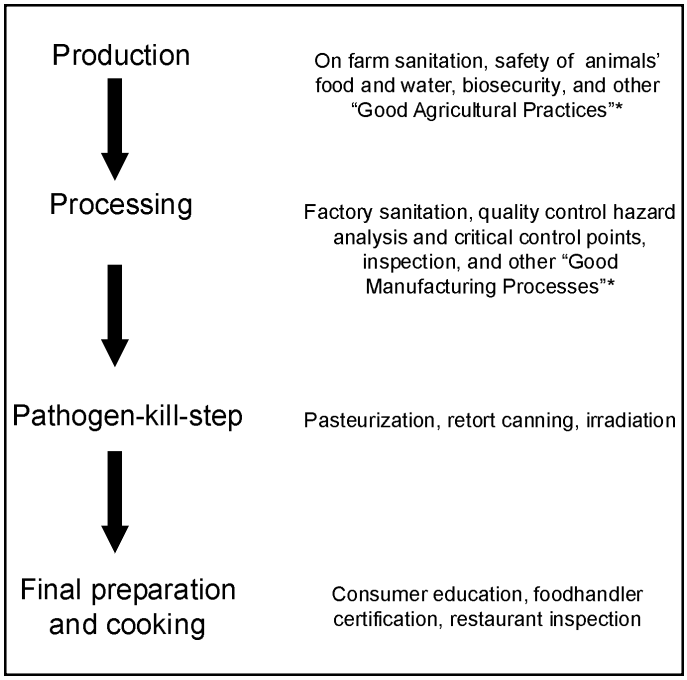

Figure. The chain of food production and foodborne disease prevention from farm to table. *These are terms used by FDA as guidelines for agriculture and food manufacturing practices.

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pathogens, we as a society have demanded that additional protective measures be taken to eliminate those pathogens from the food altogether. A definitive food safety measure is necessary when contaminated food products put the general population at risk for severe illness and when typical food production practices do not eliminate the risk reliably, especially those pathogens that cause severe illness to humans exposed to even small amounts. It is not sufficient to rely on routine foodhandling practices in the kitchen to prevent illness if the pathogen is already present in the food. In the past, it has often been a catastrophe resulting from a large foodborne outbreak that spurs the demand for new measures to completely eliminate the pathogen from food.

Implementation of definitive new measures for food safety has historically been slow. For example, canning was widely practiced as a means of preserving food in the 19th century, but methods were not standardized. The principal risk associated with eating improperly canned foods is botulism, a devastating paralytic illness that follows ingestion of food containing botulinum toxin. Botulinum is an extremely potent toxin produced by the bacterium *Clostridium botulinum* under certain anaerobic conditions, such as those that may be found inside a hermetically sealed can. This bacterium can live inside a can because it forms a hardy spore that can survive the temperature at which water boils at ordinary air pressure. It takes temperatures higher than 100 degrees Celsius to kill spores in canned food.

Before the invention of artificial ventilation and intensive care, half of those who contracted botulism died, and even now, botulism means many weeks in intensive care. Large outbreaks during and following World War I drew attention to the public health hazard of poorly canned foods. A 1919 multistate outbreak that resulted in 15 deaths was traced to canned ripe olives from California (8,9). This outbreak led to the development in 1923 of an industry standard method for cooking food at high enough temperatures to kill the botulinum toxin, the so-called botulism retort cook. This method reliably reduced clostridial spore counts by 12 decimal logs, the highest conceivable level of contamination (10). In 1930, a federal standard for quality of canned foods was developed, because of concern that vegetables that were canned might be of inferior quality (11). However, it was not until 1973, following an outbreak of botulism traced to defectively canned commercial vichysoisse soup (12), that the current federal regulation of canned foods was passed.

Pasteurization of milk, another fundamental technology used to prevent foodborne disease, was also adopted slowly over many years. At the turn of the last century, cows' milk was recognized as the source of a large number of different infections, including typhoid fever, bovine tuberculosis, diphtheria, and severe streptococcal infections (13). A commercial pasteurizer was patented in Germany in 1893, and, by 1900, a standard set of pasteurization conditions were defined, based on the time and temperature required to inactivate *Mycobacterium tuberculosis*, which was thought to be the most heat-resistant pathogen. However, pasteurization was opposed because it was believed that it might be used to market dirtier milk and also because of fears that it might affect the nutritional value of milk (14); therefore, the technology was implemented slowly. For some, the best way to prevent infections spread through milk was to pay scrupulous attention to the health of animals and to create sanitary conditions for the milk production process. This "certification movement" led to substantial improvements in dairy conditions. However, recurrent outbreaks of illness traced to some certified dairies clearly indicated a need for pasteurization. Initially, different jurisdictions adopted either improved sanitation or pasteurization. The requirements of the Public Health Service Standard Milk Ordinance in 1927 combined the two strategies: first, milk was to be graded based on a variety of sanitation measures; second, only Grade A milk could be pasteurized (15). By the end of the 1940s pasteurization was heavily promoted throughout the industry and became the norm. Now, 99% of fresh milk consumed in the United States is pasteurized, Grade A (16).

The use of both retort canning and milk pasteurization took decades to gain universal acceptance. Many were concerned that the use of these technologies would lead to slippage of standards for quality and sanitation. These concerns were ultimately addressed by using formal grading processes to assure the public that only clean milk would be pasteurized, and only vegetables of clearly defined quality would be canned. Concerns that loss of nutrients would be an important issue were found to be unwarranted. Although a wide variety of times and temperatures were initially used, clear microbial target endpoints were ultimately defined for both canning and pasteurization so that milk pasteurization and botulism retort cook have standard meanings everywhere in the United States. Quality grading standards and pathogen elimination processes were first developed by the industry and then formally adopted via federal regulation. Both processes are generally applied to foods that are either packaged or that will be immediately packaged. This method minimizes the opportunity for posttreatment recontamination.

Use of these processes has eliminated outbreaks of botulism in commercially canned food and has made outbreaks of illness spread through milk extremely rare. Foodborne botulism is now a rare illness, with approximately 25 cases a year that are almost always associated with home-canning or home-preserving (16). Bovine tuberculosis, typhoid fever, and septic sore throat resulting from milk tainted with bacteria have disappeared. Outbreaks of infections due to unpasteurized milk still occur (16). The rare outbreaks that are traced to pasteurized milk are usually the result of breaks in postpasteurization hygiene (16,18).

Today's Technology: Food Irradiation

The use of high energy irradiation to kill microbes in food was evaluated in this country as early as 1921, when scientists at the United States Department of Agriculture reported that it would effectively kill trichinae in pork (19). Irradiation has become a standard process used to sterilize many consumer and medical products, from adhesive strips to surgical implants. Three different technologies that can be used to treat food have been developed by the sterilization industry.

Gamma Irradiation

Gamma irradiation technology uses high energy gamma rays that are emitted by radioactive cobalt 60 or cesium 137. These radioactive sources are produced in commercial nuclear reactors and have a long half-life that makes them useful for commercial installation. Food or other products are brought into a heavily-shielded chamber and exposed to gamma rays for a defined length of time. When the source is not in use, it is

stored in a pool of water that absorbs all irradiation, effectively turning it off. These high energy rays can penetrate deeply, making it possible to treat bulk foods on shipping pallets.

Electron Beam Irradiation

Electron beam technology uses a stream of high energy electrons, also known as beta rays, that are emitted from an electron gun. The technology is analogous to an electron beam in a television tube, though far more powerful. Electrons can only penetrate several centimeters of food, and for this reason, foods are treated in relatively thin layers. Modest metal shielding of the treatment cell is sufficient to prevent the escape of stray electrons. When not in use, the electron source is turned off by switching off the electric current. No radioactivity is involved.

X-Irradiation

The most recently developed technology, X-irradiation, mixes properties of both of the above. High energy X-rays can be produced if an electron beam hits a thin metal foil target. Like gamma rays, a beam of X-rays can penetrate foods to a much greater depth than electron beams and requires heavier shielding. However, like electron beams, X-ray sources can be switched on and off and do not use a radioactive source.

Effect of Irradiation on Microbes

The high energy rays of irradiation directly damage the DNA of living organisms, inducing cross-linkages and other changes that make an organism unable to grow or reproduce. When these rays interact with water molecules in an organism, they generate transient free radicals that can cause additional indirect damage to DNA. An absorbed dose of irradiation energy is now measured in units called Grays, rather than an older measure called a rad. One Gray equals 100 rads, and 10 kiloGray equal 1 megarad. Complex life forms with large DNA molecules are affected by relatively low doses. Simpler organisms with smaller DNA can take progressively higher doses. Thus, a low dose of under 0.1 kiloGray kills insects and parasites and inhibits plants from sprouting. A medium dose, between 1.5 and 4.5 kiloGray, kills most bacterial pathogens other than spores, and a higher dose of 10 to 45 kiloGray will inactivate bacterial spores and some viruses. Prions, which do not contain nucleic acid, are difficult to inactivate by irradiation. For humans, the lethal dose is 4 Gray.

The actual dose required to treat food varies with the specific pathogen and the specific circumstances of the food. It generally takes a higher dose to kill the same number of organisms in frozen food than it does to kill them in refrigerated food. A D-dose is the amount of irradiation that it takes to destroy 90% of the organisms or one decimal log. Thus, a one log kill would reduce a million bacteria to 100,000. Getting rid of more bacteria takes more irradiation as they are small targets and it is not easy to hit each of them. To eliminate 99.999% of the bacteria (a so-called 5-logarithm kill) takes 5 times the irradiation dose needed for a 1 log kill and would reduce a million bacteria to 10. For example, it takes 0.2 kiloGray to reduce *Campylobacter* in meat by one decimal log or 1 kiloGray to reduce it by 5 decimal logs (Table 1).

Irradiation has been approved for use on a broad range of foods for different purposes (Table 2). By an historical quirk, the use of irradiation on food was formally approved as though it were something added to food, rather than a process to which the food is subjected. This means that for meats and poultry, approval is required from both the FDA and USDA. The effect of irradiation on food itself is usually minimal at doses up to 7.5 kGray. Treated food does not become radioactive, and, in general, shelf life is prolonged because organisms that cause spoilage are reduced along with pathogens. Irradiation has been used effectively in meats, poultry, grains, and produce. However, not all foods can be irradiated without changing their quality. Meats with a high fat content may develop off-odors; the whites of eggs may go milky and liquid; and grapefruit gets mushy. Alfalfa seeds do not seem to sprout as well if they are irradiated, and raw oysters may die, which shortens their shelf life substantially.

Table 1. Doses required to decrease selected pathogens at refrigerator temperatures by one decimal log/90% (D-dose)

Pathogens	D-dose in kGray*	5-log reduction dose in kGray
Campylobacter	0.20	1.00
Toxoplasma cysts	0.25	1.25
E. coli O157	0.30	1.50
Listeria	0.45	2.25
Salmonella	0.70	2.80
Cl. botulinum spores	3.60	18.00

* 1 Gray = 100 rad; 10 kGray = 1 megarad

Nutritional and other chemical changes induced in food by irradiation have been studied extensively. In general, these changes are limited to modest declines in the quality and amount of a few vitamins, particularly thiamine (vitamin B1), that are not likely to change the overall adequacy of dietary intake, and to production of transient free radical oxidants, which react almost immediately in the food and do not persist. Similar oxidants are also produced by cooking,

Table 2. Irradiation approved for foods in the United States

Year	Food	Dose (kGy)	Purpose
1963	Wheat flour	0.20-0.50	Control mold
1964	White potatoes	0.05-0.15	Inhibit sprouting
1986	Pork	0.30-1.00	Reduce cases of trichinosis
1986	Fruits and vegetables	1.00	Increase shelf life and control insects
1986	Herbs and spices	30.00	Sterilize
1990 (FDA)	Poultry	3.00	Reduce bacterial pathogens
1992 (USDA)	Poultry	1.50-4.50	Reduce bacterial pathogens
1997 (FDA)	Fresh meat	4.50	Reduce bacterial pathogens
2000 (USDA)	Fresh meat	4.50	Reduce bacterial pathogens

and, in any event, would be hydrolyzed immediately in the stomach if any are present. Other radiolytic products are difficult to detect and are present in only trace amounts. It is important to remember that the processes of cooking, such as grilling or frying, themselves induce profound chemical changes in foods, which we depend on to make them edible and tasty. The safety of consuming irradiated foods has been evaluated in large scale trials in animals, some of which lived for several generations (19). No ill effects were observed, and, in particular, no teratogenic effects were seen in mice, hamsters, rats, or rabbits. Formal feeding trials were also conducted with human volunteers without ill effects, and NASA routinely uses irradiated meats in the diet of astronauts.

Acceptance of Irradiated Foods

Will the public accept irradiated foods? Surveys conducted recently by the Food Marketing Institute and one conducted at FoodNet sites on the general population have had results similar to those obtained in the studies mentioned above (20,21). About 50% of the population is ready to buy irradiated foods, if asked. Acceptance will be greater if irradiated food is not much more expensive than nonirradiated food. The rate of acceptance can increase from 50% up to 80% to 90% if customers understand that irradiation reduces harmful bacteria in food. Similar results have been observed when test marketing irradiated products. Since 2000, irradiated ground beef has been for sale in many markets, and the medical and public health communities can respond to this with enthusiasm.

Candidates for Food Irradiation

E. coli O157 and other Shiga toxin–producing *E. coli* cause more than 100,000 cases of illness per year (2). This infection is untreatable and can lead to severe complications, including hemolytic uremic syndrome, chronic renal failure, and death (22). Ground beef is the most commonly identified source of infection. Pooling the meat of many thousands of animals into ground beef may increase the rate of contamination. Just a few organisms are sufficient to cause severe illness, and efforts to decrease the contamination of ground beef have probably reduced but not eliminated the risk. Irradiating ground beef would effectively destroy *E. coli*.

Campylobacter jejuni, the most common of all bacterial foodborne infections, causes an estimated 2,000,000 cases of illness per year (2), and has been associated with Guillain-Barré syndrome (GBS), an acute neurologic disorder (23). Treatment of a *Campylobacter* infection does not prevent its progression to GBS. Poultry is the most commonly identified source of infection. Cross-contamination during slaughter may lead to nearly universal contamination of poultry meat. It takes only a small number of organisms to cause infection. Current efforts to reduce cross-contamination may be responsible for a decrease in *Campylobacter* infections, but these efforts are not likely to eliminate the risk altogether. Irradiating poultry meat would effectively eliminate *Campylobacter* from that food.

Salmonella, whose many serotypes are harbored by mammals, birds, and reptiles, causes an estimated 1,400,000 cases of illness and 16,400 hospitalizations per year (2). Up to 2% of humans develop reactive arthropathy after being infected. Foods of animal origin have been the most commonly identified sources, including meat, poultry, eggs, and raw milk (24). Improvements in the safety of egg production and handling have been associated with a recent substantial reduction in the incidence of one common egg-associated serotype, *Salmonella* Enteritidis. Further progress is possible with increased use of eggs pasteurized in their shells, which reached the market in 2000. Improvements in meat and poultry slaughter practices under HACCP may have also had an impact, but they have not eliminated the risk of salmonellosis from raw meat. Irradiating meat and poultry would eliminate *Salmonella* from those foods.

Listeria monocytogenes is an opportunistic pathogen that causes an estimated 2,600 cases per year of severe invasive illness (2). This infection affects those who have compromised or undeveloped immune systems, particularly the elderly, the immunocompromised, and pregnant women (25). Approximately 25% of infections lead to death of the immunocompromised patient or loss of the fetus. The number of organisms sufficient to cause infection has not been clearly established. In a healthy host, exposure to extremely high numbers of *Listeria* can result in nothing more than febrile gastroenteritis; in a high-risk individual, a low amount may be sufficient to cause severe infection. The most frequently identified sources are ready-to-eat processed meats and soft cheeses made from unpasteurized milk. Ready-to-eat meats, such as hot dogs, have already been subjected to a pathogen-killing step when the meat is cooked at the factory, so contamination is typically the result of in-plant contamination after that step. Improved sanitation in many plants has reduced the incidence of infection by half since 1986, but the risk persists, as illustrated by a large hot dog–associated outbreak that occurred in 1999 (26). Additional heat treatment or irradiation of meat after it is packaged would eliminate *Listeria* that might be present at that point.

Toxoplasma gondii is the most common of all parasitic foodborne infections. As with *Listeria monocytogenes*, the consequences of infection with *T. gondii* are most evident in an immunocompromised person or a pregnant woman (27). Toxoplasmosis causes an estimated 400 to 4,000 cases of congenital disease each year, including hydrocephalus, mental retardation, blindness, and sometimes even death, as well as more than 200,000 noncongenital illnesses, leading to approximately 750 deaths per year, 375 of which may be the consequence of foodborne infections. Consumption of or contact with undercooked meat, especially pork, is an important source of infection, as is contact with feces of an infected cat. Up to 3% of market pigs show serologic evidence of infections or have *Toxoplasma* cysts. Irradiation would inactivate parasites in meat.

Potential Health Benefits of Irradiating Meat and Poultry

We can roughly estimate the potential benefit of irradiating meat and poultry with a simple calculation. Let us assume that 50% of poultry, ground beef, pork, and processed meats is irradiated. Let us also assume that these foods are the source of 50% of foodborne *E. coli* O157, *Campylobacter*, *Salmonella*, *Listeria,* and *Toxoplasma* infections. The potential benefit of the irradiation would be a 25% reduction in the morbidity and mortality rate caused by these infections (Table 3). This estimated net benefit is substantial, as the measure could prevent nearly 900,000 cases of infection, 8,500 hospitalizations, over 6,000 catastrophic illnesses, and 350 deaths each year. With this estimate we assume that

heavily contaminated meat is just as likely to be treated with irradiation as meat which is less contaminated. This estimate does not include the impact on other known pathogens contained in these foods, such as *Yersinia enterocolitica,* or those yet to be identified. This estimate also does not account for the benefits of using irradiation to treat other foods, such as fresh produce that can also be a source of infection.

Potential Public Concerns about Irradiation

Just as in the early days of milk pasteurization and retort canning, several concerns about this technology have been expressed. Some may ask whether irradiated food is safe to eat. The safety of irradiated food has been studied for four decades, making it the most intensively assessed of any food safety process. Extensive nutritional assessments, toxicity studies, and feeding trials have not indicated a risk, and the process has been approved by many regulatory agencies around the world (28). The nutritional changes produced by irradiation are fewer than those produced by canning or pasteurization.

Others may question the safety of the technology. Irradiation is also used to sterilize products such as surgical implants and instruments; this is a well-established procedure that has been practiced for many years. With regulatory oversight by the Nuclear Regulatory Commission and the Department of Transportation, these procedures are models for how to safely use radioactive sources. Electron beam facilities, which do not have radioactive sources, at this point require less regulatory oversight. Others may wonder if "radiophobia" will prevent the acceptance of irradiation. Actually, most of the American public is prepared to accept irradiation when the benefit of pathogen elimination is clear. For example, it makes sense to use irradiation to sterilize surgical supplies. The population has shown that it is also generally willing to accept X-rays and microwaves as essential to medical diagnosis and convenient cooking, respectively. In the future, a logo indicating that a food product has been irradiated will make it easy for consumers to identify food that has been treated.

Some people may be concerned that gamma ray sources cobalt 60 and cesium 137 are produced in nuclear power reactors. However, this is true of many other radionuclides used routinely in industry, science, and medicine as tracers and treatment agents. Even if nuclear energy is no longer used for commercial power generation in the future, these radionuclides will still be produced in smaller scale reactors. Electron beams and X-rays, of course, do not use radioactive sources and have no link to nuclear energy.

Finally, some people may object to the use of irradiation because it might allow standards to slip in the food industry if irradiation is substituted for other efforts to sanitize the food supply. Actually, combining irradiation with increased sanitation is advantageous because less contamination means lower doses of irradiation would be needed, decreasing the chance of changes in taste or smell of a product. This concern may not be fully resolved until the food industry demonstrates that irradiation is only used in concert with other methods that maintain food sanitation.

Many concerns about irradiation harken back to earlier objections to pasteurization and retort canning. Progressive development in processes and regulations of both technologies ultimately brought about a high measure of safety. The debate between those advocating improved sanitation and those advocating a definitive pathogen reduction technology was finally resolved when both strategies were combined. Instituting pretreatment standards and meat grading would ensure that meat would be clean enough to irradiate. Both milk pasteurization and retort canning became codified with a defined log kill against specific organisms, so that treatment in one place was comparable to treatment in another. Similarly, as the food irradiation industry becomes organized, the process should be defined so that the word "irradiated" will have a standard meaning, thereby ensuring uniform applications. Finally, both pasteurization and retort canning are used to treat food just before or in the final packaging step, at a point when the opportunity for recontamination of the food is minimal. Irradiating food in the same manner will increase confidence that it is not contaminated.

The Centers for Disease Control and Prevention, along with the World Health Organization and many other health organizations, welcomes the use of food irradiation as an important technology that can protect the public against foodborne diseases (28-30). Like pasteurization and retort canning, irradiation is a safe and effective food processing step. Preventing foodborne diseases requires a "farm-to-table" strategy with multiple control steps used along the way. For some foods, this includes a measure that eliminates pathogens definitively. Defined standards and norms for the process of irradiation could enhance general acceptance of this technology, and it would benefit the food industry to begin developing them. Irradiation procedures can be monitored and regulated as are procedures for pasteurization and medical sterilization. The potential benefit of irradiating meat and poultry alone is substantial; it could prevent hundreds of thousands of foodborne illnesses, thousands of hospitalizations, and hundreds of deaths each year. Using these promising technologies is critical to meeting national goals for foodborne disease prevention by 2010.

Table 3. Potential number of health problems prevented annually if 50% of meat and poultry is irradiated

Pathogen	Cases	Hospitalizations	Major complications	Deaths
E. coli O157:H7 and other STEC	23,000	700	At least 250 cases of hemolytic uremic syndrome	20
Campylobacter	500,000	2,600	250 cases of GBS	25
Salmonella	330,000	4,000	6,000 cases of reactive arthropathy	140
Listeria	625	575	60 miscarriages	125
Toxoplasma	28,000	625	100-1,000 cases of congenital toxoplasmosis	94
Total	881,625	8,500	6,660 catastrophic illnesses	352

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Public/Private Sector Partnership For Emerging Infections

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This paper gives examples of public/private partnerships that support research, support drug development and that advance policy development, suggesting that such partnerships can advance our understanding and control of emerging infections. The investment in emerging infectious diseases from government and from industry is currently much larger than that from philanthropy. Nevertheless philanthropy, even with limited dollars, is able to play a catalytic function and provide risk capitol for innovative partnerships and could in the future play an even larger role if the value of such investment is better defined and argued to recruit additional dollars to this area.

Emerging infectious diseases cause a disproportionate burden in developing countries, hindering economic and political advancement. Because of global interdependence, modern transportation, trade, and changing social and cultural patterns, emerging or reemerging infections are also threats to the United States (1). Therefore, the United States has a vital and direct stake in the health of people around the world, both out of humanitarian concern and enlightened self-interest.

In the battle against infectious diseases, drugs, vaccines, and pesticides are important weapons. Given the lack of substantial markets for such products in developing countries, public/private partnerships are essential to the development of new ways to prevent and treat infectious diseases in those populations that are poor and where the burden of disease lies, largely in the developing world.

Kinds of Partnerships

Our understanding and control of emerging infections can be advanced by several types of public/private partnerships, such as those that support research, develop drugs and vaccines, or advance policy development.

The role of philanthropical investments in partnerships that support medical research is also an important consideration. Strong investments in public health agencies, both in facilities and programs, will enable public/private partnerships to reach their full potential.[1]

Partnership To Support Research: Sequencing the Malaria Genome

Sequencing the genomes of microorganisms, those that either afflict millions of persons primarily in poorer parts of the world or those that afflict persons in both the developing and developed worlds in much smaller numbers, needs champions among public or philanthropic funders of biomedical research because corporations will generally not be interested in research on such organisms.

Sequence information can be essential to identifying therapeutic approaches to diseases such as malaria. In response to the rising global incidence of malaria, the Burroughs Wellcome Fund (BWF) has joined with the Wellcome Trust in the United Kingdom, the U.S. Department of Defense, and the National Institute of Allergy and Infectious Diseases (NIAID) to support the Malaria Genome Project, an international effort to sequence the genetic code of *Plasmodium falciparum*, the major causative agent of malaria. This partnership has supported not only the sequencing of the parasite's 14 chromosomes but also the development of databases and new tools for studying the expression of the newly identified genes. The sequencing is being carried out at the Sanger Center, at The Institute for Genomic Research (TIGR) (with colleagues from the Department of Defense) and at Stanford University. Data from this project already have provided new insights into the parasite's biology and have helped advance vaccine research (2). The malaria research community will participate in a "jamboree," similar to that held by scientists working on *Drosophila*, at the end of 2001 to finish the sequencing of the malaria genome.

The experience of funding this large-scale project has been even more rewarding than BWF had anticipated. Along with the new scientific insight it has provided, the Malaria Genome Project has grown into a model consortium that comprises a collaborative and interactive group able to work with scientists locally and globally. Funders and scientists have developed a strong relationship that allows "just in time" acquisition of resources and an agile, responsive approach to planning. In addition, flexibility in the project's funding (because different organizations can support different aspects) has allowed the consortium to take advantage of new technologies and techniques. For example, the Burroughs Wellcome Fund was able to provide seed dollars to convene the scientific community to

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[1] For more information about public/private partnerships, see Roy Widdus' work at www.ippph.org. The site identifies all significant public/private partnerships and their origins, aims, governance structures, modus operandi, degree of success, constraints, and difficulties. The goal of the project is to assist in the creation of new, effective partnerships.

discuss needs, select the strain to be sequenced and provide funding for pilot work. The National Institute of Allergy and Infectious Diseases and the Wellcome Trust provided funds for sequencing while the Department of Defense provided expertise and tool development.

Partnership for Drug Development: Medicines for Malaria Venture

The Medicines for Malaria Venture (MMV) is a new public/private partnership developed under the umbrella of the World Health Organization's (WHO) Roll Back Malaria Program (Ridley, pers. comm.). Like the Malaria Genome Project, the driving force behind the creation of MMV is the disease burden of malaria in the developing world. The lack of vaccines and increasing problems of drug resistance to the available drugs mean that new antimalarial drugs are urgently needed.

Under the leadership of Win Gutteridge (WHO/TDR), a strategic planning group was assembled from persons representing large pharmaceutical companies, development agencies, foundations, and international health organizations. The planning group confirmed the urgent need for new antimalarial drugs but also noted that the market could not support the high cost and risk associated with pharmaceutical drug company research and development (R&D) (as much as $500 million per drug candidate). Since expertise in pharmaceutical R&D resides with industry, the planning committee sought to combine public and private sector resources and expertise to "lower the risk" of drug development and encourage industry to make new drugs.

MMV is set up as a not-for-profit business with a mission of fostering and financing the discovery and development of new, affordable antimalarial drugs. The organization's goal is to have one new product granted regulatory approval every five years and to make arrangements for the products' commercialization. With each project, appropriate intellectual property would be owned by MMV with commercialization through out-licensing.

To carry out its mission, MMV created a "public venture capital fund" to support R&D projects on a competitive basis. MMV accesses knowledge, experience, gifts-in-kind, and, if appropriate, money from the private sector. However, MMV seeks most of its financial resources from the public and philanthropic sector.

As of May 2000, MMV has been constituted as an independent foundation in Switzerland, the board has been appointed, a CEO selected, the business plan completed, and a portfolio of R&D projects funded. Although $30 million per year by 2004 is required to meet the business plan, $15 million was raised for 1999/2000. MMV has received support from development agencies, foundations, industry, and health agencies. The organization is now selecting a second round of projects after a promising selection of three first-round projects from 101 applications.

Besides public/private partnerships to develop new drugs, some groups have programs to control infectious diseases in the developing world using donations of existing drugs. Industry has made substantial donations to programs against onchocerciasis, lymphatic filariasis, drug-resistant malaria, trachoma, and leprosy that involve donations of Mectizan, Albendazole, Malarone, Zithromax, and Leprosy MDT, respectively. Sixty percent of corporate contributions to philanthropy were product donations (3). In all cases the contribution from the company has gone far beyond the provision of the drug to supporting development of systems that will ensure the efficient distribution and effective use of donated drugs.

The incentive for this kind of project is altruism, which has obvious limits in the competitive commercial environments. Ventures that provide both "push" and "pull" interventions are more likely to be sustained. A company or partnership underwriting the cost of research and development is a push intervention. Creating a market for the drugs or vaccines being developed is a pull intervention. MMV uses both strategies, supporting drug development and working through WHO's Roll Back Malaria activity and other partners to assure the existence of a market for the drugs.

Partnership for Policy Development: Institute of Medicine Emerging Infections Activities

In May 1989, Rockefeller University, the National Institute of Allergy and Infectious Diseases, and the Fogarty International Center cosponsored a conference on emerging viral agents. Although the conference focused on viruses, it spurred interest in the emergence and resurgence of all classes of infectious agents. Subsequently, the Institute of Medicine (IOM) convened a panel and carried out a study under the leadership of Joshua Lederberg and Robert Shope that resulted in the 1992 report Emerging Infections: Microbial Threats to Health in the United States (4). Funding for this study was provided by the Centers for Disease Control and Prevention (CDC), the Fogarty International Center, Lederle-Praxis Laboratories, the Lucille B. Markey Charitable Trust, the National Institute of Allergy and Infectious Diseases, the Rockefeller Foundation, and the U.S. Army Medical Research and Development Command. From the beginning, the broad support from private and public agencies provided the foundation for an ongoing partnership for policy development.

The report called for increasing investments in the public health infrastructure, especially in surveillance, research, and training; in the development and deployment of vaccines and antimicrobial drugs and the control of resistance; vector control; and research on personal and community health practices relevant to disease transmission. Perhaps the most effective response to the report came CDC under the leadership of Walter Dowdle, James Hughes, and Ruth Berkelman, who put together a CDC plan for addressing the issues raised in the report (5). This plan galvanized congressional attention, and with the advocacy of groups like the American Society for Microbiology and others, drew attention to the need for additional resources and investments in treating and preventing emerging infections. Subsequently, in partnership CDC and NIAID asked the IOM to establish the Forum on Emerging Infections as a convening ground for public and private agencies to address continuing issues and problems related to emerging infections. The Burroughs Wellcome Fund also supports this activity in which policy makers can address the problem of emerging infections.

Philanthropic Investments

What role can philanthropy play in public/private partnerships to address emerging infections? Foundations are uniquely qualified to initiate thought and action, experiment with new and untried ventures, dissent from prevailing attitudes, and act quickly and flexibly (6). Several kinds of foundations are common. Independent foundations, such as the Bill and Melinda Gates Foundation, are usually established by an individual. Company-sponsored foundations include the

Merck Foundation. The American Cancer Society is an example of an operating or special interest foundation. The Research Triangle Community Foundation is a community foundation, which usually raise and manage money from different donors and direct the contributions locally.

Foundation type as much as size influences patterns of giving and growth. In 1999 there were 47,000 foundations in the United States, many of them small family foundations with assets of about $1 million. In the United States, foundations with assets of $50 million or more represent 2% of foundations, yet control 71% of total assets (7). In 1999, total giving in the United States amounted to $190.16 billion (8). Most contributions, nearly $160 billion, came from individual donations; foundations contribute nearly $20 billion, and corporations and their foundations more than $11 billion (60% in the form of product donations). Health care received only 9.4% of philanthropic donations (Figure 1) (8). In contrast, in 1996 U.S. health research and development expenditures were close to $38 billion, with industry investing more than half of those dollars and the foundation contributions amounting to only 4% of the total (Figure 2) (9). A 1997 survey of private funders of biomedical research in the United States showed that $1.3 billion was invested that year. Thus philanthropic support of $1-2 billion (10) for medical research is small in comparison with that from NIH (1999 budget $15.6 billion) or that from industry ($22 billion).

The entry of the Bill and Melinda Gates Foundation in support of international health R&D has had a stunning effect because of the comparatively large amount of money it has committed to the philanthropically undervalued area of international health. Perhaps, other foundations could be recruited to support this area during the anticipated transfer of wealth projected over the next decade if the public health community makes a cogent case for the need and value of such investment.

Philanthropic organizations can move quickly to fill a gap, function as neutral conveners, model successful approaches, develop information for policy debate, fund

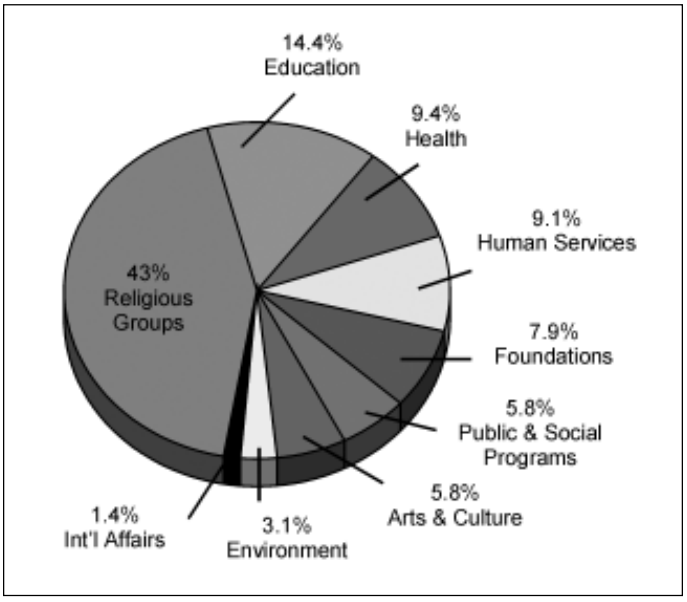

Figure 1. Giving in 1999: contributions received by type of recipient organization in the United States (8)

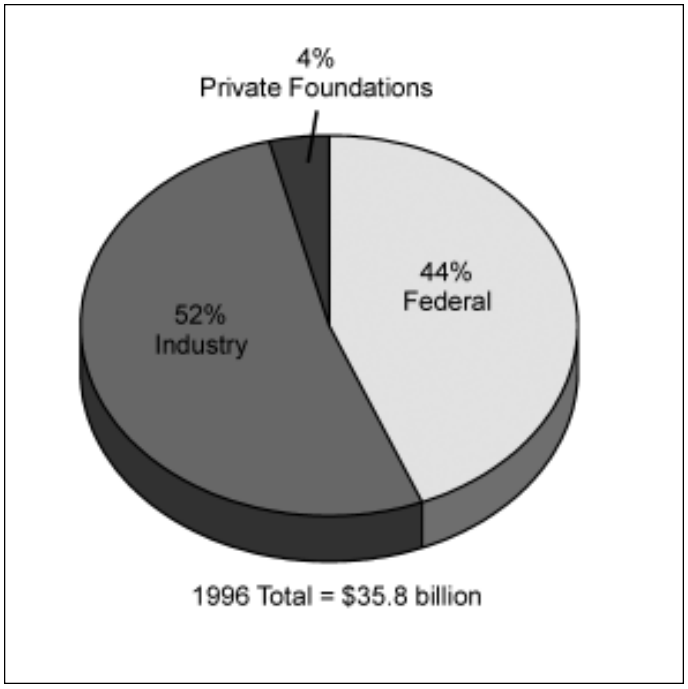

Figure 2. U.S. health research and development expenditures (9)

politically unpopular areas of research, and take risks. Drawbacks of philanthropic support include limited funds for research, less willingness to support overhead or infrastructure, the desire to model programs and move on, and the tendency to resist collaborative ventures. Thus, philanthropic organizations can be catalysts for developing public/private partnerships, but these groups do have limitations because they cannot commit as much money to emerging infections research as industry or government agencies.

Conclusions

The following lessons can be derived from these examples of public/private partnership and an examination of philanthropic capacity:

1. Philanthopic support, though important as risk capital in the system, is modest in comparison to industrial and government support for medical research.
2. The amount of wealth expected to be transferred during the period 1990-2040 has been estimated to exceed $10 trillion (11), thus providing opportunities to capture additional dollars for medical/health research and international health.
3. Industry is an essential partner but needs both "push" and "pull" mechanisms to participate in drug and vaccine development for diseases that largely affect poor people.
4. Public health and government agencies need long-term, increased investments to advance knowledge, to develop vaccines and drugs, and to control emerging infectious diseases.
5. Owing to the complexity and global nature of the issues in emerging infections, partnerships are more important today than ever before.

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Adaptation of *Bordetella pertussis* to Vaccination: A Cause for Its Reemergence?

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In the Netherlands, as in many other western countries, pertussis vaccines have been used extensively for more than 40 years. Therefore, it is conceivable that vaccine-induced immunity has affected the evolution of *Bordetella pertussis*. Consistent with this notion, pertussis has reemerged in the Netherlands, despite high vaccination coverage. Further, a notable change in the population structure of *B. pertussis* was observed in the Netherlands subsequent to the introduction of vaccination in the 1950s. Finally, we observed antigenic divergence between clinical isolates and vaccine strains, in particular with respect to the surface-associated proteins pertactin and pertussis toxin. Adaptation may have allowed *B. pertussis* to remain endemic despite widespread vaccination and may have contributed to the reemergence of pertussis in the Netherlands.

Bordetella pertussis and *Bordetella parapertussis* are the etiologic agents of whooping cough or pertussis, a respiratory disease that is most severe in infants and young children. Compared to *B. pertussis*, *B. parapertussis* is isolated less frequently from pertussis patients (1% to 5% of pertussis patients in The Netherlands) and generally causes less severe symptoms. Pertussis is highly contagious, and, in the prevaccination era, nearly every child contracted this disease. The clinical course of pertussis is characterized by paroxysms, or bursts, of numerous, rapid coughs followed by a long inspiratory effort, which may be accompanied by a characteristic high-pitched whoop (hence the designation whooping cough). During such an attack, patients may turn blue due to lack of oxygen. In serious cases, this oxygen deprivation may lead to brain damage. The most common complication of pertussis, however, is secondary pneumonia. Young infants are at highest risk for pertussis-associated complications. More than 50% of infants less than 6 months old who contract pertussis require hospitalization. Treatment of pertussis is primarily supportive, and adequate control of the disease depends on effective immunization. Before vaccination was introduced in the 1950s, pertussis was a major cause of infant death throughout the world (1). Widespread vaccination of young children has been successful in controlling the disease (1).

Vaccination

The high rate of illness and death caused by pertussis stimulated the early development of vaccines composed of whole, killed bacteria. These whole-cell vaccines were introduced in many countries in the 1950s and 1960s and have been highly successful in reducing the incidence of pertussis (1). The desire to avoid the side effects of whole-cell vaccines has stimulated the development of less reactogenic, acellular, vaccines composed of purified *B. pertussis* proteins (2). Acellular vaccines are replacing whole-cell vaccines in many countries.

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Despite vaccination, pertussis is an endemic disease. Various sero-epidemiologic studies have shown that the frequency of infection may be as high as 1% to 4% (de Melker and Schellekens, pers. comm.) (3). Further up to 30% of people with a persistent cough were found to have been infected with *B. pertussis* (4). It is possible that vaccination initially reduced the circulation of *B. pertussis* and that adaptation allowed the *B. pertussis* population to restore its high circulation rate. This assumption predicts a change in the makeup of the *B. pertussis* population after the introduction of vaccination, a phenomenon that has indeed been observed in The Netherlands.

Changes in *B. pertussis* in a Highly Vaccinated Population

In some countries with highly vaccinated populations such as Australia (5), Canada (6), and The Netherlands (7) (Figure 1), pertussis has reemerged. Such a phenomenon may have been caused by changes in the accuracy of notifications, decreases in vaccine coverage, or changes in vaccine quality. These possibilities have been excluded for The Netherlands (7), and we have proposed another possible cause: adaptation of *B. pertussis*

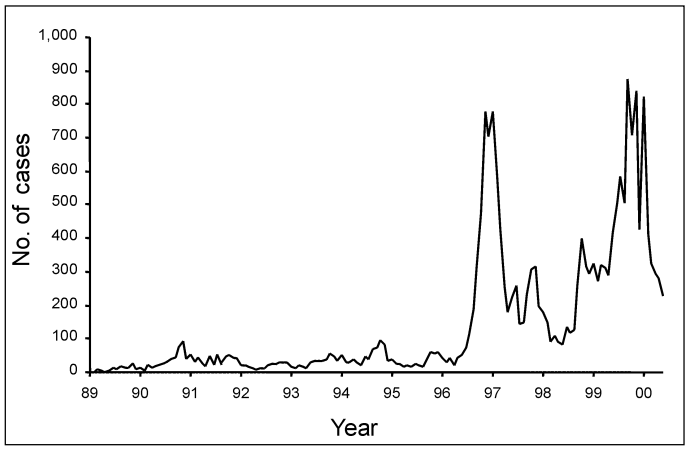

Figure 1. Notifications of pertussis in the Netherlands. Source: National Institute of Public Health and the Environment

to the vaccine. To investigate this hypothesis, *B. pertussis* strains collected in The Netherlands from 1949 to 1996 were characterized by DNA fingerprinting and sequencing of genes coding for surface proteins (8,9).

Initially, we studied changes in the *B. pertussis* population by *IS1002*-based DNA fingerprinting (9,10). Strains collected from 1949 to 1996 were stratified in periods of 5 to 8 years, and the frequency of fingerprint types in each period was determined (Figure 2). Widespread vaccination was introduced in The Netherlands in 1953, and we assumed that the *B. pertussis* population was not significantly affected by vaccination from 1949 to 1954 (defined as the prevaccination period in Figure 2). Notable differences were

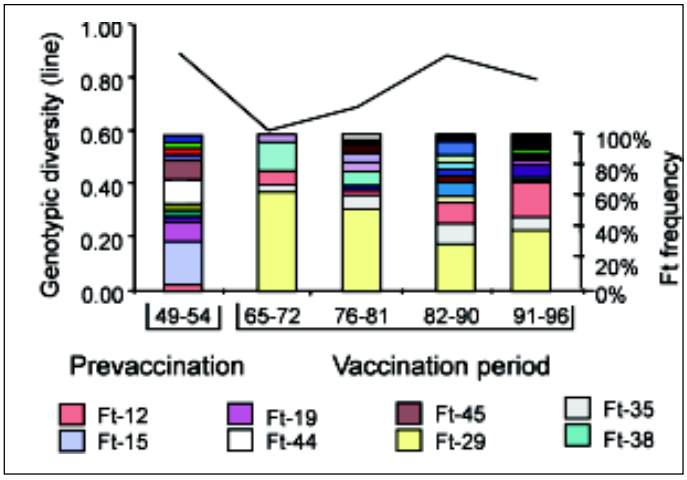

Figure 2. Changes in the population structure of *B. pertussis* in The Netherlands as determined by IS*1002*-based DNA fingerprinting. Strains isolated from Dutch patients from 1949 to 1996 were stratified in periods of 5 to 8 years. The frequency of fingerprint types (Ft) in each period was determined and is displayed by colored bars. Genotypic diversity was calculated according to Nei, using fingerprint data (11). Adapted from (10).

found between the populations from the prevaccination era and the subsequent period, both in the type and frequency of fingerprint types (e.g., the major fingerprint type found in strains collected from 1965-1972 [Ft-29] was absent during the prevaccination period). These qualitative observations were confirmed by the trend in genotypic diversity (Figure 2). Genotypic diversity decreased significantly after the introduction of vaccination and subsequently increased to prevaccination levels. In the 1980s, a second decrease in genotypic diversity occurred. Apart from sampling artifacts, a drop in genotypic diversity may be caused by a decrease in population size or clonal expansion. Indeed, we found that the reduction in genotypic diversity in the 1960s and 1980s was associated with the expansion of antigenically distinct strains.

In a second study of changes in the *B. pertussis* population, we investigated whether antigenic shifts had occurred in surface proteins (8). Very little polymorphism was observed in most proteins studied. However, two virulence factors, pertussis toxin and pertactin, were polymorphic. Interestingly, antibodies against these proteins correlate with protection against disease, which suggests they have an important role in inducing host immunity (12,13). Essentially, all DNA polymorphisms observed were nonconservative, indicating Darwinian selection. Three pertactin and three pertussis toxin variants were found in the Dutch *B. pertussis* population (Figure 3). Polymorphism in pertussis toxin was restricted to the S1 subunit (PtxS1), which carries the toxic activity. Variation in PtxS1 was observed in two regions. Significantly, one of the polymorphic residues has been implicated in binding to the T-cell receptor (14).

Polymorphism in pertactin was confined to a region comprised of tandem repeats located proximally to the RGD

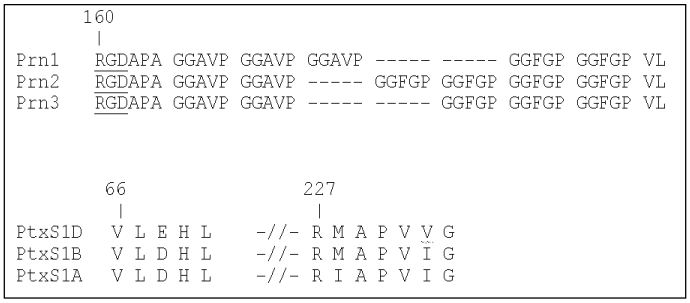

Figure 3. Polymorphism in pertactin (Prn) and the S1 subunit of pertussis (PtxS1) toxin found in Dutch strains. The RGD sequence in pertactin, involved in binding to host receptors, has been underlined. Dashes indicate gaps. Numbers refer to positions of amino acids relative to the N-terminal methionine.

motif involved in adherence to host tissues (15). Regions with tandem repeats are known to undergo rapid variation due to slipped-strand mispairing during replication (16). Pertactin and PtxS1 variants, identical to those included in the Dutch whole-cell vaccine, were found in 100% of the strains from the 1950s, when the whole-cell vaccine was introduced in The Netherlands (Figure 4). However, nonvaccine types of pertactin and PtxS1 gradually replaced the vaccine types in later years and were found in 90% of strains collected from 1990 to 1996. These results suggest that vaccination has caused strains that are antigenically distinct from vaccine strains to be selected. The drop in genotypic diversity observed in the 1960s and the 1980s coincided with the emergence of nonvaccine-type pertussis toxin and pertactin variants, respectively, suggesting that the drop was caused by clonal expansion. Antigenic divergence between vaccine strains and clinical isolates was also observed in other

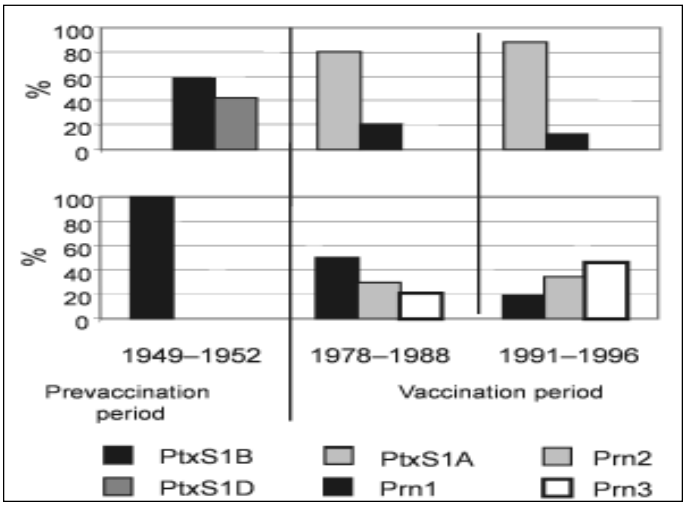

Figure 4. Temporal trends in frequencies of pertussis toxin and pertactin variants in The Netherlands.

countries with a long history of pertussis vaccination, such as Finland and the United States (17,18), and also in Italy, where vaccine coverage has varied considerably.

Discussion

Is polymorphism in pertactin and pertussis toxin driven by host immunity, or is it the result of random fixation due to genetic drift? The latter possibility is highly unlikely since essentially all DNA mutations we detected in the pertactin and pertussis toxin genes were nonconservative. In contrast, random genetic drift is characterized by a high degree of conservative mutations in protein coding regions (20). Further, the polymorphic regions interact directly with the immune system. The polymorphic region of pertactin induces a protective immune response (unpublished data). One of the polymorphic residues in PtxS1 has been implicated in binding to the T-cell receptor (14). Finally, the fact that the same temporal trends in allele frequencies are observed in geographically distinct regions such as Finland, the United States, and The Netherlands argues against random genetic drift. It is possible that the polymorphic loci we have identified are linked to other, as yet unknown, polymorphic loci that increase fitness of strains in vaccinated populations (hitchhiking).

Strains carrying nonvaccine-type pertactin or pertussis toxin variants were not found in the prevaccination era. Although the number of strains analyzed from this period was limited, these data suggest that the nonvaccine-type variants are not able to displace the vaccine-type strains in unvaccinated populations (i.e., they have a lower fitness level, or reproductive rate, in unvaccinated communities). Alternatively, the nonvaccine-type strains may have evolved relatively recently. Consistent with the first hypothesis, we have observed that nonvaccine-type strains are less fit in naive mice than vaccine-type strains. In immune mice the difference in fitness between the two types of strains was much less pronounced (unpublished data). Thus vaccination has acted to shift the competitive balance between strains.

An important question to address is whether adaptation of the *B. pertussis* population has affected vaccine efficacy, i.e., contributed to the reemergence of *B. pertussis*. Animal experiments have indicated that variation in pertactin affects vaccine efficacy (unpublished data). Further, we found vaccine-type pertactin variants less frequently among vaccinated persons than among unvaccinated persons, which would be expected if the vaccine protects differentially against strains with distinct pertactin types (8). However, the extent to which polymorphism affects vaccine efficacy is probably dependent on the vaccine used. It is conceivable that the increase in fitness associated with nonvaccine types of pertactin and pertussis toxin in vaccinated populations is substantial enough to drive expansion of strains carrying these protein variants but that the effect is too small to result in a measurable drop in vaccine efficacy. Further studies are required to assess the effect of the observed adaptations on the efficacy of pertussis vaccines. In this period, when whole-cell vaccines are being replaced by acellular vaccines in many countries, continued strain surveillance is of paramount importance.

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Strengthening Capability for Malaria Research in Africa

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The Task Force on Malaria Research Capability Strengthening (RCS) in Africa, coordinated by the United Nations Development Programme, the World Bank, and the World Health Organization (WHO) Special Programme for Research and Training in Tropical Diseases (TDR), represents a collaborative funding strategy in which multiple agencies and governments promote capacity-building activities in the context of the Multilateral Initiative for Malaria in Africa (MIM). This task force was established to promote human resource development and strengthen research capacity in malaria-endemic countries.

A total of 112 proposals involving 42 countries (25 from Africa) and over 200 partner institutions/research groups have been reviewed since the program's inception in 1998. The proposals covered a wide range of aspects of malaria research, including the clinical and molecular basis of drug resistance, drug policy, immune responses to *Plasmodium* infection, evaluation of natural products for antimalarial activity, parasite diversity, home management, vector biology, and epidemiology of malaria. The task force has recommended for funding, 23 full proposals involving 24 African and 8 European countries and the USA, with annual budgets ranging from US $60,000 to US $250,000. Twenty Ph.D. and 17 M.Sc. training grants were also approved in connection with the funded projects. In addition, support was also recommended for a few proposals that would promote interactions between partners for improving protocols and collecting preliminary data.

Task Force Objectives

The objective of the malaria RCS grants is to develop or strengthen, core African research groups' (engaged in basic or applied science) capacity for producing effective control tools for malaria and improving relevant health policy strategies. The task force believes there is an urgent need to attract scientists with new skills to foster genuine partnerships based on national and regional priorities, mutual and complementary scientific objectives, expertise, and shared responsibility. The partnerships will also provide opportunities to study specific aspects of malaria at multiple sites.

Research Priorities

The task force encourages research projects or programs to help establish networks focusing on the following areas, including multidisciplinary cross-cutting innovative approaches.

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Antimalarial drug policy and chemotherapy—development of strategies for rapid mapping of drug resistance; innovative approaches for preventing, retarding, and reversing drug resistance; definition of criteria for replacing first-line drugs.

Epidemiology—the use of new technologies to identify parasite diversity and its effect on immune responses; development of methods to measure the impact of interventions; and development of simple and rapid epidemiologic methods for mapping malaria morbidity and mortality.

Pathogenesis and immunology—studies on parasite-vector-host factors involved in severe disease and malaria in pregnancy, with the aim of developing and promoting improved control and management strategies and of evaluating potential vaccine candidates.

Entomology and vector studies—screening of natural local products for insecticidal and repellent properties, application of molecular tools for studies on vector biology, feeding behavior, vector capacity, insecticide resistance, and population genetics with the aim of identifying and developing effective strategies for vector control in settings of low and high transmission.

Health systems research, including social science—improvement of the home management of malaria on the basis of community knowledge, community practices, and the development of new products.

Natural products and antimalarial drug development—promotion of systematic identification and chemical and biological screening with in vitro and in vivo systems for isolating antimalarial compounds from natural products used by the indigenous populations for treatment of fevers.

Project Profile

Proposals should be submitted and coordinated by an African national scientist working in a research group in Africa, and should include at least two African research partner institutions (one established and one emerging) and at least one non-African partner, which could be an international institution with a base in Africa. All proposals must describe in detail a plan for strengthening research capability. The grants are awarded on the basis of scientific merit, relevance, and quality of partnerships that promote capacity building and human resource development in Africa.

Enhancing Factors

Activities of the task force have been enhanced by the use of unique strategies for identifying and managing the research projects. These include the following:

Strengthening research capability through sound research projects.

Developing a select group of projects around the interface of bench work/control operations.

Focusing the research agenda in priority areas.

Maximizing training opportunities.

Working for more rapid funding and implementation.

Encouraging research collaboration among developing country scientists and among developing and developed country scientists.

Promoting higher funding levels, including salary supplementation, for principal investigators to enhance long-term career commitment to research in Africa.

Task Force Progress

The activities of the MIM/TDR-RCS have produced the following results:

1. Continental networks for malaria mortality/demographic surveillance have been enhanced, including mapping the risk of malaria and determining the relationship(s) between transmission intensity and control activities in Africa. These networks have hubs in South Africa, Mozambique, Burkina Faso, and Ghana with 31 collaborating sites in 15 countries in Africa.

2. A model for collaboration between research scientists, malaria control personnel, and policy makers has been developed (Nigeria).

3. The impact of environmental modification for agricultural activities on malaria transmission and morbidity has been evaluated (Benin and Ivory Coast)

4. Simple molecular assays have been developed for the surveillance of drug-resistant malaria, and results have been used to make evidence-based malaria control policy decisions (Mali and Tanzania)

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5. Information has been obtained on the contributions of tumor necrosis factor and soluble receptors to the pathogenesis of cerebral malaria and severe malarial anemia (Ghana).

6. National capacity has been strengthened in African countries for rational selection of insecticides used in vector control (South Africa, Benin.)

7. Biodiversity prospecting (discovery and development of biochemical and genetic resources from plants, animals, and microorganisms to be used in biotechnology applications) has begun for new antimalaria compounds and insecticides (Kenya and Nigeria).

8. Five African research centers have been established to develop strategies for rapid mapping and control of drug-resistant malaria (Ghana, Nigeria, Malawi, Mali, and Tanzania).

Other accomplishments of the MIM/TDR-RCS include conducting the following group learning activities between March 1988 and March 2000:

Workshop on Research Capacity Development in Africa, March 1999.

Workshop on Markers of Antimalarial Drug Resistance: Practical, Clinical, and Epidemiological Applications, June 1999.

Laboratory Training on Molecular Markers of Antimalarial Drug Resistance, January-February 2000. Jointly organized by the MIM/TDR–RCS, National Institutes of Health (NIH), Malaria Research and Reference Reagent Resource Center (MR4), and Malaria Research Training Center, Bamako.

Workshop on Handling and Managing Biological Materials, March 2000. Jointly organized by the MR4, American Type Culture Collection, MIM/TDR-RCS, and Centre Nationale de Lutte Contre le Paludisme, Burkina Faso.

Mini-Symposium on Biological Resource Centers in Africa: Creating New Research Opportunities in Malaria, March 2000. Jointly organized by the MIM/TDR–RCS and MR4.

The success of this initiative will be confirmed by the existence of a sustainable malaria research community, which is capable of implementing relevant public health interventions and policies across Africa. The MIM/TDR program represents an innovative approach to accomplish this goal.

Toward a National Laboratory System for Public Health

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Currently, there is no cohesive national system of laboratories to support community health activities. One vision for such a system would be a cooperative arrangement of public health, hospital and independent laboratories that address community needs (R.A. Martin, pers. comm., 2000; 1-3). The rationale for this system includes the following:

- Laboratory services for infectious and toxic agents are vital to community health.
- Information technology has made real-time data sharing on a large scale possible.
- Most communicable disease diagnosis and reporting occurs in private sector laboratories.

Public health laboratories provide these essential services: case finding in high-risk groups, outbreak detection, emergency response, environmental monitoring, and disease surveillance. Public and private laboratories have complementary (not competitive) roles in ensuring the health of their communities.

A national laboratory system would ensure the availability of consistent laboratory capacity for public health across the nation. Public health, hospital, and independent laboratories currently have a loose, inconsistent association; relationships between public and private partners are underdeveloped, and multiple barriers prevent information sharing. Federal initiatives have been categorical, rather than system building. Disease reporting is inadequate, and public health surveillance and response are compromised.

State and local public health laboratories can serve as a focal point for a national system, through their core functions (3): 1) disease prevention, control, and surveillance; 2) integrated data management; 3) reference and specialized testing; 4) environmental health and protection; 5) food safety; 6) laboratory improvement/regulation; 7) policy development; 8) emergency response; 9) public health related research; 10) training and education; and 11) partnerships and communication.

Hospital and independent laboratories have an important role to play as well, by participating in the system development process, submitting samples and isolates of public health importance, cosponsoring local, state, and national meetings, and helping develop standard methods and procedures for public health situations.

Professional organizations can contribute by helping develop and maintain state and regional databases of services, convening meetings of state and national laboratory network constituents, and working to produce a consensus process to determine which services will be available.

Federal agencies, especially CDC, must provide national leadership for system development in several ways: developing funding and reimbursement mechanisms; developing and promoting best practices guidelines; establishing an advisory body; developing and maintaining a Web-based information system that links CDC, public health, hospital, and independent laboratories; and focusing training needs on identified gaps.

Implementation of a national laboratory system for public health will hinge on state and regional initiatives and coordination. A consensus must be developed around the need for such a system, and the cooperation of public and private laboratories must be secured. Agreement must be reached about the roles of the respective participants, and federal agencies will need to provide active leadership. Examples of state and regional coordination activities might include (R.A. Martin, pers. comm., 2000):

- Establishing a collaborative network.
- Creating a menu of available services to support public health.
- Developing a state or regional database of constituent laboratories.
- Coordinating emergency response planning.
- Standardizing test methods for disorders and exposures of public health importance.
- Implementing active (vs. passive) surveillance systems
- Creating shared specimen delivery systems.
- Linking to other states, regions, and CDC.
- Building relationships with managed care organizations to ensure the flow of information and provision of specimens of public health importance.
- Addressing home and point-of-care testing issues that have public health implications.
- Establishing links to infectious disease physicians and infection control practitioners.
- Serving as an authoritative source on laboratory testing, as part of a national network.

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Early Opportunities for Prevention: Infections of Pregnant Women and Young Infants

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Infectious agents are a leading cause of pregnancy complications and contribute to serious illness, death, and disability in infants. Substantial prevention opportunities exist.

Role of Infectious Agents in Adverse Consequences of Pregnancy

A variety of studies have assessed the role of sexually transmitted infections (e.g., *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*) and other genital tract infections (e.g., those caused by group B streptococcus [GBS], bacterial vaginosis [BV]) in preterm deliveries. Although numerous studies have shown that the presence of each of these agents can increase the risk of preterm delivery, nearly every antimicrobial treatment trial failed to show a beneficial effect in reducing preterm deliveries or numbers of newborns of low birth weight. A consistently strong association between BV and preterm delivery has been identified, yet only two randomized controlled trials, out of approximately 15 clinical trials, found that antimicrobial treatment during pregnancy had a positive impact. The typical intervention trial targeted a single infectious agent, even though the agents used have broad effects on multiple organisms and interactions exist among genital tract flora. Future research approaches may benefit from abandoning the paradigm of the randomized controlled trial that attempts to vary a single parameter and instead adopting holistic approaches to address multiple pathogens in concert.

Maternal Immunization

Vaccination of pregnant women has a long history, and several vaccines are now routinely recommended during pregnancy. An essential factor to consider is that the placenta is an extremely complex immunologic organ, with highly selective placental transport of immunoglobulin G that is receptor-mediated and active. Vaccinations are given in the last trimester to avoid effects on organogenesis. Excellent safety profiles were observed for several vaccines given during a large perinatal project conducted by the National Institutes of Health from 1957 to 1966. Nevertheless, legal liability for manufacturers remains a barrier. The high background rate of congenital anomalies and fetal loss is compounded by the challenges of distinguishing temporal relationships from causal ones. Despite these factors, active research is proceeding on maternal immunization against GBS, *Streptococcus pneumoniae*, and respiratory syncytial virus (RSV) using polysaccharide and protein conjugate vaccines as well as RSV-subunit vaccines (PFP-2).

Control of Infectious Diseases Through Breast-Feeding

Breast-feeding reduces illness and death from infectious diseases in all socioeconomic settings. Maternal exposure and antibodies generally negate any impact of passage of infectious agents. The one rare, but important, exception is passage of retroviruses, including HIV, in cells. Breast milk contains cellular (e.g., T and B lymphocytes, neutrophils, macrophages) and noncellular (e.g., immunoglobulins, hormones, enzymes) components, both of which contribute to preventing infection. Exclusive breast-feeding is associated with the greatest reduction in illness and death from infectious agents when compared to results from partial breast-feeding. Models have estimated tremendous economic and health benefits of increasing the proportion of women who breast-feed; increases will only occur where breast-feeding skills are supported by both the health and social systems. Studies need to differentiate between exclusive breast-feeding, partial breast-feeding, and exclusive formula feeding to carefully determine risk-benefits for women and infants in particular settings.

Prevention Success Stories

Several features differentiate perinatal infections from other infectious disease syndromes: 1) the time during which disease transmission can occur is limited; 2) eradication of the pathogen from the mother is not essential in preventing transmission; 3) researchers have avoided testing in pregnant women and newborns because they are vulnerable populations; and 4) pregnancy provides opportunities to integrate public health response. During the 1990s, efforts to prevent perinatal transmission of HIV and GBS in the United States were highly successful, with an 86% reduction in perinatally acquired AIDS and a 70% reduction in early-onset GBS disease. Racial disparity was reduced significantly in both circumstances. Keys to success included advocacy, surveillance, support by professional organizations, and the integration of perinatal prevention programs.

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Teaming Up To Prevent Foodborne Disease

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Preventing foodborne disease requires the efforts of different segments of our "global" society. This session featured presentations on emerging issues across the spectrum of food safety activities, from science and technology to policy development and regulation.

Comprehensive Surveillance of Human Caliciviruses in The Netherlands

Norwalk-like caliciviruses (NLV) are increasingly recognized as a significant cause of food-related illness. In recent years, The Netherlands has conducted four NLV studies: i) a physician-based case-control study, 1996-99; ii) a population-based cohort study with a nested case-control design, 1999; iii) NLV testing of stools obtained from patients who became ill during gastroenteritis outbreaks reported through municipal health services; and iv) NLV testing in samples from animal surveillance systems. NLVs were the causative agent in 5% of patients with foodborne illness seen by a general practitioner and 16% of patients in the community cohort. In the past 6 years, NLVs have caused more than 80% of all gastroenteritis outbreaks. Multiple NLV variants cocirculate in the population, and their diversity allows researchers to trace outbreaks to a common source. Highly related viruses have been found in herds of calves and swine, suggesting that animals may be a reservoir. A project has been initiated to study transmission patterns of these viruses across Europe.

Reducing the Risk of Pathogens in Foods

Under Hazard Analysis and Critical Control Point (HACCP) systems, food manufacturers identify points where contamination is likely to occur and implement process controls to prevent it. A current limitation of the HACCP is that very few CCP practices are available for on-farm use, although several interventions appear to have promise. These practices include probiotic bacteria (benign bacteria that can be used to out-compete pathogenic bacteria) that prevent colonization by pathogens, edible vaccines that stimulate IgA production in an animal's gut, and improved farm management practices, such as reducing pathogen contamination in watering systems. Food processors have a larger array of current and promising control measures available, but most measures have limitations. For example, irradiation may cause undesirable "off" flavors in meat and poultry or undesirable texture characteristics in vegetables such as lettuce.

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Some treatments such as probiotic bacteria may be especially useful for protecting high-risk populations, such as the immunocompromised. Inevitably, consumers must also play a role in preventing foodborne disease. Consumers must be more active in such practices as avoiding undercooked and uncooked high-risk foods, refrigerating perishable foods, and disposing of hazardous foods that have been recalled.

Science as Basis for Regulations

The food industry bears responsibility for providing safe foods for consumption. A framework of laws, regulations, and an inspection system facilitates production of safe foods. Food safety regulations fall into two basic classes: process-based and performance-based. Future legislative and regulatory requirements will focus more on performance-based standards, leaving the specifics of how the standard is achieved to individual processors, although most will likely develop HACCP programs. Future regulations, however, will also consider international agreements, such as those that enable the World Trade Organization to impose obligations that nations must fulfill to enter into free trade.

Both international and domestic policy will increasingly rely on the discipline of risk analysis for decision-making. The need for better data to conduct risk assessment will spur increased emphasis on foodborne illness surveillance systems like FoodNet (1) and PulseNet (2). Results of risk assessments must be analyzed through the risk management process to yield food safety policies that lead to development of performance criteria that the food industry can use to develop safer food processes.

Borders? What Borders?

There has been a globalization of the food supply. Although no evidence supports the idea that imported food is less safe than domestic food, the Food and Drug Administration's (FDA) imported foods plan recognizes that some food safety issues, such as lack of regulatory authority and basic infrastructure, are specific to developing nations. Along with important surveillance and sampling activities, FDA provides international training and fosters technical cooperation aimed at prevention at the source of production. FDA's international partners are often from industry, nongovernment institutions, and universities. Other key partners include Food and Agricultural Organization, Pan American Health Organization, Instituto Interamericano de Cooperacion para la Agricultura, as well as the Centers for Disease Control and Prevention and the Foreign Agricultural Service. In 1999, FDA tested 1,000 samples of imported celery, cantaloupe, cilantro, green onions, parsley, loose-leaf

lettuce, strawberries, and broccoli for *Salmonella*, *Shigella*, and *Escherichia coli* O157:H7. To date, 95.4% of these samples have been free of contamination. FDA has worked closely with several countries (e.g., Costa Rica, Trinidad and Tobago, Honduras, and Jamaica) to determine their needs and capacities. Examples of training and outreach include a Regional Outreach Meeting on Food Safety in Mexico City, Mexico, and one in Santiago, Chile.

To paraphrase The Future of Public Health, the 1988 Institute on Medicine report, food safety is not just what government regulators or industry quality assurance managers do. Food safety is what society does to ensure the conditions under which people can consume food that is safe, as well as wholesome and nutritious. Safe food requires the work of producers and consumers; industry and government; local, state, federal, and, increasingly, international partners.

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Ethical and Legal Issues in Infectious Disease Research and Control

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Ethical issues in international research have received an increasing amount of publicity over the past decade, as more attention is focused on global health and expanded funding is provided for research in the developing world. Much of the discussion has focused on issues that arise when researchers from countries rich in resources collaborate with researchers from poorer countries. Some of the controversies result from research on vaccines and drugs for emerging infectious diseases. This panel addressed ongoing challenges to conducting ethical research.

Christine Grady from the National Institutes of Health reviewed existing international codes and guidelines for conducting research. Ethical concerns in international research often stem from the potential for study communities in the developing world to be vulnerable to exploitation because of their social and economic circumstances. Several international codes provide guidance on the ethical conduct of clinical research including the Declaration of Helsinki, Council for International Organizations of Medical Sciences (CIOMS), International Guidelines for Biomedical Research, and the UNAIDS Guidance Document on Ethical Considerations in HIV Vaccine Research. However, these codes are recommendations, not legal imperatives. In addition, they do not address how disagreements might be resolved. Dr. Grady mentioned some current ethical dilemmas, including determination of treatment provided to participants during the course of a clinical trial, the obtaining of informed consent, obligations of researchers to the study community, and investigators' responsiveness to local health needs. Although there are no easy answers, she suggested that basic ethical principles should be applied globally, with local interpretation and implementation.

Gita Ramjee from the South African Medical Research Council discussed ethical problems and solutions used by South African researchers conducting a phase-III trial of a vaginal microbicide to prevent HIV infection. Researchers realized that participants did not understand the details of the study. Investigators then modified the process to include role playing and to allow time for trial participants to consult with peers. Researchers also added a knowledge testing component to the trial. These changes suggest that informed consent is not a one-time event but is instead an ongoing process.

The need to minimize the risk of participants' contracting HIV during the study necessitated an intensive counseling component. Participants were informed about local HIV counseling services, and sex workers were encouraged to set standard prices and to refuse clients who would not use condoms. This study shows how practical solutions can be used to reduce risks associated with research.

Finally, Jean Pape from Les Centres GHESKIO in Port-au-Prince, Haiti, highlighted the major challenges to conducting international research, which center on obtaining ethical review or institutional review board clearance. See page 547 for a more detailed article concerning this discussion.

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Injection Safety

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Mathematical modeling of available epidemiologic data suggests that each year unsafe injection practices are responsible for 8 to 16 million persons contracting hepatitis B virus (HBV), 2.3 to 4.7 million persons contracting hepatitis C virus (HCV), and 80,000 to 160,000 persons contracting HIV worldwide. In most cases, the transmission of these agents goes unrecognized because the infection is initially subclinical.

An estimated 12 billion injections are administered annually worldwide. Injections are not the most efficient way to transmit HBV, HCV, and HIV, but because so many injections are given, and enough of them are unsafe, they account for a much larger proportion of bloodborne disease transmission than does unsafe transfusion. Global estimates of the percentage of unsafe injections range from 15% in Eastern Europe to 50% throughout Asia.

Injections are popular in many settings because they have important social meaning. Dispensing an injection communicates that the patient's problem is serious and that the provider is concerned and reinforces the special status of the healer in the community. The injection healing ritual usually brings comfort even if it appears irrational from a Western perspective. Because biomedical considerations do not motivate the injection, biomedical concerns with injection safety are often not emphasized.

Safe Injection Global Network (SIGN) was established in October 1999 as a voluntary association of stakeholders who share a common interest in safe and appropriate use of injections. SIGN associates agree to collaborate with other members for developing a common strategic framework and communication strategy. SIGN recommends a three-part, multidisciplinary approach to achieve safe and appropriate use of injections. First, behavior of health care providers and patients must be changed to decrease injection overuse and achieve safety. Second, sufficient quantities of appropriate injection equipment and infection control supplies should be available. Third, a sharps waste management system should be set up to ensure that disposable equipment is destroyed and not reused.

Before SIGN, a number of successful efforts have reduced injection overuse and improved safety. In Indonesia rates of injections decreased from 73% to 14% after group discussions between health care providers and patients. In Tanzania, avoidable injections decreased from 16% to 6% after guidelines to improve injection practices were developed and communicated. In Hafizabad, Pakistan, the proportion of injections conducted with a new sterile syringe increased from 24% to 60% after a health education program was conducted in mosques. Indeed, wherever a plan to promote injection safety has been implemented, it has brought about at least some success.

Vaccine programs are centrally important in promoting safe injections. By adopting safe injection practices, these programs can minimize adverse events resulting from their own intervention. These groups can also share their experience in developing reasonable, safe standards and practices and help catalyze change by being high profile early adopters of injection safety.

Persons interested in learning more about injection safety can visit the Web site www.injectionsafety.org.

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West Nile Virus: A Newly Emergent Epidemic Disease

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West Nile (WN) virus is a mosquitoborne virus of the genus *Flavivirus*, family *Flaviviridae*. WN, Japanese encephalitis, St. Louis encephalitis, Murray Valley encephalitis, and Kunjin viruses (along with other viruses) belong to the Japanese encephalitis serocomplex and are closely related to each other genetically and ecologically. The Japanese serocomplex of viruses has a near global distribution with some overlap. All the viruses are maintained in cycles involving birds as vertebrate hosts and mosquitoes (principally *Culex* species) as vectors.

WN virus has a wide geographic distribution in Africa, west and central Asia, the Middle East, and Europe. Historically, epidemics have been infrequent and not associated with severe disease. In the past decade, however, epidemics/epizootics have occurred in several countries, including Romania (1996, humans), Morocco (1996, horses), Tunisia (1997, humans), Italy (1998, horses), Israel (1997, 1998, 1999, domestic geese), Russia (1999, birds and humans), and the United States (1999, humans, birds, and horses). Severe neurologic disease and fatalities have occurred in all these outbreaks.

The 1999 epidemic/epizootic in New York affected humans (62 laboratory-positive cases of neurologic disease with 7 deaths), birds (thousands of bird deaths, with illness and death documented in 26 species), and horses (25 cases with 9 deaths). The epicenter of the outbreak was the Queens section of New York City, where more than half of the laboratory-positive human cases occurred. The outbreak in humans peaked in late August, with the first patient experiencing onset of symptoms on August 2, and the last on September 22. Virus transmission became widespread, and WN virus–positive birds, mosquitoes, or both ultimately were documented in New York, Connecticut, New Jersey, and Maryland. Virus isolation data suggest the 1999 outbreak was transmitted by *Culex* species mosquitoes, principally *Culex pipiens*. Overwintering mosquitoes of this species collected in January and February 2000 were found to be positive for WN virus.

Comparison of sequenced virus isolates from birds, horses, and mosquitoes and viral sequences amplified from human brain tissue in the 1999 New York outbreak confirmed that the viruses infecting all species were identical. Moreover, these viruses were identical (99.9% nucleotide homology) to a virus isolated in 1998 from domestic geese in Israel. Analysis of amino acid and nucleic acid sequences for a portion of the envelope gene of 40 WN virus strains from wide geographic areas produced pylogenetic trees showing that WN viruses segregate into two lineages. Lineage I includes viruses from Africa, all strains from north Africa, Europe, Israel, the United States, and Kunjin virus from Australia. Lineage II is composed only of strains from west, central, and east Africa and Madagascar. The data strongly suggest that the virus causing the 1999 New York epidemic/epizootic was introduced from Israel or the Middle East.

The epizootics in domestic geese in Israel over 3 years (1997, 1998, and 1999) and the strong genetic similarity among WN virus strains suggest that the virus may have persisted in the area in mosquitoes, ticks, or chronically infected birds. Alternatively, WN virus could have been reintroduced in migrating birds from Africa or from Europe. Israeli data provide strong evidence that WN virus is introduced in white storks. It is possible that both mechanisms are correct.

The recent epidemics/epizootics of WN virus in north Africa and Europe and the unprecedented epidemic/epizootic in the northeastern United States underscore the ease with which exotic pathogens can move between continents and regions today. These epidemics/epizootics also reinforce the need to rebuild the public health infrastructure to deal with epidemics of vectorborne diseases and to develop effective surveillance, prevention, and control strategies for these diseases.

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Emerging Zoonotic Diseases

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Emerging Zoonotic Bacterial and Parasitic Diseases

Corrie Brown from the University of Georgia discussed 15 diseases common to humans and animals, with a brief synopsis of how each disease is transmitted from animals to humans, the major animal reservoirs, and factors influencing the emergence of these diseases as human pathogens. The most important factors for emerging zoonotic diseases are (1) the transportation of humans and animals to new areas, (2) increased contact between animals and humans, (3) changes in the environment and husbandry practices, (4) a larger immunocompromised population, (5) increased recognition of diseases as zoonotic in origin, and (6) the discovery of new organisms not previously recognized. Domestic pets can transmit *Bartonella henselae*, *Sporothrix schenkii*, *Capnocytophaga carnimorsus*, *Echinococcus multilocularis* (alveolar hydatid disease), leishmaniasis, *Yersinia pestis* (plague), and ehrlichiosis. Emerging zoonotic disease agents transmitted by food animals include enteropathogenic *E. coli*, *Salmonella DT104* (which may have human origins), *Campylobacter* spp, and *Streptococcus iniae* (from farmed fish). The most common rat-transmitted disease in the United States is leptospirosis, which has a variety of serovars. Many of these diseases are difficult to diagnose and all of them can be fatal.

Pathology of Animal Models for Filoviruses

Nancy Jaax, Colonel of the Veterinary Corps, USAMRIID, discussed Marburg and Ebola viruses, the only known filoviruses. Both Marburg and Ebola viruses have several serotypes, and all are African viruses, with the exception of Reston Ebola virus, which was traced to the Philippines. The natural histories, reservoirs, and epidemiologies of the viruses are largely unknown. In humans, persons with illness caused by filovirus infection usuall have influenzalike symptoms, with subsequent disseminated intravascular coagulopathy (DIC) and often generalized bleeding from body orifices. Pathologically, there is early and sustained infection of the mononuclear phagocyte system. There is no cure or vaccine, and treatment is symptomatic. The infections are transmitted by direct contact, and all body fluids contain large amounts of the rapidly replicating virus. Nonhuman primates have introduced the virus into human populations, but the animals appear to be amplifiers rather than reservoirs of the disease. The outbreaks associated with the filoviruses have been infrequent and temporally widely spaced, and few pathologic tissues are available for study. Only a few animal models are suitable for research, and research requires biosafety level (BSL) 4 containment. Guinea pigs and mice have been experimentally infected, but are poor predictors of primate response to treatment.

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Exotic Birds as Sentinels for Human Disease

Tracy McNamara from the Wildlife Conservation Society discussed the West Nile virus outbreak that occurred in New York in the summer of 1999. More than 40 dead crows were recovered on the zoo grounds in the Bronx, New York. Necropsies ruled out bacteria, toxic pathogens, and several viral pathogens as the cause of death, but the exact cause remained elusive. Several North and South American birds died unexpectedly at the zoo in the following weeks. Abnormal pathologic changes were found in multiple organ systems in the birds, and encephalitis was only one of many manifestations of disease. The only common condition of the birds was that they were housed outdoors rather than indoors, but the petting zoo chickens and turkeys were unaffected. It was determined that a flavirus was causing the deaths, but further evaluation by the zoo was hampered by the lack of specific serologic tests for exotic animals and lack of adequate containment facilities. Emus are exquisitely susceptible to eastern equine encephalitis virus (an alphavirus), but the emus were healthy. From samples sent to USAMRIID, the birds were determined to have West Nile Virus. Birds are reservoirs for arboviruses but rarely die of them. In September 1999, several New York City residents were diagnosed with encephalitis caused by a flavivirus (originally thought to be St. Louis encephalitis virus but later determined to be West Nile Virus), and the connections were made. Zoo pathologists routinely take extensive tissue sections and blood samples on necropsied zoo animals, and these samples have been archived for more than 30 years. This archive may be valuable for future animal and human research.

Plague in the Americas

Alfonso Ruiz from the Pan American Health Organization discussed plague, which is caused by *Yersinia pestis* and has caused three major pandemics in the history of civilization. Please see "Plague in the Americas" on page 539 for a more detailed article about this portion of the panel discussion.

Electronic Laboratory-Based Reporting: Opportunities and Challenges for Surveillance

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Public health surveillance has been defined as the ongoing, systematic collection, analysis, interpretation, and feedback of outcome-specific data used for public health practice (1). Laboratory reports are critical to public health surveillance because they initiate investigations of cases of reportable diseases or outbreaks of infections. The current system of laboratory reporting, which often relies on paper reports delivered by mail, is slow and incomplete. Electronic laboratory-based reporting (ELR) is likely to be more timely and complete (2). A number of challenges must be addressed before ELR can be used effectively, but recent activities are encouraging, and new opportunities for ELR implementation have arisen.

ELR Challenges

The laboratory landscape is changing. Large national and regional laboratories have developed advanced information technology (IT) capabilites and use standardized test codes, making ELR possible. However, many smaller laboratories do not have the technology necessary for ELR. Additionally, many states have reporting regulations that are not structured for electronic reporting, and health department staff often have limited knowledge of electronic data interchange technology. In the past, public health agencies have focused more on epidemiology and statistics, and less on IT.

ELR Activities

In 1997, the Centers for Disease Control and Prevention (CDC) and other public health partners sponsored a meeting at which standards for pilot implementation of ELR were determined (3) (i.e., the electronic message format uses the national clinical information standard Health Level Seven [HL7](4), tests are coded with the Logical Observation Identifiers Names and Codes [LOINC] (5), and results are coded with the Systemized Nomenclature for Medicine [SNOMED](6) terminology. At a follow-up meeting in 1999 with broader participation from informatics specialists and representatives from the laboratory community, the group affirmed the ELR approach and planned further implementations (7).

ELR pilot activities have provided valuable lessons. In Hawaii, ELR increased the number of reports 2.3 times, reports arrived 4 days earlier, and demographic data were more complete (2). In Washington and Texas, the feasibility of using HL7 with standardized codes was demonstrated. In Minnesota and Oregon, commercial off-the-shelf messaging software has been used to receive standard electronic messages from national laboratories. Other projects are under way at various sites.

Recently, CDC initiated the National Electronic Disease Surveillance System (NEDSS) to improve public health surveillance through enhanced IT infrastructure and capability. Electronic messaging for clinical and laboratory reports is one of eight elements that will be implemented by using the NEDSS information architecture (8).

ELR Opportunities

A few large national laboratories contribute a substantial proportion of reports to health departments. Focusing on these laboratories and ensuring their ELR capacity early will likely produce important results. Some of these laboratories can report from a single data repository, which may allow more efficient and possibly enhanced surveillance. Another component of the NEDSS architecture, Web-based reporting, may permit smaller laboratories to more easily participate in ELR. CDC is working closely with standards organizations to ensure that public health needs are represented in national data standards.

Conclusion

Effective use of ELR will require overcoming several challenges: standardizing message format and coding, developing policy, and improving knowledge and skills for implementation. As part of the larger surveillance system integration initiative of NEDSS, ELR will likely be more accessible to public health partners and providers of public health reports. Opportunities exist for developing public-private partnerships with national laboratories. Public health issues are being incorporated in national standards development. NEDSS support to states will identify current ELR capabilities and provide an opportunity for health officials to examine IT issues.

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Plague in the Americas

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Plague, caused by the bacteria *Y. pestis,* is a disease of rodents and their fleas that occasionally is transmitted to other animals and humans. Three worldwide pandemics, causing millions of deaths, have been recorded. The last one, which began in the 19th century, reached the Americas.

A few natural plague foci are found in the Americas: in the western United States; in northern Peru; in Chimborazo Province, Ecuador; and in the Department of La Paz, Bolivia. Several foci are also located in the semi-arid regions of northeastern Brazil. The population at risk in these areas is estimated at more than 16 million.

From 1994 through 1999, plague was reported in five American countries: Bolivia, Brazil, Ecuador, Peru, and the United States; approximately 1,700 cases were recorded, with 79 deaths (Table 1).

Several rodents have been identified as reservoirs. The main vector is the rodent flea *Xenopsylla cheopis*, although other species have been identified, particularly, in the United States (Table 2).

Three clinical forms of plague are recognized: bubonic, septicemic, and pneumonic. The septicemic and pneumonic forms are usually secondary to the bubonic form, and the bubonic form is the most common in the Americas. It is characterized by swelling of cervical, axillary, and inguinal lymph nodes, depending on the location of the portal of entry of the bacteria. The incubation period is from 3 to 6 days. Hematogenous dissemination of the bacteria to other organs and tissues may cause intravascular coagulation and endotoxic shock, producing dark discoloration in the extremities (so-called black death).

Laboratory confirmation of all forms is encouraged, either by microbiologic methods or serologic demonstration of antigens or antibody titers.

Infection mostly occurs through flea bites; however, infection can become airborne when a patient with pneumonic plague coughs. Humans are exposed to infection in the outdoor or household environment. Infections in the wild usually cause isolated or sporadic cases; this occurs in the United States and Brazil, where most infected persons are Indians, hunters, miners, and tourists.

Household infections occur when people, domestic animals (especially cats), and peridomestic rodents bring infected fleas into the house, exposing more persons. Raising guinea pigs inside homes, as they do in the Andean countries, is an additional risk factor for outbreaks. These animals become infected and multiply the infection by sharing their fleas with humans. Persons might also become infected through skin injuries when preparing the guinea pigs for cooking. Houses constructed with thatched walls and roofs or adobe walls are highly vulnerable to rodent activities (seen in plague-endemic areas of Andean countries). Improper storage of crops in patio areas or in the roof provides easy food access for rodents, facilitating transmission of plague.

Increased rainfall in a geographic area causes extensive changes to the surroundings, which can lead to the displacement of wild fauna, including rodents. The resulting soil moisture may improve crop production, or any other mammal food resources, and lead to an increase of plague hosts. These effects are difficult to register and correlate because plague events occur years later after the meteorologic phenomenon and ecologic modification. This has been the case with the El Nino-Southern oscillation phenomenon, which caused an unusual amount of precipitation in northern Peru. The ecology was modified over a wide area, resulting in the development of new crops, which then helped the rodent population increase.

In addition, deforestation to gain new lands for agriculture in areas known as natural foci of plague will eliminate most of the rodent predators and provide additional food and shelter to wild rodents, facilitating their rapid reproduction. Such was the case in the province of Chimborazo, Ecuador, where the inhabitants planted wheat crops after deforestation.

Epidemiologic characterization of areas where plague is prevalent, a potential risk, or silent will help establish surveillance and prevention measures. Obtaining serologic specimens from dogs is an effective tool for identifying areas

Table 1. Plague: Reported Cases and Deaths, 1994–1999

	1994		1995		1996		1997		1998		1999	
Country	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
Bolivia	0	0	0	0	26	4	1	0	0	0	0	0
Brazil	0	0	0	0	1	0	0	0	0	0	0	0
United States	13	1	9	1	5	0	4	1	9	0	9	0
Peru	1,122	51	97	2	33	0	39	0	20	0	151	5
Ecuador	0	0	0	0	0	0	0	0	*160	14	0	0
Total	1,135	52	105	3	55	4	44	1	189	14	160	5

*Estimate

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Table 2. Plague in the Americas: Reservoirs and Vectors

Country	Reservoirs	Vectors
Bolivia	*Akodon* sp *Rattus rattus*	*Xenopsylla cheopis* *Pulex irritans*
Brazil	*Akodon* sp *Oryzomys* sp *Callomys* sp *Bolomy* sp *Monodelphis deomestica*	*X. cheopis*
Ecuador	*R rattus* *R. norvegicus* *R. alexandrinus* *Akodon mollis* *Oryzomys* sp *Phyllotis* sp *Scirurus stramineus*	*P. irritans*
United States	Marmot (*Cynomys* sp) Rabbits Rats (*Dipodomys* sp) Mice (*Peromyscus* sp) Terrestrial squirrel (*Citellus* sp)	*Orchopeas sexdentatus* *Oropsylla montana* *Haplosyllus* sp *Diamanus* sp *Thrasis* sp
Peru	*Akodon* sp *Oryzomys* sp *Sigmodon* sp *Phyllotis* sp *R. rattus* *Cavia porcellus*	*X cheopis* *Polygenes* sp *Tiamastus* sp *P. irritans*

where plague is prevalent because dogs are susceptible to *Y. pestis* infection. Although they rarely develop the disease, they can maintain detectable titers of antibodies for extended periods. Trapping rodents can also be used for surveillance to detect *Y. pestis* infection by microbiologic or serologic testing and for identifying the flea vectors.

Identifying and treating infected persons are priorities in plague-endemic areas. Streptomycin is the most effective antibiotic for treating plague. Tetracyclines are preferred for prophylactic use. Vaccination is not possible because no effective vaccines currently exist.

Education is appropriate in the areas where infection is known and where people are at risk. Messages can be delivered that take into account the local, cultural, and ethnic characteristics of the communities.

Flea surveillance and control with proper insecticides could be carried out by a local community. Periodic application of insecticides inside and outside homes is important in reducing the flea population in infected areas. Other prevention measures could be implemented on the basis of local risk assessments; for example, in Peru when improper storage of grains attracted rodents inside the houses, small silos were designed to store the goods.

We recognize that plague is still in the Americas and human population is rapidly growing. New lands are being used for new settlements, and new crops are being grown for food production. Many species of rodents can serve as reservoirs, not only for *Y. pestis* infection but also for other emerging infections, and at any moment a new outbreak might appear. Local, cross-cutting, and interdisciplinary approaches are encouraged to implement adequate surveillance of rodentborne diseases.

Intercontinental Transmission of West Nile Virus by Migrating White Storks

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In September and October 1998, West Nile (WN) virus was isolated from a flock of 1,200 migrating white storks (*Ciconia ciconia*) that had landed in Eilat, a town in southern Israel. Inclement weather conditions of strong, hot westerly winds had forced them to fly under considerable physical stress to reach Eilat. The storks were fledgelings, less than 1 year old, that had hatched in Europe. Analysis of blood samples taken from several birds within days of their arrival showed the presence of WN virus–neutralizing antibodies. Sequence analysis of the envelope glycoprotein gene of the stork isolate showed almost complete identity with a sample isolated from a dead goose in Israel in 1998.

Because this Eilat flock was migrating southward for the first time and had not previously flown over Israel, we assume that it became infected with WN virus in Europe. The presence of virus-neutralizing antibodies in stork serum samples collected from German flocks provided additional evidence that the birds contracted WN virus in Europe. These findings indicate that the recent epizootic of WN virus in Israeli geese had its origin in Europe, where the virus had been circulating in epidemic proportions since 1996. Epidemiologic studies of eastern European epidemics indicate that WN virus may now be endemic in southern Europe.

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Opportunistic Infections in Persons with HIV or Other Immunocompromising Conditions

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Henry Masur, from the National Institutes of Health, Bethesda, Maryland, discussed the changing nature of opportunistic infections (OIs) in HIV-infected persons in the United States in light of the use of highly active antiretroviral therapy (HAART). While the incidence of nearly all OIs has decreased since 1996, several AIDS-related malignancies have maintained stable incidence rates and will likely assume greater importance. Complications resulting from infection with hepatitis C virus (HCV) will become more prevalent, since as many as 30% of HIV-infected persons are coinfected with HCV. Several "reconstitution syndromes," illnesses attributed to improved T-cell immunity, have been described as unusual manifestations of OI in the first few weeks following initiation of HAART. Antimicrobial resistance is an increasing problem for bacterial and fungal OIs and threatens to diminish the efficacy of trimethoprim-sulfamethoxazole against *Pneumocystis carinii* pneumonia, although evidence of this effect is incomplete at present. OIs also occur because many persons remain undiagnosed with HIV and therefore do not receive appropriate prophylaxis against OIs. The fact that only a minority of persons receiving HAART maintain undetectable virus levels for a sustained period suggests that HAART will not be effective in many of these patients and that OIs will increase in incidence.

Thira Sirisanthana, from the Chiang Mai Medical School, Chiang Mai, Thailand, discussed the importance of *Penicillium marneffei* (PM) infection among HIV-infected persons in Southeast Asia. Please see "*Penicillium marneffei* Infection in Patients with AIDS" on page 561 for a more detailed discussion of this topic.

Mark Russo, from Cornell University Medical College, New York, New York reviewed the consequences of HCV infection in recipients of solid organ transplants. Approximately 21,000 solid organ transplants are performed each year in the United States, and that number is increasing. In liver transplantation, persons who receive transplants to treat liver disease due to chronic hepatitis C may have lower graft survival, and retransplantation rates may be as high as 20%. Treatment of hepatitis C with interferon after liver transplantation has been disappointing, but new formulations and combination therapy with ribavirin may lead to increased efficacy. The use of hepatitis C–positive organs in hepatitis C–infected recipients is being explored. A small study of patients with chronic hepatitis C undergoing liver transplantation showed increased graft survival in those who received a hepatitis C–positive liver, compared with those who received a hepatitis C–negative liver.

Chronic hepatitis C infection is a health care problem in recipients of organs other than the liver, such as kidney transplants. Although short-term studies of less than 5 years demonstrate no difference in liver-related illness and death between kidney transplant recipients with chronic hepatitis C and those without infection, long-term studies of 10 years or more show higher illness and death rates from liver disease in HCV-infected recipients. Less is known about the effect of chronic hepatitis C in heart or lung transplant recipients, but up to 10% of recipients are hepatitis C–positive. Outcomes in recipients of these organs and the role of treatment needs to be further defined.

Louisa Chapman, from the Centers for Disease Control and Prevention in Atlanta, Georgia, spoke on xenotransplantation and xenogeneic infections. Please see "Xenotransplantation: Benefits and Risks" on page 545 for a more detailed article on this portion of the panel.

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International Partnerships in Infectious Diseases Research, Training, and Control

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Partnerships are sine qua nons for effective work in international health. While individuals, institutes, and agencies comprise the usual coalitions, linkages between research, training, and control activities are also essential in international science and public health, and a balance between these components must be fostered. Support for research is particularly important when effective disease control interventions do not exist or are not available for managing emerging or reemerging infectious diseases. The five presentations in this panel represent outstanding examples of the need for close links between research, training, and control activities.

In 1998, the Burroughs Wellcome Fund (BWF) and the Wellcome Trust launched a joint research effort focused on infectious diseases of the tropical developing world. This grant program addresses parasitic, bacterial, fungal, and non-HIV/AIDS related viral infections of importance to developing tropical countries and their collaborators in developed nations. The North American and United Kingdom institutions that have funded projects must make the tropics their center of operations. The program is an experiment for the fund, allowing the BWF to explore a new, collaborative approach to health philanthropy compared to prior experience when focus was on a specific scientific topic, often investigated outside of tropical areas. (See Victoria McGovern's article in this issue on p. 564.)

The Fogarty International Center (FIC) of the National Institutes of Health (NIH) advances health research through international scientific cooperation and is the center for NIH international activities. The Multilateral Initiative on Malaria (MIM) is an alliance of organizations and individuals that aim to facilitate international collaboration and cooperation in scientific research that will lead to the control of malaria. The rotating secretariat of MIM was moved from the Wellcome Trust to the FIC in 1999 on recommendation of the partners. To ensure that research findings are applied to malaria treatment and control, scientists in malaria-endemic countries must be at the forefront of research addressing the local malaria situation.

MIM supports research that will lead to better use of current control methods and development of new and sustainable methods of malaria control in endemic countries. MIM works to strengthen and sustain malaria research capacity in endemic countries through regional and international scientific collaboration and training. It promotes regional and international communication and cooperation to maximize the impact of resources and to avoid the duplication of effort. MIM also aims to facilitate dialogue between researchers and control program personal in malaria-endemic countries to promote research that will address the needs of malaria control programs and eventually encourage collaborative research between these two groups. Finally, MIM facilitates communication among scientists, public health professionals, and policymakers to ensure that research findings lead to policy changes at the government and international levels.

The research grant component of MIM remains with the Special Programme for Research and Training in Tropical Diseases (TDR)/World Health Organization program. The task force on malaria Research Capability Strengthening (RCS) in Africa, coordinated by the United Nations Development Programme/World Bank and WHO Special Programme for Research and Training in Tropical Diseases (TDR), represents a collaborative funding strategy involving multiple agencies and governments to promote capacity-building activities carried out by MIM in Africa. (See Fabio Zicker's article in this issue on p. 529.)

Members of the East African AIDS Training Initiative have developed a model for HIV/AIDS education and training for community-based health- care workers at the grass roots level. The goal was to implement a community-owned program which could be readily adapted for the needs of any resource-poor community. Two factors led to developing the program. First, requests were received for education and training from members of the health-care community in Nairobi; second, education and training delivered at the grass roots level is believed to be the most effective vehicle for introducing rapid social change. This program involved a 3-day residential workshop and continues to be monitored with quarterly site visits in support of participants. Outcomes demonstrate the positive effects of partnerships among community members, funding organizations, and individual charitable donors. The careful development of individual action plans coupled with ongoing support of training mentors via site visits has contributed to the success of this program.

The International Trachoma Initiative is focusing on the world's leading cause of preventable blindness. An estimated 6 million people are blind or visually impaired due to trachoma, and an additional 150 million have the disease. Trachoma is an infectious disease caused by the bacterium *Chlamydia trachomatis*. The disease is most common in children but causes blindness in adults, particularly women. Poverty is the fundamental determinant of trachoma. It results in a lack of basic sanitation, medical care, drugs, and education on prevention and cure in trachoma-endemic areas. Pfizer, Inc. and the Edna McConnell Clark Foundation founded the International Trachoma Initiative (ITI) in

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November 1998 with the explicit mission of working to advance the elimination of trachoma and the blindness it causes. A WHO-approved strategy called SAFE is simple, sustainable, and addresses both cure and prevention:

Surgery for trichiasis—the immediate precursor to blindness
Antibiotics to treat active disease
Facial cleanliness to reduce transmission
Environmental improvement to control the agents of the disease

In ITI countries the antibiotic used is Zithromax (azithromycin), donated by Pfizer. A single oral dose of Zithromax once a year is as effective as the standard treatment of tetracycline eye ointment 2 times a day for 6 weeks. The ITI is currently working in five countries: Morocco, Tanzania, Mali, Ghana, and Vietnam. The ITI works with ministries of health to devise an operating plan and joins WHO, United Nations Children's Fund, and nongovernmental organizations to carry out this work.

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Emerging Infectious Diseases and the Law

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In the 1960s, the United States began to lose interest in public health. The development of effective vaccines and antibiotics, combined with the long-term benefits of sanitary reforms begun 100 years earlier, fostered the belief that communicable diseases had been conquered and that it was time to focus the nation's resources on chronic diseases such as cancer and heart disease. This shift led to the deterioration of the public health infrastructure, including public health law training and practice. At the same time, bioethics and the legal specialty of health law began to evolve. Both of these fields were individual-centered: bioethics concentrated in individual autonomy and health law concentrated on the delivery of, and reimbursement for, personal health services. By the 1980s, legal discourse and training on health and public health was dominated by an individual-centered jurisprudence that subordinated the public's interest to that of the individual. Although this approach resulted in important advances in patient autonomy, it undermined the public's understanding and acceptance of the traditional role of public health law—the protection of the health of the population. Many states weakened their communicable disease-reporting laws and otherwise made it more difficult to identify and manage communicable disease threats. More critically, public health professionals began to believe that they do not have the legal authority to restrict individual behavior to protect the public health and that their role is to provide personal health services on the same basis as private health care providers.

The threat of emerging infectious diseases and bioterrorism is forcing the states and the federal government to reassess the U.S. public health infrastructure and the provision of public health services, as well as to review international treaties and trade agreements to ensure that they are consistent with effective public health measures. As part of this process, it is critical to ensure that each jurisdiction has adequate legal authority to protect the health of the public and to act quickly in the face of bioterrorism or a disease outbreak. This will require the restoration of more traditional public health laws in some jurisdictions and the training of lawyers, judges, and public health professionals in public health jurisprudence. The federal government should help coordinate state efforts and should ensure that there are no federal law impediments to effective public health enforcement.

The restoration and expansion of the public health infrastructure and the development of more effective public health legal services will have many benefits beyond improving the response to emerging infectious diseases and bioterrorism. Achieving these goals is also essential to the improvement of the delivery of routine public health services such as food sanitation, immunizations, and the abatement of hazardous environmental conditions.

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Waterborne Diseases

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Waterborne Disease Outbreaks

In the United States, 127 drinking water outbreaks, most of them associated with groundwater systems, were reported to CDC from 1990 through 1998. The number of outbreaks has declined over the last 20 years, probably as a result of actions by the U.S. Environmental Protection Agency (EPA), water utilities, and public health officials; however, changes in reporting practices may also have contributed to this trend.

World Water Issues

Paul R. Hunter, consultant medical microbiologist and director of the Chester Public Health Laboratory and honorary professor of epidemiology and public health at the University of Central Lancashire, presented World Health Organization data that showed high morbidity and death rates worldwide due to consumption of unsafe drinking water. Currently, about 20% of the world's population lacks access to safe drinking water, and more than 5 million people die annually from illnesses associated with unsafe drinking water or inadequate sanitation. If everyone had safe drinking water and adequate sanitation services, there would be 200 million fewer cases of diarrhea and 2.1 million fewer deaths caused by diarrheal illness each year.

Dr. Hunter noted the wide variety of microbes recognized since 1980 as waterborne disease agents, including *Cryptosporidium*, *Cyclospora*, *Escherichia coli* O157:H7, *Legionella*, *Helicobacter pylori*, hepatitis E virus, *Toxoplasma*, and others. The factors that contribute to the emergence and spread of disease agents are ecologic changes (including those caused by human activity), international travel and commerce, technology, human demographics and behavior, microbial evolution, and the breakdown of public health systems. Dr. Hunter warned that global freshwater consumption rose sixfold between 1900 and 1995, and that this places increasing stress on available drinking water reserves. This increasing stress will result in ecologic damage from over-extraction from rivers, saltwater intrusion into groundwater from over-extraction of groundwater, more highly contaminated water sources, and the potential struggle for access to water. Dr. Hunter concluded his presentation by discussing the threat of biological terrorism via microbes that could be used for deliberate contamination of the water supply.

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Methodologic Issues in the Evaluation of Waterborne Disease

Jack M. Colford, assistant professor of epidemiology, at the University of California-Berkeley, discussed methods for estimating the incidence of infectious diseases attributable to the consumption of tap water. In a previously published study, investigators in Canada compared the incidence of gastroenteritis in homes with and without a reverse-osmosis filter. The study showed that 35% of gastrointestinal illness in the community studied was attributable to drinking water. The study was randomized, but participants knew in which group they were enrolled. As a partial consequence of this study, when Congress amended the Safe Drinking Water Act in 1996, it required that EPA and CDC develop a national estimate of waterborne disease occurrence in the United States. In response, CDC and EPA jointly convened a series of workshops and consensus panel meetings to develop an approach to meet this mandate.

As a result of these meetings, EPA and CDC are supporting several studies, the largest of which will be a randomized, blinded, placebo-controlled trial, involving treatment of in-house drinking water. A pilot study of this intervention trial was recently conducted with residents in 74 homes in northern California; some residents received an active water treatment device containing a 1-µm filter and ultraviolet (UV) light for disinfection, and others received a placebo—the same device without a filter or UV light. Results from this study and a similar study in Australia should be released in fall 2000. Other studies employing the same design will include persons using a groundwater system in Davenport, Iowa; HIV-positive persons in San Francisco; and elderly persons in Sonoma, California. The results of these studies will be used to estimate the percentage of gastrointestinal disease associated with different types of drinking water. When compared with the total prevalence of gastrointestinal disease in the United States, these results will provide an estimate of the national burden of waterborne disease caused by drinking water.

Biofilms

Mark W. LeChevallier, director of research at the American Water Works Service Company, discussed health concerns regarding biofilms in the drinking water distribution system. Biofilms are coatings of organic and inorganic materials in pipes that can harbor, protect, and allow the proliferation of several bacterial pathogens, including *Legionella* and *Mycobacterium avium* complex (MAC). Factors that affect bacterial growth on biofilms include water temperature, type of disinfectant and residual concentration, assimilable organic carbon level, biodegrad-

able organic carbon level, degree of pipe corrosion, and treatment/distribution system characteristics. Chloramine is considerably more effective than chlorine for controlling *Legionella* in biofilms, presumably because chloramine is more stable and thus less reactive than chlorine, allowing it to penetrate the biofilm more deeply.

An important factor in distribution system contamination and bacterial growth on biofilms is transient water pressure fluctuations that create pressure waves that pass through pipes in the distibution system. During the negative portion of the pressure wave, a substantial amount of contaminated water (>1 gal per minute) from the outside can be pulled into pipes through a small leak. This problem is aggravated when sewer lines are placed close to water pipes. Dr. LeChevallier stated that a number of waterborne disease outbreaks have been linked to distribution system deficiencies. Among the agents of nosocomial waterborne disease is MAC. This opportunistic bacterial pathogen lives in water, is resistant to water disinfection (much more so than *Giardia* cysts), and grows in pipe biofilms.

Xenotransplantation: Benefits and Risks

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During transitional periods new scientific understandings bring new questions. The pivotal issues in xenotransplantation concern biohazards. The U.S. Public Health Service defines xenotransplantation as ". . . any procedure that involves the transplantation, implantation, or infusion into a human recipient of either A) live cells, tissues, or organs from a nonhuman animal source or B) human body fluids, cells, tissues, or organs that have had ex vivo contact with live nonhuman animal cells, tissues, or organs."

Prior to 1990, xenotransplants were largely whole organs; recipients survived only days or weeks. However, in most recent xenotransplantation trials, immunoprotected porcine neurologic, pancreatic, and hepatic cells are used to treat degenerative neurologic disorders, diabetes, or hepatic failure. Increasingly, xenotransplantation products function for prolonged periods in recipients who survive months or years.

Xenotransplantation is a public health concern because it has the potential to infect human recipients with agents that do not ordinarily infect humans, thereby introducing new infections to humans. Therefore, xenotransplantation combines a potential benefit with a potential risk to humans that is presently unknown.

Xenogeneic infections belong to a larger category of "bioproduct-acquired" infections, an example of which is simian virus 40 (SV40). SV40, a polyomavirus, contaminated polio vaccine stocks in the 1950s. Investigations into whether SV40 infection is associated with an increased risk of cancer have been inconclusive. This lingering uncertainty about the long-term significance of apparently innocuous persistent human infection with nonhuman viruses underscores the potential for therapeutic use of bioproducts to have unintended consequences.

The PHS Guideline on Infectious Disease Issues in Xenotransplantation describes a system of safeguards built around two key concepts: pretransplant screening of some animal herds, source animals, and xenotransplantation products to minimize the risk of xenogeneic infections with recognized pathogens and posttransplant surveillance of recipients for previously unrecognized xenogeneic organisms.

Endogenous retroviruses exist as inactive proviral DNA in the germline of all mammals adequately studied to date. However, inactive genomic endogenous retroviruses can often express active virus capable of infecting human cell lines in vitro. Thus, xenotransplantation products contain benign genomic DNA that, on transfer into a human, may express infectious retrovirus capable of creating active, persistent infection. The importance of this infectious potential of animal tissue devoid of any identifiable exogenous microorganisms has been the subject of much concern and scientific inquiry over the past 5 years.

To date, limited studies on humans exposed to pig cells and tissues have produced no evidence of porcine endogenous retrovirus infection. However, the persistent presence of microchimeric xenogeneic pig cells in the human recipient confirms that even temporary exposure to xenotransplantation products may continuously expose humans to infectious agents contained within them. For this reason, it is argued that humans should not be exposed to xenotransplantation products containing infectious agents whose ability to infect, cause disease in, or be spread among humans is incompletely defined.

Xenotransplantation is a process that occurs under controlled circumstances; thus, measures can be implemented to minimize associated iatrogenic biohazards. Studies performed in the service of developing policies on xenotransplantation can model other approaches to science-based risk minimization used for other bioproducts.

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Malaria

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Malaria remains a major killer of young children and an enormous economic drain on developing countries. The purpose of this conference panel was to explore two major initiatives to build capacity for prevention and control of malaria.

Roll Back Malaria

Awash Teklehaimanot, acting project manager for Roll Back Malaria (RBM), described its initiative. Each year, more than 300 million clinical cases of acute malarial illness occur, mainly affecting the world's poorest populations. More than 1 million people die each year from malaria, and 90% of these deaths occur in children in sub-Saharan Africa. Malaria is also a substantial impediment to human development in poor countries. It slows economic growth in Africa by up to 1.3% each year; the short-term benefits of malaria control have been estimated at U.S. $3 to $12 billion per year. Malaria is a growing concern as antimicrobial resistance against multiple drugs becomes more widespread and malaria develops in areas previously malaria-free.

The RBM partnership, launched by World Health Organization (WHO) Director-General Grö Harlem Brundtland in October 1998, is committed to cutting the global malaria burden in half by 2010. In Africa, where most malaria occurs, the RBM partnership builds on a history of malaria control and a political commitment to eliminating the disease, which has never been higher. For example, the African Heads of State Summit to Roll Back Malaria, held in Abuja, Nigeria, on April 25, 2000, marked the first meeting of African political leaders to discuss the human and economic consequences of malaria on their continent. At the summit, heads of several development agencies pledged $750 million in new money and discussed concrete action to be taken over the next decade.

The core elements of RBM strategy include 1) ensuring rapid diagnosis and early treatment within or near the home; 2) making insecticide-treated mosquito nets (ITNs) available and increasing access to other vector control measures, such as environmental management to control mosquitoes; 3) making pregnancy safer through preventive intermittent malaria treatment for pregnant women; 4) improving epidemic preparedness through improved surveillance and appropriate rapid response; and 5) supporting focused research to develop new medicines, vaccines, and insecticides.

To implement these core interventions on a large-scale, the RBM partnership recognizes the need to 1) strengthen the capacity of health systems and services; 2) work with and through other sectors such as education, public works, women's development, agriculture, and local government; 3) involve other groups, such as those in the private sector, and 4) sponsor focused applied research and development of effective tools and approaches. In addition, technical support networks comprised of experts with practical experience and from various institutions have been established to provide a link between universities, disease control operations, and international experts.

Some recent promising developments include ITNs with long-lasting insecticide; initiatives to create commercially sustainable markets for ITNs; more effective and less expensive antimalarial drug combinations; concerted efforts to reduce tariffs and taxes on antimalarial commodities, such as drugs and nets; and partnerships with other international health programs, such as the Integrated Management of Childhood Illness program, to both ensure more efficient health systems that address all diseases of poverty and to improve medical treatment of children. For further information about RBM, please visit their website at www.rbm.who.int.

Multilateral Initiative on Malaria

Gerald Keusch described the Multilateral Initiative on Malaria (MIM) as an alliance of organizations and individuals working together to increase malaria research in Africa and to facilitate global collaboration, coordination, and capacity-building. MIM's roots can be traced back to 1995 when the National Institutes of Health (NIH) organized an initial planning meeting. This was followed in 1997 by an international conference in Dakar, Senegal, which was notable for the prominent role played by African malaria research scientists. After follow-up meetings in The Hague and in London, MIM was officially launched in late 1997, with the first secretariat housed at the Wellcome Trust. In 1999 the 1st International MIM Conference was held in Durban, South Africa, to bring the malaria research and control communities together. MIM's secretariat is intended to rotate among member organizations; since June 1999, it has been housed at the Fogarty International Center of NIH.

MIM has several objectives: 1) to raise international public awareness of the problem of malaria; 2) to promote global communication and cooperation on malaria; 3) to develop sustainable malaria research capacity in Africa; and 4) to ensure that research findings are applied to malaria treatment and control.

To date, MIM has had several notable accomplishments. With funding from NIH, the World Bank, the Rockefeller Foundation, WHO, and the governments of Norway, France, and Japan, MIM and WHO's Tropical Disease Research (TDR) program formed a MIM-TDR Research and Capacity-Building Grants Program. To date, 20 grants have been given through which $6 million has been distributed. The grants embody several of the guiding principles of MIM, such as an

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emphasis on partnerships, decision-making by African scientists, and a strong scientific basis for the funded research. To support a variety of research programs, MIM has also developed the Malaria Research and Reference Reagent Resource Center, which provides high quality reagents and materials to investigators who are, or wish to be, involved in malaria research. NIH's National Library of Medicine has taken responsibility for enhancing the capacity of African scientists to do research by establishing and supporting access to communications and information resources. A number of research networks are online using very small aperture telecommunications (VSAT) technology for Internet access. This allows for shared databases, electronic mail and discussion groups, access to published literature, and use of remote sensing technologies. Information about the progress of MIM is shared through meetings, a newsletter, and on the internet at http://mim.nih.gov

Future goals of MIM include stabilizing funding for the MIM-TDR grant program, developing new partnerships, and creating new training opportunities, such as training on research management. Scientific research on *Plasmodium vivax* and on malaria-related anemia is being conducted. Interactions with RBM are well-established and coordinated. The 2nd International MIM Conference is scheduled for 2002 in Tanzania.

Institutional Review Boards: Consideration in Developing Countries

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Institutional review boards (IRBs) play an essential role in protecting the rights of volunteers involved in research projects. Their function has become more complex, particularly concerning projects conducted in developing countries. But can IRBs in the United States guarantee the protection of human subjects involved in research projects in developing countries?

IRBs have no effective way of controlling what goes on in the field. The complex ethical clearance process does not determine whether persons engaged in research projects in developing countries are fully aware of the major aspects of the studies they participate in. The clearance process includes the IRB approval and consent forms. Required U.S. consent forms are too long and the language too complicated to be certain all participants have a full understanding of the study. The forms also appear to be intended more to offer legal protection to sponsoring agencies than to protect the welfare of the volunteer. Most importantly, the forms do not guarantee that volunteers have fully understood the objectives, risks, and benefits of the study and the extent of their voluntary participation. To protect volunteers as well as all persons and institutions involved, these forms must not only communicate necessary information concerning the study to be conducted but also evaluate volunteers' knowledge and their desire to participate. To achieve this goal, we propose to use a simple questionnaire administered by a team not involved in the volunteer recruitment process. We have used such a questionnaire to evaluate potential volunteers for a phase-II HIV vaccine trial. Although volunteers had three intensive, 2-hour counseling sessions, only half responded correctly to all 21 questions. The others were referred for additional counseling and reevaluation.

The IRB process requires that collaborative projects with U.S. institutions have clearance from multiple IRBs. Each IRB meets generally once a month and uses its own consent forms. Each has its own set of rules. Each will respond with different concerns that must be addressed. The approval process may create a lag time of 3 to 12 months to obtain ethical clearances for a project lasting 12 to 24 months.

The ethical clearance process can be simplified in several ways: 1) All studies supported by NIH should have a unique IRB application form and a unique IRB consent form. 2) A certain percentage of the research grant should be allocated to support the ethical clearance process. Ethical support should be available at the grant's initiation. 3) While waiting for the formal ethical clearance and final consent, potential volunteers could be counseled and evaluated. 4) The primary responsibility of local and national IRBs should be clearly determined. IRBs must share responsibilities to achieve the greatest benefit for volunteers. 5) A mechanism must be developed to resolve conflicts between IRBs from developed and developing countries. Yearly meetings of IRBs from host and sponsoring institutions should take place to facilitate the exchange of documents and other information.

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Antimicrobial Resistance

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This symposium highlights selected topics in antimicrobial resistance: community-acquired methicillin-resistant *Staphylococcus aureus*, malaria, reducing inappropriate antimicrobial drug prescribing for outpatient respiratory infections, and addressing the human health impact of antimicrobial drug use in food animals.

Dr. Timothy Naimi discussed community-acquired methicillin-resistant *Staphylococcus aureus* (CMRSA) infections, which have been reported in the Western Pacific and North America with increasing frequency. Few incidence data are available, but reports show a disproportionate distribution among racial minorities and groups with low socioeconomic status. CMRSA infections resembled community-acquired methicillin-susceptible *S. aureus* infections more than MRSA infections acquired in health-care facilities.

Patients with CMRSA were young, healthy, and lacked risk factors for MRSA (recent hospitalization, dialysis, and injection drug use), and they had predominantly skin and soft tissue infections; four deaths due to invasive CMRSA infections were reported in the United States. Unlike nosocomial MRSA, CMRSA is sensitive to multiple non-beta-lactam antibiotics. CMRSA isolates have the mec A gene and distinct pulsed-field-gel electrophoresis patterns. CMRSA presents a clinical challenge because most community-acquired *S. aureus* infections are diagnosed without taking a culture, and the patient is treated with beta-lactam drugs. Improved surveillance and efforts to define risk factors are under way.

Dr. Pascal Ringwald reviewed antimalarial drug resistance, which greatly hinders malaria control since no vaccine will be available in the near future. Resistance of *Plasmodium falciparum* to chloroquine, the most frequently used antimalarial drug, is found in nearly all malaria-endemic countries. Resistance also affects all other antimalarial drugs; cross-resistance occurs against drugs in the same group.

In recent years, chloroquine-resistant *P. vivax* has been reported in Southeast Asia and in South America. Drug resistance increases illness and death, especially among children. As the number of new antimalarial agents is limited, the use of drug combinations is under evaluation. WHO supports national malaria control programs to monitor antimalarial drug resistance in sentinel sites. Decisions to change drug policy should be based on the results of monitoring in these sites. The establishment of a new policy must entail a rational use of drugs and improved compliance.

Dr. Richard Besser discussed efforts to reduce inappropriate antibiotic prescribing for outpatient respiratory infections in the United States. Over 40% of outpatient antibiotic prescriptions are for viral illnesses, which are not affected by antibiotics. CDC's educational campaign, targeted to clinicians and patients, involves partnerships with health departments, health-care delivery organizations, health-care purchasers, medical societies, and others. Materials and information are available at www.cdc.gov/drugresistance. Controlled trials of interventions demonstrated notable reductions in antibiotic prescriptions written in Denver, central Wisconsin, and rural Alaska.

Successful interventions were multifaceted and directed at both clinicians and patients. Clinician interventions included peer education using opinion leaders, improving diagnosis of acute otitis media, and feedback on group-prescribing practice. Patient interventions included education in the community (day care centers, health fairs), homes (reminder refrigerator magnets), and physician offices (videos in waiting rooms). CDC is expanding this campaign and seeking to assess its impact in larger areas.

Dr. Sharon Thompson described Food and Drug Administration (FDA) initiatives to address the human health impact of antimicrobial drug use in food animals. These initiatives include regulatory changes, antimicrobial resistance monitoring, risk assessment, promoting judicious drug use, and research. FDA has concluded that past regulations are no longer adequate to preserve important antimicrobial drugs for human use when their use in food animals may contribute to antimicrobial resistance. A proposed framework would classify antimicrobial agents according to their importance in human medicine and the likelihood that human exposure to them from consuming food animals containing them would create more resistant bacteria. Preapproval studies would assess resistance development and pathogen load in treated animals. Specified thresholds of resistance in human and animal isolates would provide risk management tools—as these thresholds were approached, drug use in animals could be restricted.

FDA is currently finalizing a risk assessment of fluoroquinolone-resistant *Campylobacter* infection and its relationship to fluoroquinolone use in poultry and is beginning an assessment of quinupristin/dalfopristin-resistant *Enterococcus faecium* and its relationship to virginiamycin use as a growth promotant. Visit the FDA website at www.fda.gov.

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Preventing Polio from Becoming a Reemerging Disease

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The global effort to eradicate polio has become the largest public health initiative in history and is spearheaded by the World Health Organization, Rotary International, the Centers for Disease Control and Prevention, and UNICEF (United Nations Children's Fund). During 1999, extraordinary progress continued, with the number of polio-endemic countries declining to 30 from 50 in 1998. Of the three poliovirus types, poliovirus type 2 has reached the verge of extinction, with the only known remaining foci existing in northern India. Polio incidence declined to the lowest levels ever in 1999, although the number of reported cases (7,012) increased slightly due to improvements in surveillance and polio outbreaks in Angola and Iraq. Existing challenges in the initiative include maintaining effective activities, gaining access to children in conflict-affected countries, and sustaining political and financial support until certification is achieved in 2005. Maintaining sufficient supplies of oral polio vaccine emerged as an additional challenge during 1999, resulting from marked acceleration of immunization activities. The public-private sector partnership supporting the initiative expanded in 1999 to include the Bill and Melinda Gates Foundation, Ted Turner's United Nations Foundation, the World Bank, Aventis Pasteur, and De Beers.

Cessation of Polio Vaccination

Following certification of polio eradication by the year 2005 or shortly thereafter, the public health community and policy makers will be faced with the decision of how and when to stop polio vaccination. The benefits of ceasing vaccination are well defined (i.e., annual savings of U.S. $1.5 billion in direct global vaccination costs; the possibility of directing these savings to other health priorities; and eradication of vaccine-associated paralytic poliomyelitis cases). The risks are obvious. If poliovirus is reintroduced into a susceptible population, a catastrophic epidemic of paralytic disease, disability, and death could ensue. Poliovirus could reemerge through 1) reintroduction of poliovirus from a laboratory; 2) prolonged replication in immunodeficient patients; and 3) persistent transmission of vaccine-derived virus in populations. The probability of vaccine-derived poliovirus remaining in circulation is impossible to estimate directly. However, the basic reproductive number or BRN (derived from proportion excreting virus, duration of excretion, virus titer in stool) for vaccine-derived poliovirus is lower than for wild-type polioviruses (typically between 2 and 5 in industrialized countries and 10 and 15 under conditions of poor hygiene in tropical countries). Additional data exist from outbreak investigations, molecular sequencing of polioviruses, and studies of poliovirus persistence following mass vaccination campaigns (e.g., Hungary, Finland, and Cuba). Although the currently available data are encouraging (i.e., decreasing BRN and 3 to 6 months' duration of circulation), substantial gaps in knowledge still exist, including the probability of continued virus circulation in populations with poor hygiene. These gaps need to be addressed to ensure that the best available scientific data will be available for decision-making.

Laboratory Containment of Wild Polioviruses

Global documentation of laboratory containment of materials infected or potentially infected with wild poliovirus is a key component in the decision to stop vaccination. The last smallpox case occurred not in Somalia in 1977, but in England in 1978. The virus was transmitted through a faulty ventilation system from a laboratory to a nearby office, where it infected a person. Like smallpox virus, the only remaining sources of wild virus will be in the laboratories once the virus has been eradicated from the natural environment. The reported transmission of wild poliovirus from a vaccine production facility, presumably through an infected worker, to the community underscores the need for increased containment once wild poliovirus has been eradicated (1).

Infectious or potentially infectious poliovirus materials may be present in a wide range of laboratories, including clinical diagnostic, environmental, research, and teaching. The types of materials that might contain wild poliovirus include clinical (e.g., diagnostic specimens or unidentified enteroviruslike isolates), research (e.g., wild poliovirus strains or derivatives, full-length poliovirus RNA, or cDNA containing full capsid sequences), and environmental (sewage). Because of its high rate of subclinical infections, poliovirus may be found in fecal specimens collected for other purposes. For example, fecal or sewage samples collected in polio-endemic countries for nutritional and environmental studies or studies of other viral, bacterial, or parasitic diseases may contain wild poliovirus.

The *WHO Global Action Plan for Laboratory Containment of Wild Polioviruses* (2) was published in 1999. The plan is linked to the eradication progress. In the preeradication phase (the present), laboratories are required to implement safe handling procedures for materials infected or potentially infected with poliovirus (biosafety level [BSL] 2/polio). Countries must establish national inventories of laboratories holding such materials. Completion of preeradication activities is required before a region can be certified polio-free.

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The posteradication phase (high containment) begins 1 year after identification of the world's last case. At that time, laboratories holding wild poliovirus stocks and potentially infectious materials must either place all materials under appropriate biosafety conditions, transfer important virus isolates to WHO interim repositories, or render all wild poliovirus materials noninfectious. Documentation of containment compliance by all regions is required for global certification of poliovirus eradication. For countries that intend to stop all poliovirus vaccination, work with materials that could cause infection with wild poliovirus must be conducted under BSL 4 containment. High containment (BSL 3/polio) will be required for work with vaccine-derived viruses.

High-level political involvement and multi-sector commitments, including departments of health, defense, education, environment, and private industry are essential to achieving and maintaining global containment of wild poliovirus.

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GIDEON: A Computer Program for Diagnosis, Simulation, and Informatics in the Fields of Geographic Medicine and Emerging Diseases

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Over 300 infectious diseases occur and are challenged by over 250 drugs and vaccines. Fifteen hundred species of pathogenic bacteria, viruses, parasites, and fungi have been described, and printed media can no longer keep up with the dynamics of diseases, outbreaks, and epidemics in "real time." Although electronic media have given us unlimited information access, the search for meaningful data is confusing and time-consuming. Global Infectious Diseases and Epidemiology Network (GIDEON) is a computer software program that was developed for disease simulation and informatics in the fields of geographic and travel medicine.

GIDEON is currently used in 1,500 sites in 45 countries: health ministries, military installations, travel clinics, libraries and student teaching modules, clinical departments, laboratories, and missionary agencies. The program consists of four components. The first generates a Bayesian ranked differential diagnosis based on signs, symptoms, laboratory tests, country of origin, and incubation period and can be used for diagnostic support and simulation of all infectious diseases in all countries. In a blind trial conducted on 495 patients, the correct diagnosis was included in the differential diagnosis list in 94.7% of cases (sensitivity) and displayed as the first disease in the list in 75% (specificity).

The second component presents the epidemiology of individual diseases, including their global effects and status in each of 205 countries and regions. All past and current outbreaks are described in detail, and a web-based version under development will allow for daily updating online. The user may also access a list of diseases related to any agent, vector, vehicle, reservoir or country or any combination of all five (i.e., a list of all mosquito-borne viruses of Brazil which have an avian reservoir).

The third module is an interactive encyclopedia which includes information on the pharmacology, use, testing standards, and global trade names of all antiinfective drugs and vaccines.

The fourth module is designed to identify all species of bacteria, mycobacteria, and yeasts. The database includes 50 to 100 additional taxa that may not appear in standard texts and laboratory databases for several months. Other options allow the user to add data relevant to his own institution, electronic patient charts, material from the Internet, important telephone numbers, drug prices, antimicrobial resistance patterns, and other information. This form of custom data input is particularly useful when running GIDEON on institutional networks because software administrators can use it to disseminate and file information relevant to their own institution for use by all computers on their network. The data in GIDEON are derived from all peer-reviewed journals in the fields of infectious diseases, pediatrics, internal medicine, tropical medicine, travel medicine, antimicrobial pharmacology, and clinical microbiology; a monthly electronic literature search based on all relevant terms in GIDEON (e.g., diseases, drugs, etc.) all available health ministry reports (both printed and electronic); standard texts; and abstracts of major meetings. Further details regarding the program are available at http://www.cyinfo.com.

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Migrating Populations—A Closer View of Who, Why, and So What

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In the last decade, human migration increased fourfold; destinations now involve all points on the globe, with religious persecution and political conflict as common reasons to migrate. Environmental disasters and economic factors, causing rapid depletion of natural resources, also impel people to seek job opportunities and an improved standard of living in cities (1).

Since 1985, 36 countries have been involved in conflicts that caused the uprooting of 100 million persons—more than 35 million in 1999 alone (2). These conflicts result in massive displacement of people and communities; increased illness and death; and major social, cultural, ethnic, and material disruption. To mitigate the impact of these detrimental effects exceptional measures need to be taken. However, the number of migrating workers seeking temporary lodging, estimated at 42 million, overshadows the number of people forced to immigrate as a result of conflicts (3). Visitors comprise the largest group of migrating people, with 25 million traveling to Australia, Canada, and the United States annually.

Immigrants bring with them their cultural and health beliefs (4). For instance, Ukrainians believed that positive tuberculin skin tests meant that the bacillus Calmette-Guérin vaccine was effective. However, after resettling in Seattle, they learned that people who test positive have latent tuberculosis. Other societies, for example, Bosnia, teach that pharmaceuticals weaken the body and should be taken only when a person feels ill—making the concept of treating latent infection difficult for Bosnian immigrants to grasp.

Speaking different languages is a substantial barrier to immigrants receiving appropriate health care. In a California school, 1,000 students speak 15 languages. Medical interpreters are usually scarce. Family members are often recruited to translate, but this can lead to misunderstandings. When intervention is available, fear of consequences prevents many immigrants from seeking medical advice and treatment.

Even immigrants securely resettled may be reexposed to diseases when they return home to visit friends and relatives or associate with newly arrived members of their ethnic group. The incidence of malaria and typhoid fever is greater among immigrants returning home for visits than among North American-born travelers (5,6) because the former tend not to obtain pretravel health advice—physicians are not consulted, and sometimes physicians do not provide appropriate advice (7,8). The lack of medical infrastructure in countries of origin and the lack of medical surveillance after resettlement are additional problems.

Current national and international immigration policies are insufficient, in part because conflicting social, political, and health issues impede progress. Public health policies should be more flexible and proactive. Greater understanding is needed regarding the dynamics of human migration and its long-term health consequences. Solutions for development aid and conflict prevention and resolution must be found; health problems must be mitigated through timely responses. As public health officials, we must promote an international vision of migration as an inevitable phenomenon that is key to economic development. Health policies and development aid to mitigate the adverse effects of migration are essential.

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Emerging Infectious Disease Issues in Blood Safety

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Improvements in donor screening and testing and viral inactivation of plasma derivatives together have resulted in substantial declines in transfusion-transmitted infections over the last two decades. Most recently, nucleic acid testing techniques have been developed to screen blood and plasma donations for evidence of very recent viral infections that could be missed by conventional serologic tests. Nonetheless, the blood supply remains vulnerable to new and reemerging infections. In recent years, numerous infectious agents found worldwide have been identified as potential threats to the blood supply. Several newly discovered hepatitis viruses and agents of transmissible spongiform encephalopathies present unique challenges in assessing possible risks they may pose to the safety of blood and plasma products.

Nucleic Acid Testing

The risk of transfusion-transmitted viral infections is primarily due to the failure of serologic screening tests to detect recently infected donors in the preseroconversion "window" phase of infection. To reduce this window period, European Union regulators began to require in 1999 that all plasma be tested by nucleic acid testing (NAT) techniques for hepatitis C virus (HCV) if derivatives made from such plasma were to be sold in Europe. This announcement was a major impetus for developing and implementing NAT of blood and plasma from donors in the United States and other developed countries.

Virtually all whole blood and plasma donations collected in the United States are being screened for both HCV and HIV-1 by NAT. The testing is being done as a part of investigational new drug applications approved by the U.S. Food and Drug Administration. Due to the complex and labor-intensive nature of the testing, it is being implemented by using a pooled strategy-–namely, donations are being tested in pools of 16 to 24. At this nascent stage, NAT can require several days more to complete than conventional serologic tests. Certain components, particularly platelets because they become outdated in 5 days, are being released by some blood centers before NAT has been completed and on the basis of serologic testing alone.

The pooled NAT procedure is expected to reduce the preantibody seroconversion window period from the current 22 days to about 12 days for HIV and from 70 days to 10 to 14 days for HCV. Organisms are potentially detectable only during a portion of the pre-NAT window period, even when using single donor NAT. It is this portion of the window period when donations are thought to be viremic (i.e., infectious) that is critical to transfusion safety.

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Testing of whole blood donations in the United States from April 1999 through March 2000 revealed 42 NAT-positive/HCV antibody–negative donations (of 1.04×10^7 tested) and 4 NAT-positive/HIV-1 antibody–negative/HIV-1 p24 antigen–negative donations (of 0.76×10^7 tested). The rate of HCV-infected donations positive by only NAT was 40.3 (approximately 1 in 250,000) and HIV-infected was 5.2 (approximately 1 in 2,000,000) per 10 million donations. These testing advances, however, are associated with substantial costs: the mini-pool NAT procedure has been estimated to cost $1.2 million dollars per quality-adjusted life year.

As the technology evolves, NAT testing of all blood components will be completed prior to transfusion. The ultimate goal is to progress from mini-pool testing to single donor testing, but this change appears to be several years away. Future applications of NAT technology may include expanding testing platforms to enable direct detection of additional viruses (e.g., hepatitis B virus, parvovirus B-19, and cytomegalovirus) and other infectious agents (e.g. *Trypanosoma*, *Babesia*, and *Plasmodium* species).

Novel Hepatitis Agents

Although important advances have been made in our understanding of transfusion-transmitted hepatitis over the last several decades, some persons with acute posttransfusion hepatitis test negative for all known hepatitis agents. This observation has fueled concerns about the existence of one or more as-yet-unidentified hepatitis viruses that can be transmitted by blood transfusion. Through advances in molecular virology several "candidate" viruses have been identified as the cause of non-A-E hepatitis.

TT virus (TTV), named for the patient from whom it was first isolated in Japan, is a novel, single-stranded, circular DNA virus. Although TTV can be transmitted by transfusion, similar rates of infection have been observed among blood recipients tested posttransfusion who did and did not develop transfusion-associated hepatitis. TTV can result in persistent infection; however, studies to date have not shown an

associated pathologic condition, and no current evidence suggests that TTV is an agent of hepatitis in humans.

Recently, much attention has been focused on a diverse family of viruses called SEN-V that were isolated by using degenerate primers from TTV. To date, preliminary, limited studies have found that approximately 2% of current and pre-1990 blood donors test positive for SEN-V. Testing of archived serum samples at the National Institutes of Health (NIH) showed that the proportion of cardiac surgery patients with evidence of new infection with SEN-V was 10 times higher among those who had received blood transfusions (30%) than among those who had not (3%). Further, a SEN-V-positive donor could be identified for about 70% of SEN-V-positive recipients. Although these data clearly indicate that SEN-V is transmitted by transfusion, many important questions remain unanswered; for example, is SEN-V the long-anticipated primary agent of non-A-E hepatitis? The NIH study found new SEN-V infections in 11 of 12 patients (92%) with non-A-E hepatitis and 60 of 252 patients (24%) who did not develop hepatitis ($p<0.001$). In addition, the level of viremia generally paralleled the alanine aminotransferase level. These data suggest, but do not prove, a causal association with transfusion-transmitted non A-E hepatitis. Early evidence suggests that SEN-V can replicate in the liver, but there are no data to show that it is a cause of fulminant liver failure, and its role in cirrhosis and chronic cryptogenic hepatitis is uncertain.

Transmissible Spongiform Encephalopathies

Creutzfeldt-Jakob disease (CJD) is a human transmissible spongiform encephalopathy believed to be caused by an unconventional agent—a prion protein—which is an altered form of a normal protein found in many tissues of the body. Most cases of CJD are classified as sporadic, since they are thought to result from spontaneous generation of the abnormal prion protein, which then continues to replicate and accumulate in the brain. Between 10% and 15% of persons with CJD have familial disease, caused by one of more than 20 known mutations. A small number (250) of iatrogenic cases of CJD have occurred in persons who received contaminated pituitary growth hormone, corneas, or dura mater from human cadavers or who were operated on with contaminated neurosurgical instruments.

Concerns regarding the transmissibility of the agent of CJD by blood are supported primarily by laboratory and experimental studies. These studies have demonstrated that rodents with several experimental transmissible spongiform encephalopathies (TSE) have small amounts of infectivity in blood during both the asymptomatic incubation period and clinically overt disease. Transfusion transmission of an experimental TSE in hamsters has been demonstrated, albeit rarely, when known infected blood was administered intravenously.

Despite the findings that suggest a potential for bloodborne transmission of CJD, accumulating epidemiologic data support the view that such a risk, if it exists at all, remains theoretical. First, there are no confirmed reports of CJD transmission by blood or blood products, despite intensive efforts to identify such cases. Second, five case-control studies involving nearly 2,500 patients have not shown blood transfusions to be a risk factor for CJD. Third, CJD has not been detected in recipients of blood from donors who developed CJD months to years after donating blood and were presumably incubating CJD at the time of donation. Finally, active surveillance was conducted among an estimated 12,000 hemophilia patients in hemophilia treatment centers in the United States since 1995, plus additional centers in other countries, and no cases of CJD have been found among them.

In 1996, a new variant of CJD (nvCJD) was first recognized in the United Kingdom. The cause of this new human transmissible spongiform encephalopathy appears to be the same agent responsible for an outbreak of bovine spongiform encephalopathy (BSE) among cattle in the United Kingdom. The BSE epizootic is thought to have resulted from inclusion of carcasses of sheep infected with scrapie in the meat and bone meal fed to cattle in the early 1980s. Features of nvCJD are distinctly different from those of sporadic CJD; for example, patients infected with nvCJD are younger and have prominent early psychiatric and behavioral manifestations, and nvCJD has a distinctive neuropathology. As of July 2000, public health officials had reported 75 cases of confirmed or probable nvCJD in the United Kingdom; 2 cases were found in France and 1 in Ireland.

No cases of transmission of nvCJD by blood transfusion have been reported; nonetheless, because its agent is newly discovered, its degree of infectiousness is unknown. Further, important differences have been noted between sporadic CJD and nvCJD, and it is therefore impossible to extrapolate about nvCJD based on what is known about sporadic CJD. For example, the abnormal prion protein is detectable in tissues taken from spleens and tonsils of patients with nvCJD but not in those taken from patients with sporadic CJD. In view of this uncertainty, U.K. health officials have taken several precautions, which include retrieving all blood and blood products made with blood or plasma obtained from donors subsequently identified with nvCJD, importing all plasma used to produce plasma-derived products, and implementing leukofiltration of all blood donations. Persons who resided in or traveled to the United Kingdom for a total of 6 months or more between 1980 and 1996 are not permitted to donate blood and plasma in the United States.

Conclusion

The high safety level of the blood supply is the result of continued refinements and improvements in donor screening and testing. Continued vigilance is critical to protect the blood supply from known pathogens and to detect the emergence of new infectious agents. As potential new threats are discovered, the need for both the safety of the blood supply and the availability of lifesaving blood and blood products must be balanced. Finally, because the margin of benefit is likely to be very small in some cases, especially when the risk of transmitting some infectious agents via transfusion is very low, policymakers may need to consider economic factors as well as health factors when making decisions involving the blood supply.

Emerging Infectious Diseases among Indigenous Peoples

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Many indigenous peoples are at higher risk for emerging infectious diseases compared to other populations. This conference panel focused on diseases of particular concern to Native Americans (American Indians and Alaska Natives), Australian aboriginal peoples, and the Maori of New Zealand. Important emerging diseases among these groups include respiratory tract infections, infections with antimicrobial-resistant organisms, zoonotic diseases, viral hepatitis, *Helicobacter pylori* and respiratory syncytial virus infections, diseases caused by Group A and B streptococcus, tuberculosis, and bacteremia and meningitis caused by *Streptococcus pneumoniae, Haemophilus influenzae* type b, and *Neisseria meningitidis*. Although the populations discussed are diverse, they have many things in common, including a high risk for many emerging infectious diseases, the requirement for culturally appropriate prevention and control strategies, and the need for increased leadership within communities of indigenous peoples.

Native Americans

Native Americans comprise over 500 American Indian and Alaska Native tribes of unique ethnic and anthropologic origin. Although Native Americans account for only about 1% of the total U.S. population, American Indians account for a larger percentage of the population in western states, and Alaska Natives make up 17% of persons living in Alaska.

Hospitalization rates among American Indians are 20 to 40 times greater than rates in the general U.S. population for a number of zoonotic and vectorborne diseases, such as hantavirus pulmonary syndrome, plague, and Rocky Mountain spotted fever. Multiple factors may contribute to higher risks for American Indians, but a likely explanation is that they live in rural areas or do some type of agricultural work, both of which increase their chance of contact with small mammals and arthropods capable of transmitting these diseases. Greater understanding of the transmission of these diseases will help in the development of prevention strategies that also preserve traditional practices.

As with hantavirus pulmonary syndrome, methicillin-resistant *Staphylococcus aureus* (MRSA) infection acquired outside of healthcare settings is an emerging infectious disease first recognized among Native Americans. At some rural clinics serving Natives, over 60% of *S. aureus* isolates are methicillin-resistant. In one rural American Indian community, 74% of MRSA infections could not be linked to any of the known risk factors for MRSA such as hospitalization within the prior year, residence in a long-term care facility, hemodialysis, or injecting drug use.

Tuberculosis is an example of a disease that recently reemerged, is now in decline, but continues to disproportionately affect Native Americans, both in number of cases and severity of disease. Annual incidence for Native Americans remains twice that of the overall U.S. population, and mortality rates are six times higher. Possible reasons for the persistence of tuberculosis among Natives include living in crowded households, high rates of type 2 diabetes mellitus, and deterioration of the public health infrastructure.

The epidemiology of invasive pneumococcal disease has been characterized for three groups of Native Americans—Alaska Natives, members of the Navajo Nation, and White Mountain Apaches. Rates of invasive infection among Native American children <2 years old are some of the highest reported in the world. Across all age groups, rates are generally higher for Native Americans compared with those for white or black persons in the United States. The distributions of pneumococcal serotypes that cause invasive disease among Navajo and Alaska Native children differ from distributions among the total U.S. population. From 1989 to 1996, only 68% of sterile site isolates collected from Navajo children <2 years old were serotypes in the licensed 7-valent pneumococcal conjugate vaccine. Similarly, only 74% of isolates from Alaska Natives <2 years old were vaccine serotypes. This compares with 83% for non-Native children living in Alaska and 82% for the total U.S. population. Few studies have been conducted to determine the reason for increased disease rates or different serotype distribution for Native Americans. Among Alaska Native infants, group childcare, living with someone who chews tobacco, and lack of breastfeeding were factors associated with invasive pneumococcal disease. A study of risk factors among Navajo adults is underway.

It is not clear whether data from one Native American group can be applied to other tribal populations. Currently, no published data identify specific genetic factors among Native Americans that may contribute to an increased risk of contracting pneumococcal disease. Efforts to reduce the burden of pneumococcal disease among Native Americans include use of 23-valent polysaccharide vaccine in adults and 7-valent conjugate vaccine in infants, judicious antimicrobial

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drug use to limit spread of drug-resistant strains, reducing the incidence of conditions and activities associated with greater risk of infection (diabetes mellitus, alcohol abuse, cigarette smoking), and support of programs to increase knowledge about and use of protection measures against acute respiratory infection such as breast-feeding.

Australian Aboriginal Peoples

Aboriginal peoples account for 2% of the population of Australia. Aboriginal children have extremely high rates of chronic suppurative otitis media (CSOM) and purulent rhinitis. In a longitudinal study in one remote community, 60% of infants developed otorrhea by 6 months of age. As a result, substantial hearing loss occurs in at least 20% to 25% of school-age children and is associated with diminished school performance. Longitudinal studies of aboriginal infants have shown that otitis media often develops within weeks of birth, only days after initial colonization with the three principal bacterial pathogens *S. pneumoniae, H. influenzae,* and *Moraxella catarrhalis*. Multiple serotypes and molecular subtypes cocolonize infants and contribute to persistent and progressive middle ear disease. This multiplicity of colonizing organisms, and the high density of colonizing bacteria in the nasopharynx, may create a vicious cycle of inflammation in the upper airway and clinically manifest as CSOM and purulent rhinitis. The density and diversity of pathogens may also limit the success of antimicrobial therapy.

Colonization by bacterial respiratory pathogens among aboriginal children in the absence of selective pressure from antimicrobial agents is characterized by chronic infection from an early age. Bacterial competition, genetic exchange among cocolonizing organisms, and limited immune responses influence which colonizing strains dominate. Selective pressure from antibiotics provides a window of opportunity for resistant, "hidden" clones to become dominant and spread among members of the community. In a study utilizing nasopharyngeal swabs and microbiologic media, which permitted drug-resistant and susceptible pneumococcal colonies to be quantitated, densities were 2 to 3 log lower for resistant strains compared with susceptible strains in children not treated with antibiotics. However, during antibiotic treatment, the resistant strains became dominant and remained the dominant strains for several months, providing increased opportunity for transmission to susceptible persons.

In another aboriginal community, widespread use of single-dose azithromycin for trachoma was followed by a rapid increase in carriage of macrolide-resistant pneumococci. Compared with persons not colonized with pneumococci, those colonized before use of azithromycin were at greater risk of having macrolide-resistant strains isolated from the nasopharyngeal specimens after azithromycin use. Similar results may be expected from vaccine-induced immunologic selective pressures, although the clinical impact of this phenomenon will require careful evaluation.

Maori of New Zealand

The Maori account for approximately 16% of the population of New Zealand. Although the health of non-Maori, non-Pacific Polynesian children is comparable to that of children in other western countries, poorer health statistics, particularly for infectious diseases, are noted for Maori children. Humanitarian government policies (Social Security Act of 1938, free public hospitals, subsidized housing) likely contributed to improvement in the Maori infant mortality rate, but some disparity continues, most likely because of fewer educational and economic opportunities. Epidemics of vaccine-preventable diseases continue because vaccination rates are lower for the Maori than for other residents of New Zealand. Hospitalizations for pneumonia and bronchiolitis, mainly caused by respiratory syncytial virus, overload the healthcare system each winter.

A continuing, 10-year clonal epidemic of *Neisseria meningitidis* serogroup B disease (B4:P1.4) involving mostly Maori and Pacific Island children (more recent immigrants to New Zealand) highlights the importance of overcrowded households as a factor in transmission of meningococcal infection. Provision of more suitable yet affordable housing may reduce transmission of meningococcal and other infectious diseases. Preliminary data look promising that a strain-specific vaccine may control the epidemic.

Rheumatic fever has continued to be a problem among the Maori, as well as Pacific Island children. Prevention of rheumatic fever is being addressed by study of an intensive program in schools where throat swabs are collected for culture and penicillin therapy is provided for children with *Streptococcus pyogenes*.

Community Perspective and Future Directions

All too often, health research conducted among indigenous peoples has not incorporated their local worldview, cultural beliefs, or practices. As a result, research questions more often reflect researchers' interests and needs rather than those of the community. Study designs and data collection may have little meaning to community residents and therefore limit scientists' ability to obtain informed consent and collect accurate data. Researchers may have no sense of accountability for either the community's or the participant's data, and, after data collection is complete, the research team is never seen in the community again. Finally, research conducted and data collected have not always been used to bring about positive outcomes within the community and improve the health of indigenous peoples. Thus, community residents may feel that research is conducted *on* them rather than *with* them.

Participation by members of indigenous communities in research is increasing. However, to date, their contribution is mostly limited to providing community access, assisting with data collection, and facilitating ethical and institutional review board approvals. To perform research that will address the health issues of greatest concern among indigenous peoples, increasing community involvement is needed to select research topics, develop hypotheses and research questions, identify optimal study designs, create and implement data collection instruments, and analyze and interpret data. To this end, professional development of health researchers in indigenous communities is imperative.

Migration, Refugees, and Health Risks

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Migration—both voluntary and forced—is increasing all over the world. People are moving in larger numbers faster and further than at any other time in history. This is happening at a time when many countries are ill-prepared to deal with a changing demography and when policies and attitudes to population movement and immigration are hardening. The health implications of this are many, and, in some cases, illness and death rates associated with migration are exacerbated by a lack of policies needed to make migration a healthy and socially productive process. From a public health point of view, this is having—and will continue to have—serious ramifications for the people that move, the family they leave behind, and the communities that host the newcomers.

On Sunday June 18, 2000, 54 Chinese would-be immigrants suffocated to death while trying to enter the United Kingdom in a sealed truck designed to carry fruit from continental Europe. The path they took started in Fujian, a Province in southeastern China. From there they traveled to Beijing and then to Kazakhstan or Russia, the Czech Republic, Austria, France, and across the English Channel to Dover where they died.

They covered thousands of miles, and the trip cost each of them an estimated $30,000 as well as their lives. About 100,000 people from Fujian Province attempt to emigrate each year, most of them unofficially (illegally). Fujian is just one of thousands of areas throughout the developing world sending people who believe that a better quality of life and greater opportunities exist elsewhere. Modern methods of communication have made the world a far smaller place and introduced people everywhere to different concepts and increasingly shared values and expectations.

Migration is not a new phenomenon, of course. Early hunting and gathering societies migrated constantly, and nomadic herdsmen in many parts of the world still move routinely. The United States, Canada, and Australia were built on migration, and most European countries were saved by being able to send millions of people to other places when confronted with massive agricultural, political, or economic crises.

Today as many as 190 million people are thought to cross borders every year, and migration has become an integral and inevitable part of global social and economic development. More importantly, the possibility of moving elsewhere is becoming an increasingly key part of how people view the world they live in. Growing poverty in some regions is pushing more and more people to look for opportunities elsewhere at the same time as politicians and social forces are trying to prevent more immigration. The public health implications of this emerging dynamic, so replete with political contradictions, are enormous.

At the same time that voluntary migration is increasing, so are far more tragic types of uprooting and displacement. Wars, natural disasters, and complex emergencies that destroy social and cultural infrastructures are affecting more people than ever before, because as the world's population grows and becomes more concentrated, so does the number of people at risk for being affected by these events.

Despite the time and effort spent on conflict prevention and resolution, over the last 15 years alone over 36 countries have been involved in conflicts of one kind or another. These conflicts have collectively been responsible for uprooting over 60 million people—more than the combined populations of at least 13 Western European countries. Such are the vagaries of peace accords and resettlement policies that many of these refugees will remain stateless and homeless for years and, in some cases, generations to come.

Many of the same factors that influence those who migrate for economic reasons also influence the movement of refugees. Better communication and easier and faster transport systems have increased the number of places they can settle. Thus, within months of being forced from their homes in Bosnia, refugees were taken in as far afield as Malaysia, Australia, Canada, and the United States as well as many other countries closer to home. The same situation occurred with Kosovar Albanians some 7 years later, and by the end of 1999, these refugees were dispersed over at least 60 countries.

Were these mass movements simply demographic phenomena the problem might be of purely academic interest. However, the reality is that, with respect to both voluntary and forced migration, deaths occur. The 54 Chinese people who died in the back of the truck in Dover, England, were a small tip of a large iceberg. Mexicans trying to enter the United States take the same risks every day and their mortality rate is probably just as high. From Valencia, Spain, down to the Straits of Gibraltar police patrols regularly find the bodies of Africans who drowned trying to enter Europe through its southernmost door. Albanians trying to get to Italy risk meeting the same fate in the Adriatic Sea.

The number of migration-related deaths will continue to grow if policies designed to keep people out of countries are more stringently enforced just at the time when pressure on them to go to new countries is increasing. However, in addition to these mortality statistics and human rights

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questions, other public health considerations need to be addressed. Because migrants are typically poor people moving from poor economic environments, they carry with them the health profiles that result from poverty. Their understanding of health comes from having to adapt to poor ecological conditions along with limited possibilities for change and control over their own life.

Despite the magnitude of the migration process now underway, many European countries have been relatively unprepared to deal with it, and few have formulated policies needed to make immigration a healthy and socially productive process. Health indicators suggest that migrants in Europe are at considerably higher risk for contracting a number of diseases than nonmigrant populations in the same countries.

The Process of Migration

Migration, even under the best of conditions, involves a series of events that can be highly traumatizing and that can place migrants at risk. The process involves uprooting, being separated from family and traditional values, and being placed in new social and cultural situations where job and legal security may be minimal. For many migrants, social integration is rarely easy and for some impossible.

Resistance to their presence—even when their work skills are needed—often places immigrants on the periphery of society. Resistance to their participation in society results from language problems and culturally-defined behavior that often reinforce stereotypes and prejudices. Not only are migrants themselves affected, but, in many situations, their children are also discriminated against. Studies of this phenomenon, carried out by the International Centre for Migration and Health in the European Union (EU), indicate that children of migrants may be at high risk for drug abuse because they use drugs to demonstrate their rejection of, and exclusion from, so-called mainstream society.

The labile nature of health and health-related behavior makes people highly susceptible to the social dynamics and physical environments they live in, especially when there is little or no social support to compensate for exclusionary attitudes and poor working and living conditions.

The Demographics of Infectious Diseases in Migrant Workers

The incidence of new cases of tuberculosis infection (TB) in 9 EU countries fell from 34.8 out of every 100,000 persons in 1974 to 14.3 out of every 100,000 in 1995 in all 15 EU member states. In Denmark, the incidence of new cases increased steadily over the past 5 years, and the proportion of cases in foreign-born persons rose from 18% in 1986 to 60% in 1996. In England and Wales, approximately 40% of all TB infections are estimated to occur in people from the Indian subcontinent. In the Netherlands, where the incidence of TB rose 45% between 1987 and 1995, over 50% of known cases of infection occurred among immigrants. The TB profile in Germany and France is similar, and migrants are 3 times and 6 times, respectively, more likely to be diagnosed with the disease than are nonmigrants.

The irony of these figures is that, in many countries, the working and living conditions of migrants may be creating risk factors for them to contract TB. Social exclusion and language barriers, as well as cultural attitudes to seeking healthcare, often render the biomedical risks even greater. Migrants may may not be eligible for national health insurance plans, or they simply may not know where and how to seek help. In some cases, when immigrants come from high TB-endemic areas, they may have high tolerance levels for discomfort and not seek help as readily as the native population of EU countries.

Much the same can be said of hepatitis A, which, like TB, is a disease of poverty and endemic in most developing countries. Exposure to it as a result of living conditions in some EU countries cannot be excluded from the risk situations that migrants face.

The link between hepatitis B and HIV/AIDS when unprotected sexual contact is a factor presents many psychosocial questions, namely those surrounding migration and social integration. To understand this better, however, it is perhaps worthwhile to take into account the risks that travelers and tourists take because, in many ways, their behavior is often dictated by similar conditions as those experienced by migrants.

In one region of the United Kingdom, people traveling abroad accounted for more than 6% of all reported cases of hepatitis B in 1981, and from 1990 to 1994 when travel had become more popular, the proportion had more than doubled. In Germany, where an estimated 50,000 new cases occur per year, at least 7,500 are estimated to be associated with travel by Germans to other countries.

Many of these same underlying factors contribute to migrants' risk of contracting HIV/AIDS. Many EU countries require migrant workers to travel alone and leave spouses and partners behind. This, almost inevitably, places migrants at risk for unsafe sexual behavior, contact with sex workers, and infection with sexually transmitted diseases (STDs).

Yet the risk of migrant workers contracting STDs other than HIV/AIDS is an issue that has not been addressed by most national health authorities. In Belgium, illness from STDs is reported to be significantly higher among unmarried male immigrants than it is among Belgian men in general. Similarly, in Sweden, where there has been an overall decrease in the incidence of new cases of STDs, the number of cases among foreign-born persons is superseding the number among men born in Sweden.

The incidence of HIV/AIDS is also higher among migrants living in Sweden, especially those from Africa, than among Swedish nationals. In Germany a similar pattern is emerging, and it is becoming clear that the risk of HIV/AIDS among migrants reflects the global epidemiologic pattern of HIV/AIDS. Thus, in Germany where the number of AIDS cases among migrants has risen in recent years and represents approximately 14% of all reported cases, it is most commonly found among migrants from Africa, North America, Asia, and Latin America, but migrants from Turkey and Eastern Europe have been minimally affected. The picture is a complex one, however, and in Italy the risk of HIV/AIDS in migrant populations appears to be lower than among nationals.

Reproductive Health Among Migrant Women

Communicable diseases are by no means the only health problems to which migrants appear to be highly susceptible. Reproductive health in general, especially among women, seems to be affected by changes in social and economic environment, access to health care, changes in sexual behavior, and social status.

Difficult pregnancies and pregnancy-related illness among migrants are problems throughout the EU. In the United Kingdom, babies of Asian mothers tend to have lower birthweights than other babies, and perinatal and postnatal mortality rates are higher among immigrants born in Pakistan and the Caribbean than in the general population.

Data from Belgium indicate that in 1983 the highest perinatal and infant mortality rates were for babies born of immigrant women from Morocco and Turkey. By 1993, the situation had improved substantially for the domestic Belgian population and for Moroccan immigrants, but among Turkish immigrants high perinatal and infant mortality rates persisted and in 1993 were still 3.5 times higher than those for Belgians.

In Germany, perinatal and neonatal mortality rates are consistently higher in foreign-born groups, especially Turkish immigrants, than in the population as a whole. The rate of perinatal mortality for babies born to German mothers is approximately 5.2% and among nonnationals approximately 7%, and the incidence of congenital abnormalities and maternal mortality is also higher among immigrants.

In Spain, premature births, low birthweight, and complications of delivery are especially common with infants born of women who have immigrated from sub-Saharan Africa and Central and South America. African immigrant women giving birth in hospitals, for example, have an incidence of premature births almost twice as high as in Spanish women, and low-birth-weight rates are also approximately double those of women born in Spain.

Over 8% of babies born to women from Central and South America are underweight and 6.3% are born prematurely. Unwanted pregnancy and poor knowledge about contraception and where to get contraceptive devices and advice on contraception are common problems among immigrant women, and requests for abortions tend to be twice as common among them as Spanish women, especially those coming from North Africa and the sub-Saharan region.

Occupational Health and Safety Among Immigrants

Migrants tend to take jobs that are temporary, require few skills, and are largely unattractive to local labor forces. Many jobs that are available, such as those in mining, construction, heavy manufacturing, industry, and agriculture, often involve poor environmental conditions and lack of safety.

Because temporary labor markets are often seen as too short-term to justify major investments in training, employers tend not to require instruction and careful supervision. Language obstacles, poor communication, lack of familiarity with some of the technology used, and different attitudes to work safety all contribute to work-related risks.

The number of industrial accidents and injuries is higher among migrant workers than among citizens in France and Germany, especially those who work in construction and public works types of jobs. Over 30% of all accidents resulting in permanent disabilities involve non-nationals. Data from Belgium similarly indicate that Moroccan and Turkish workers in heavy industry have a higher incidence of accidents than Belgian nationals and suffer more secondary psychological sequelae.

In the agricultural sector, unprotected exposure to pesticides and other chemical products is a common problem, and chronic exposure to them has been linked to depression, neurologic disorders, and miscarriages among migrant workers in Spain. The incidence of other injuries among people working in greenhouses is also high, and muscular disease, dehydration, and heart complaints linked to high temperatures are common. Few agricultural workers receive much safety training, and few use effective protection. Because many agricultural businesses are small and harvests seasonal, health authorities tend to have limited access to them, and little routine evaluation of working conditions and safety takes place.

Domestic Accidents and Poisonings Among Immigrant Children

Immigrants also tend to be more vulnerable to other types of accidents. In Germany, non-German children 5 to 9 years old have more traffic and other accidents than German children. Children of Moroccan and Turkish migrant workers in the Netherlands also have more domestic accidents such as poisonings and burns, as well as traffic accidents, than Dutch children. Poor quality housing is often a risk factor, and in France, lead poisoning from paint in old, poorly maintained houses is a major problem for migrant children. The frequent absence of one or both parents due to heavy work schedules, along with poor childcare alternatives available to immigrants also contributes to putting children of migrants at risk.

Psychosocial Issues Among Immigrants

Underlying many of these health issues are the psychosocial determinants of health-related behavior. Whether migration is planned or not, voluntary or forced, some degree of stress is always involved. Migration means breaking with family, friends, and established social networks, departing from traditional routines, value systems, and accepted ways of behaving and having to adapt to new social and psychosocial environments.

Despite the potential magnitude of the problem, the psychosocial health of migrants remains poorly addressed. Relatively little is known about the dynamics involved or about what should and can be done to prevent or manage mental health problems related to migration. In the EU, where both the number of immigrants and the amount of internal movement is increasing, social integration and acculturation (defined as the gradual adoption of the identity, values, behaviors, and attitudes of the host community) are linked to a number of mental health problems.

Language also plays an important role in mental health, and barriers to good communication compound feelings of isolation and being "unwanted." The capacity to communicate can influence healthcare-seeking behavior, underreporting, poor explanation of health problems and symptoms, inappropriate diagnoses and the capacity of immigrants to comply with treatment regimens.

Some EU countries have adopted settlement policies that stress the social dispersal of ethnic minorities and immigrants in order to integrate them more quickly into "mainstream" society. There is little evidence that this has been effective; indeed, the feelings of isolation that follow social dispersal are often detrimental to both mental health and social integration. This has been the case with Vietnamese refugees in Finland, where younger immigrants were more able than their parents to adopt Western values and behaviors, but their "success" prompted anxiety and depression among mothers who felt they were "losing" their children.

Among rural Turkish workers in Amsterdam, only a few are able to speak Dutch, and the capacity to function and integrate into mainstream society has often been limited. Mental health problems such as neuroses are common, and over half of these immigrants say they worry and often regret their decision to move away from home. Less than 50% of those who are married are able to bring their families because work contracts and conditions do not allow it.

In Germany, an estimated 13% of immigrants seen for depressive disorders develop problems during their initial 12 months away from home. Another 25% tend to have problems within the following 2 to 5 years. Many immigrants say they "long for home" and report exaggerated memories of familial events, the ways they lived, and things they experienced as children. Fantasies about home and returning home are often described as "migrant's opium," and although these responses are not necessarily serious, they are often psychologically debilitating. In no case is this more true than when families are forced to migrate and are violently dispersed. High levels of anxiety among Bosnian refugees in Austria have been linked to their inability to trace lost family members, and this is felt to have become one of the main barriers to any real improvement in their overall quality of life in many host countries.

The relatively high incidence of depression among immigrants and their children in many EU countries has also been associated with high rates of suicide, possibly linked to unemployment. In the Netherlands, where the unemployment rate among migrants in 1994 was 31% compared to 13% for Dutch nationals, the suicide rate among children of immigrants was also considerably higher than in the general population. In Rotterdam, children of Turkish immigrants were five times as likely as Dutch children to commit suicide and Moroccan children three times as likely. Children, particularly girls, of Surinamese immigrants had a suicide rate 27.6 times higher than that of Dutch children. In the United Kingdom, suicide rates for women from the Indian subcontinent are also markedly higher than for men and are highest among girls ages 15 to 24. On the whole, suicide among this immigrant group is twice as high as the national average and in the 25 to 34 year old age group, 60% higher. Attempted suicide among young women from south Asia is also high.

For many people, migration and resettlement result in social isolation and loneliness. This is especially so when people move alone. The relief found in temporary friendships and supportive social environments at ethnic bars often heightens dependency on alcohol and other substances, and alcohol abuse is a frequent problem among single immigrants.

Substance Abuse Among Immigrants

Alcohol abuse among male Indian immigrants, especially Sikhs, is increasing and reflected in higher mortality rates associated with cirrhosis of the liver, which are twice as high as they are for men born in England. This trend is not typical of all Asian immigrants, however; Pakistani males (who tend to be Moslem) are far less likely to consume any alcohol. Irrespective of religious background, women from the Indian subcontinent are also unlikely to consume alcohol, and their death rates from alcohol-related causes are similar to those for English women.

Drug abuse also may be an emerging problem among immigrants. In the United Kingdom, immigrant drug users who have tested HIV-positive have begun to attract attention, and the migration of drug users coming to the UK in search of better living conditions has become a serious problem.

In Amsterdam, about half of people using methadone bus outreach programs are non-Dutch, and about a quarter of all young women who leave drug Youth Advice Centers prematurely are from other countries; 45% of detainees in youth penitentiaries are migrant children. The reasons for drug abuse among children of immigrants vary considerably, but in France, it is seen as a manifestation of social marginalization and an expression of anger at the problems of integration. A study of psychological stress and coping among Greek immigrant adolescents in Sweden tends to confirm this, and similar observations have been reported in Germany.

Patterns of drug abuse among migrants and refugees, however, may not necessarily be different from those of local populations, and evidence is growing that what really differentiates immigrants and their children from nationals, is the limited ability or unwillingness of immigrants to use local health and social services for fear of being reported or because they do not receive culturally sensitive support.

Conclusion

Migration nurtures economic development, encourages interdependence between countries and regions, and also provides important links for the exchange of resources between the EU and other countries. The economic need for this movement of human resources is well-established, and it is unlikely that the trend will diminish of its own accord in the foreseeable future. Indeed, the free movement of people that has become a basic principle of the EU will no doubt be extended to include other countries as they are admitted to the Union.

Despite the magnitude of the public health issues involved, relatively little attention has been given to the role of migration in changing the epidemiologic profile of receiving communities or the impact migration and resettlement have on the health of immigrants. Even less attention has been paid to conditions possibly linked to poor health in the context of migration. National health statistics rarely reflect the process or its implications, and there has been relatively little interest in the phenomenon by health and social scientists. What data are available tend to be from small studies and anecdotal reports. They nevertheless indicate that the health circumstances that characterize uprooting and migration merit more consideration.

Much, of course, depends on the type of population movement, why and where it occurs, and the profiles of the people involved. However, the fact that most migrants move because of the "push" of poverty rather than the "pull" of better living conditions means that many of them inevitably come from socioeconomically deprived backgrounds where they are not able to provide a good quality of life or health for themselves. Migrants moving because of poverty arrive with health profiles typical of those in their previous surroundings. Poverty breeds diseases of poverty no matter where or when it exists.

The range of health issues that can be associated with migration is inevitably broad. It includes communicable and noncommunicable diseases, injuries associated with work environments, and psychological problems. All or any of them can be debilitating to the health of migrants and their families. They can also have serious consequences for societies and communities into which they move and work.

Communicable and noncommunicable diseases, as well as occupational injuries, psychosocial problems, and family breakdown, can all be equally important with regard to the care and treatment required to deal with them and the work and school days that can be lost because of them. The health of migrants thus has social and economic consequences for host countries as well as for migrants and their families.

The process of migration within and into the EU includes changing and emerging trends in health. TB is a disease of poverty, and it is not surprising that migrants from poor countries are at high risk of contracting it. Much could be done to avoid TB by ensuring better standards of living for low income migrants who must often deal with poor housing and overcrowded conditions. Early identification and treatment of TB is also important and screening should be seen as a way of identifying people who need treatment. However, associating health screening with the possibility of expulsion does little to encourage migrants to participate and indeed may defeat the whole purpose of it. Illegal immigrants are possibly at particularly high risk of TB and reluctant to be tested for fear of being expelled. Screening for other diseases such as hepatitis, which also constitutes a growing threat, could also be useful if it is done in a constructive way.

Problems associated with maternal and child health among migrants have long been a matter of concern in most EU countries. Migrant women make poor use of contraceptive services and have more difficult pregnancies than other women. They have more low-birth-weight babies and tend to deliver prematurely more often. In some countries, migrant women also terminate pregnancies more often than other women. In some migrant communities, women are still regarded as second-class citizens. Different cultural practices within families and attitudes toward women often seriously limit their access to, and use of, antenatal care and other services. Conflicting pressures are brought to bear on women caught between traditional domestic values and practices and those of the social environment they work and live in and thus confront women with difficult psychological barriers.

Psychological stress may also contribute to the problems immigrants face, and the deprivation, employment difficulties, and problems of cultural and social adaptation experienced by migrants deserve more consideration. Early identification of people at risk and counseling for these people are urgently called for.

The fact that migrants are often qualified for only low-skilled jobs also means they are often confined to high-risk and irregular occupational settings. The fact that they come into them with little previous experience and receive little training and safety support means that they are often exposed to health problems and accidents associated with low-skilled jobs.

For ethical as well as public health reasons, occupational hazards for immigrants is an area that calls for much more attention than it has received to date. The fact that documentation in this area is so weak is, in itself, indicative of the little attention it has been given. As a first step, better training given by people who speak the languages of migrants would be an important contribution to solving these problems.

Culture conflict is a common and serious problem in migration. It affects people in different ways, some more overtly than others. To what extent culture conflict or clash is linked to mental health problems reported among migrants is not clear, but there is reason to believe that it is a factor. Just as with other health indicators, however, there is a paucity of information on the incidence and prevalence of mental health problems among migrants. Available data suggest that cultural background plays an important role in predisposing some immigrants to some diseases such as depression, chronic anxiety, and neuroses. Alcohol and drug abuse may also be used as coping responses that expose migrants to other health problems such as HIV/AIDS. However, in general, the trauma and exclusion that all immigrants face increase their risk of behaviors that, in turn, increases their susceptibility to all diseases.

Penicillium marneffei Infection in Patients with AIDS

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Penicillium marneffei infection (PM) is an important disease among HIV-infected persons in Southeast Asia. Discovered in 1956 from the bamboo rat, *Rhizomys sinensis*, in Vietnam (1), PM was first identified in HIV-infected persons in 1988 (2). The disease has now been reported among HIV-infected persons in Thailand, Myanmar (Burma), Vietnam, Cambodia, Malaysia, northeastern India, Hong Kong, Taiwan, and southern China (3). Cases of PM also have been reported among HIV-infected persons from the United States, the United Kingdom, The Netherlands, Italy, France, Germany, Switzerland, Sweden, Australia, and Japan after they visited the PM-endemic region (3).

PM occurs late in the course of HIV infection. Our study found that the CD4+ cell count at the time of the diagnosis of PM was consistently less than 50 cells/ml. Clinical presentation included fever (in 99% of the patients), anemia (78%), pronounced weight loss (76%), generalized lymphadenopathy (58%), and hepatomegaly (51%). However, these conditions were not specific for PM and could be caused by HIV or other HIV-related opportunistic infections. A more specific finding was skin lesions, most commonly papules with central necrotic umbilication (4), which were seen in 71% of the patients.

In 63% of the patients with PM, a presumptive diagnosis could be made several days before the results of fungal culture were available. This was done by microscopic examination of a Wright-stained sample of bone marrow aspirate, touch smears of a skin biopsy specimen, or a lymph node biopsy specimen. It was easy to culture *P. marneffei* from various clinical specimens. Bone marrow culture was the most sensitive (100%), followed by culture of the specimen obtained from skin biopsy (90%) and blood culture (76%)(4).

The fungus was sensitive to amphotericin B, itraconazole, and ketoconazole (5). The current recommended treatment regimen is to give amphotericin B, 0.6 mg/kg/day for 2 weeks, followed by itraconazole, 400 mg/day orally in two divided doses for the next 10 weeks (6). After initial treatment, the patient should be given itraconazole, 200 mg/day, as secondary prophylaxis for life (7).

P. marneffei has been isolated from several species of bamboo rats in the disease-endemic area, but epidemiologic studies have thus far failed to define an environmental exposure associated with the disease (8-10).

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Adventitious Agents and Vaccines

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A discussion of adventitious agents and vaccines is pertinent in the context of emerging infectious diseases. Many novel vaccines are produced in animal cell substrates, and emerging infectious diseases may theoretically be transmitted from animals to humans through these vaccines. The challenge of identifying potential adventitious agents in vaccines closely parallels the challenge of identifying the agents causing particular emerging infectious diseases. Thus, the major focus of this discussion will be the approaches used in a regulatory setting to ensure that vaccines are devoid of adventitious agents.

Maximal vaccine benefit is achieved when vaccination rates are sufficiently high to achieve herd immunity. Because vaccines are administered to healthy children, it is especially important that parents, pediatricians, and the public at large feel confident that the vaccines are safe. Knowing that vaccines are free from adventitious agents is a large component of this confidence. Thus, ensuring that vaccine products that are administered to the public do not contain adventitious agents is a regulatory goal. Of course, the potential for the presence of adventitious agents in any vaccine must also be evaluated in terms of the overall benefit of the product.

In the past, biologic products have served as vectors for viral diseases. Examples include the contamination of yellow fever vaccine with hepatitis B virus in the 1940s (because a human-derived excipient contained hepatitis B virus), contamination of early polio and adenovirus vaccines with simian virus 40 in the late 1950s and early 1960s, contamination of blood products with hepatitis viruses and HIV, and contamination of dura mater grafts with the Creutzfeldt-Jakob disease agent. In these examples, either human or animal materials used in production usually caused the contamination.

Production of viral vaccines generally involves inoculation of a cell substrate with a vaccine seed and purification of bulk product from these cells after a sufficient time for replication of the virus or production of vaccine proteins. Other raw materials (e.g., tissue culture reagents, stabilizers) may be added to the product at various stages of production. Thus, adventitious agents could theoretically enter a viral vaccine through any of these ingredients. Close control of the vaccine manufacturing environment (by producing vaccines in sophisticated modern facilities), appropriate testing of the raw materials, and testing of both the bulk and final products can help ensure that adventitious agents have not entered the vaccine. Most vaccines are subjected to inactivation or purification steps that can reduce the likelihood of contamination with adventitious agents.

Current research on emerging infectious diseases may help provide further assurance that new vaccines do not contain adventitious agents. Powerful methods used to discover viruses associated with emerging infectious diseases are also being adapted to ensure that new vaccines (some of which may be produced in novel cell substrates) are free of adventitious agents.

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ProMED-mail: Background and Purpose

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The online program to monitor emerging diseases (ProMED-mail) was established in 1994 with the support and encouragement of the Federation of American Scientists and SatelLife. The principal intent of ProMED-mail is to assist local, national, and international organizations in disseminating, as rapidly as possible, reports of outbreaks of emerging infectious diseases wherever they occur; these reports are taken from sources such as media reports, online summaries, local observers, official reports, and others. Subscribers are encouraged to contribute reports and to participate in the dialogue.

With a minimum amount of funding, volunteer moderators, and a bulk mailer in Newfoundland, ProMED-mail grew from 40 subscribers in 1994 to its present number of over 20,000 in more than 160 countries; there are no subscription fees. Since 1999, ProMED-mail has been administered by the International Society for Infectious Diseases, with servers and software furnished by Oracle, Inc. The e-mail service provider is located in the Harvard School of Public Health.

At a "Meet the Professor" session of the 2000 International Conference on Emerging Infectious Diseases," ProMED-mail staff described the history and purpose of the list-serve and fielded a wide range of queries from an attentive and supportive audience, most of whom were already familiar with ProMED-mail but not, it turned out, with the website <www.promedmail.org>.

"Would ProMED-mail report the occurrence of Ebola disease if it occurred in New York City?" Of course. "Has ProMED-mail felt constrained by governments or individuals in governments from reporting disease occurrences?" No. "Would ProMED-mail report a very small cluster of cases?" It depends on the etiology. "Does ProMED-mail report rumors?" No, but when we hear a rumor we might post a Request for Information. "How many hours per day, on average, do moderators spend working on ProMED-mail?" Three to six hours, depending on their area of responsibility. "Does ProMED-mail consider the World Health Organization a competitor?" Absolutely not. WHO, Office International des Epizooties, and other international and national organizations are able to report only what is officially reported to them. ProMED-mail has no such constraints, thus it is able to post preliminary and unofficial reports and summaries.

It is clear from these and other questions and comments and from the number of subscribers, many of whom are from national and international health agencies, that ProMED-mail fills a void. It is also clear that other, more official reporting systems are needed. These should follow the example of the outbreak page on WHO's website <http://www.who.int/disease-outbreak-news/index.html>, which gives short summaries of outbreaks as they are received. It would also be encouraging to see individual countries adapting the independent ProMED-mail format to supplement their own national outbreak reporting systems.

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The Wellcome Trust/Burroughs Wellcome Fund Joint Program in Infectious Diseases of the Tropical Developing World

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The Burroughs Wellcome Fund is a U.S.-based, private, independent foundation whose mission is to advance the medical sciences by supporting research and other scientific and educational activities. As a medium-sized foundation with assets of approximately $730 million, the fund invests $30 to $35 million in research each year, primarily in the United States and Canada. The foundation has been supporting basic research in infectious diseases, especially parasitic diseases, since 1981.

Since the Burroughs Wellcome Fund is relatively small, we must leverage research investments, making sure that our modest funds can have an impact beyond their face value. One way of increasing the value of our investment is to establish partnerships with other organizations. This approach allows funders to extend their support to new areas without diverting large parts of their limited resources from currently funded areas.

In 1997, the Board of Governors of the Wellcome Trust, the world's largest medical research charity, set aside $25 million to fund a 2-year collaborative venture with the Burroughs Wellcome Fund. Through this collaboration with the Wellcome Trust,[1] the Burroughs Wellcome Fund has been able to enter the international health arena. In 2000, the fund's board of directors added $1 million to the program, a sum proportionate to the trust's $25 million investment, based on each organization's respective annual research spending.

The shared program, a jointly administered effort focusing on infectious diseases in the tropical developing world, supports research collaborations between workers in the United Kingdom, North America, and the tropics. Research funded by the program must focus on diseases especially important in the developing world. It must involve a true three-way collaboration in which all the partners play interdependent and supportive roles. Importantly, it requires that the collaboration's "center of gravity" be in the tropics: the tropical world must truly contain the lynchpin of the project and not simply be the collecting ground for interesting problems to be taken away and studied elsewhere. The aim is to build true partnerships that benefit all collaborators and enhance the research strength of the developing world partner.

The problem of "brain drain" from the developing world to the developed world is significant, and it will not be solved with small investments. By strengthening collaborations and focusing on developing world research problems in situ, investors can enhance the strength of developing world scientists and stimulate further investment as their work unfolds.

The Burroughs Wellcome Fund will use the results of this program to study the fund's potential roles in this arena. The need for world health funding is vast and daunting, especially for smaller funders, but support of basic research and stimulation of research collaboration can be done with far fewer dollars than other approaches to world health.

The joint program's second and final round concluded in late 2000 with selection of six new awardee programs, in addition to the seven programs supported in 1999.

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[1] These two organizations have related names and some shared history but are completely independent of one another. The trust focuses its funding in the United Kingdom, but also supports a large international research program. In general, the trust does not fund projects in the United States or Canada.

Yellow Fever in Pará State, Amazon Region of Brazil, 1998–1999: Entomologic and Epidemiologic Findings

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Yellow fever (YF) is frequently associated with high severity and death rates in the Amazon region of Brazil. During the rainy seasons of 1998 and 1999, 23 (eight deaths) and 34 (eight deaths) human cases of YF were reported, respectively, in different geographic areas of Pará State; most cases were on Marajó Island. Patients were 1 to 46 years of age. Epidemiologic and ecological studies were conducted in Afuá and Breves on Marajó Island; captured insects yielded isolates of 4 and 11 YF strains, respectively, from *Haemagogus janthinomys* pooled mosquitoes. The cases on Marajó Island in 1999 resulted from lack of vaccination near the focus of the disease and intense migration, which brought many nonimmune people to areas where infected vectors were present. We hypothesize that YF virus remains in an area after an outbreak by vertical transmission among *Haemagogus* mosquitoes.

Yellow fever (YF) is an important arbovirus infection of humans and sylvan primates. Infection in humans is accompanied by high rates of illness and death. The causative agent, YF virus, is the prototype strain of the genus *Flavivirus*, family Flaviviridae (1). YF virus is maintained in two distinct transmission cycles. One is sylvatic: monkeys act as vertebrate hosts, forest canopy mosquitoes of the genera *Haemagogus* and *Sabethes* (chiefly *Haemagogus janthinomys* and to a lesser extent *Sabethes chloropterus)* are vectors, and human infections occur as sporadic cases or as limited outbreaks (2-4). The other transmission cycle is urban: YF virus is directly transmitted from human to human by the bite of infected *Aedes aegypti* mosquitoes, and other animals are not associated with transmission. Urban YF was eradicated from Brazil after 1942 (3-5). Sylvan YF virus is still reported in South America and Africa (6-9).

In recent decades in South America, YF cases and outbreaks have been reported, especially in Peru, Bolivia, and Brazil, where >90% of all reported episodes of disease in the 1990s occurred (10). In Brazil, YF occurs annually, causing sporadic cases, small outbreaks, or self-limited epidemics in the jungle or rural areas of the Amazon region (the natural focus of the disease), the Central-West region, and western regions of Maranhão and Minas Gerais states (3,4,11).

We report entomologic and epidemiologic findings regarding an unusual occurrence of YF cases in an area of Pará State in 1998 and 1999.

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Materials and Methods

Collection Sites

Mosquito and blood collections were made at three sites in Pará State (Figure 1). Afuá (0°06' S; 50°20' W; population approximately 30,000) and Breves (1°41' S, 50°19' W); population approximately 75,000, are municipalities of Marajó Island, in the northern region of Pará State, known for buffalo breeding and fish farming. The other site, Altamira (2° 51' S; 51° 57' W) (approximately 80,000 inhabitants), is in the central region of the state on the Xingu River delta near the Transamazon Highway; its chief products are wood, cattle, and sugar cane and cacao.

Samples

Blood samples were taken from persons who had clinical symptoms and signs compatible with YF for attempts at virus isolation, as well as from contacts (family and neighbors) for serologic examinations. Approximately 5 mL to 10 mL of blood was obtained by venipuncture. Serum samples were stored at -20°C until tested. From patients with fever and other clinical symptoms, specimens were also obtained for attempted virus isolation. Monkeys were euthanized, and blood samples and liver fragments obtained for virus isolation. All specimens were frozen in liquid nitrogen containers until processed in the laboratory.

Mosquitoes

Diurnal human-biting mosquitoes were collected in Afuá, Breves, and Altamira. The collections were made from 9:00 a.m. to 3:00 p.m. on the ground and at an elevation of approximately 15 m in the forest canopy, since these are the more active periods and places of potential YF virus vectors.

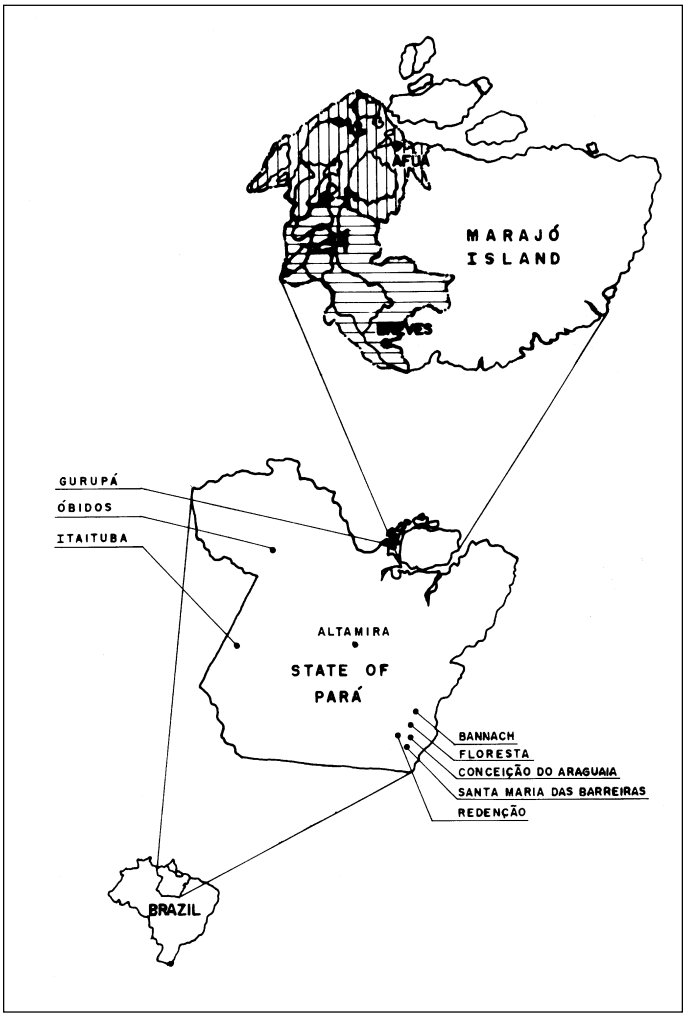

Figure 1. Map of Pará State showing Afuá, Breves and other municipalities where yellow fever was reported in 1998 and 1999, and Altamira municipality where yellow fever virus was isolated in mosquitoes and monkeys.

In Afuá, collections were made from May 17 to May 30, 1998, and from March 11 to March 25, 1999. Collections in Breves were from March 11 to March 25, 1999. In Altamira, collections were made on April 16 to April 28, May 20 to June 5, and July 10 to July 19, 1998, and from January 31 and February 13 (rainy season) and July 14 to July 27 (dry season) in 1999.

In the laboratory, mosquitoes were classified and pooled under refrigeration, by species, place, and dates of collection, and then preserved at -70°C until inoculation. Minimum infection rates (MIRs) of *Hg. janthinomys* mosquitoes were calculated by dividing the total of positive pools by the total number of specimens processed (12).

Serology

Serum samples were initially screened by hemagglutination inhibition (HI) against YF antigen (strain Be H 111). Tests were performed as described by Clarke and Casals, using a microtechnique in which serum samples were acetone extracted (13). All positive samples were later assayed by enzyme immunoassay for capture of immunoglobulin (Ig) M (MAC-ELISA) (14). All positive samples by both tests were later tested by plaque reduction neutralization test (PRNT) to confirm infection (15).

Serologic Criteria for Inclusion in Study

Positive diagnostic criteria for inclusion in our study were 1) serologic conversion by fourfold increase in antibody titers between acute- and convalescent-phase (7 to 14 days after first collection) serum samples and 2) presence of IgM without history of YF vaccination plus positive reaction (>1:10) by PRNT.

Pathology

From fatal cases, liver samples were obtained; histologic sections were stained by hematoxylin and eosin and examined by light microscopy. Specific YF antigens were detected in paraffin-embedded liver samples of fatal cases by means of an immunohistochemistry technique (16). All patients with these antigens were considered YF-positive cases and included in the study.

Virus Isolation and Identification

All samples (blood, viscera, and mosquitoes) were inoculated into suckling mice and C6/36 cells for virus isolation. Before processing, samples were thawed, triturated, and diluted in phosphate-buffered saline (pH 7.4) with 0.75% of bovine albumin and antibiotics (100 µg/mL of streptomycin and 100 IU/mL of penicillin) and centrifuged for 10 minutes at 2,100 x *g* (15). The supernatant of each specimen was then injected into suckling mice (0.02 mL intracerebrally) and into tubes of cells (0.1 mL), respectively. Isolated strains were identified by indirect immunofluorescence assay and complement fixation test (15). Isolation of YF virus from blood or tissues of human patients without history of vaccination was used as the positive criterion for inclusion in the study.

Results

Epidemiology

In 1998 and 1999 in Pará State, 23 and 34 YF cases, respectively, were diagnosed. In 1998, 17 of the 23 occurred in Afuá and 6 in municipalities not on Marajó Island (Bannach, Floresta, Gurupá, Itaituba, Óbidos, and Redenção). In 1999, 15 of the 34 diagnosed cases occurred in Afuá, 14 in Breves, and 5 in three other municipalities (Conceição do Araguaia, 2; Santa Maria das Barreiras, 2; Redenção, 1), all in the southeast region (Figure 1). The sex and age distributions of cases were determined (Figure 2). Comparison of total cases and deaths in both years shows a case-fatality rate of 34.8% in 1998 and 23.5% in 1999 (Figure 3). Most reported cases were diagnosed by serology or another technique combined with serology, with clinical and epidemiologic features taken into account (Figure 4).

Afuá

A scientific trip was undertaken to the municipality (May 17 to 25, 1998). From the 23 human samples, three isolations of the YF virus (H 603325, H 603327, and H 603797) were made. Of the monkey specimens, two samples of YF virus (AN 604552, AN 604555), were isolated from the blood and liver, respectively of monkey (PR 2968) of the species *Alouatta belzebul*. The YF cases occurred in several areas, among them

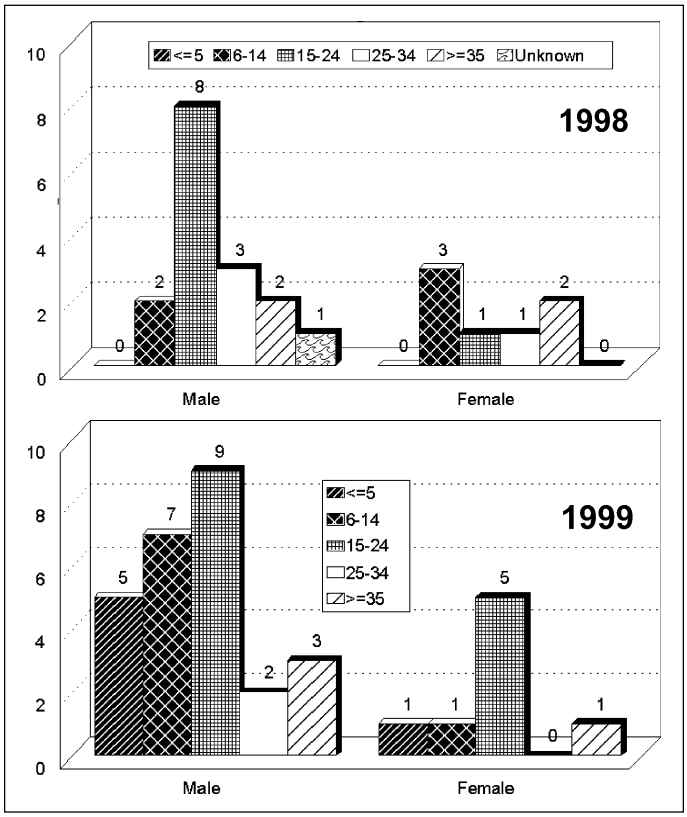

Figure 2. Distribution of yellow fever cases, Pará State, by age groups, 1998 and 1999.

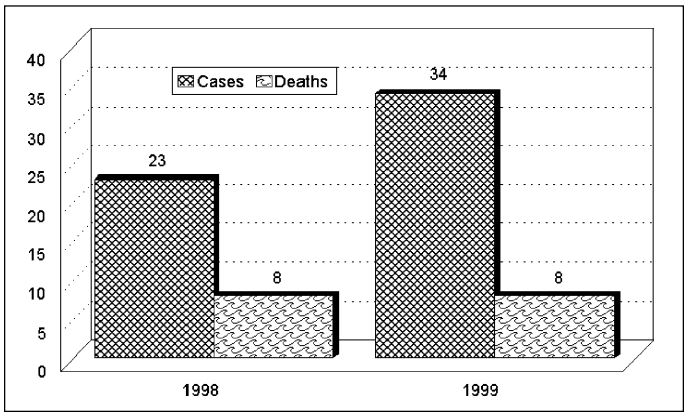

Figure 3. Yellow fever cases and deaths reported, Pará State, 1998–1999.

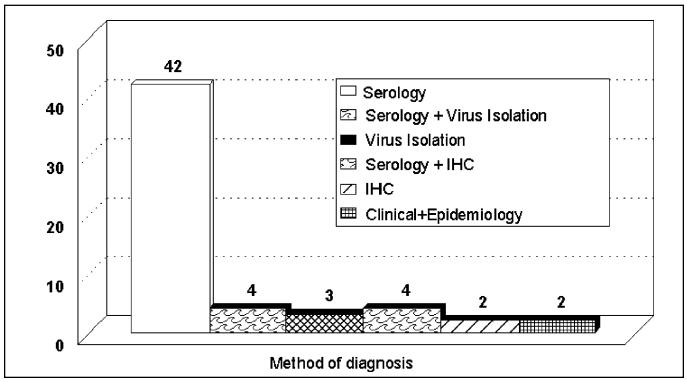

Figure 4. Diagnostic procedures used on the yellow fever cases reported, Pará State, 1998–1999. IHC = immunohistochemistry.

the river Morcego, Morceguinho, Tamanduá, Bom Jardim, and Furo da Cidade.

Entomology

Afuá

A total of 1,621 Culicidae were collected with human bait during the day in the canopy and at ground level, which after identification, provided 77 pools for inoculation. The most abundant species were *Wyeomyia* sp and *Hg. janthinomys,* with 1,119 (69%) and 296 (18.3%) specimens, respectively; after identification, these formed 23 and 14 pools, respectively, for virus isolation in tissues. Four samples of YF virus were isolated from pools of *Hg. janthinomys* (AR 605158, AR 605159, AR 605160, and AR 605161). The MIR for *Hg. janthinomys* was 1.35%.

Altamira

Deaths of monkeys in the forest on agricultural secondary roads of the Transamazon Highway in the stretch from Altamira to Marabá (km 20 and 27) motivated a scientific expedition from April 16 to 28, 1998. A total of 592 hematophagous insects were collected on human bait; 479 (80.9%) were the mosquito *Hg. janthinomys*, which after identification provided 38 and 24 lots, respectively, for virus research. The pools inoculated yielded 10 isolations of YF virus, all from *Hg. janthinomys* (MIR = 2.01%).

The high infection rate with YF virus observed in *Hg. janthinomys* motivated a second trip (May 20-June 5) to determine the spatial distribution and infection rate of *Hg. janthinomys*, as well as to evaluate the dynamics of the circulation of YF virus in the Altamira area. A total of 509 hematophagous diptera (66 lots) were captured; 312 (61.3%) (29 lots) belonged to the species *Hg. janthinomys*. Three samples were identified as YF virus, as occurred in the first trip; all positive samples were from pools of *Hg. janthinomys*, for an MIR of 0.96%.

From July 10 to July 19 (dry season), a third trip was made to the same area of Altamira to monitor circulation of YF virus. In this trip, 120 hematophagous insects were captured in 14 lots; 28 (23.3%) of them were *Hg. janthinomys* from a single lot. Injection of these lots into newborn mice did not produce virus.

In the rainy season (January 31 to February 13) of 1999, 1,105 (93 pools) mosquitoes were captured; 84 (5 pools) were *Hg. janthinomys*. During the dry season (July 14 to 27), 133 mosquitoes (14 pools) were collected; 44 (3 pools) were *Hg. janthinomys*. No virus was isolated.

Afuá/Breves

The occurrence of human cases motivated the expedition to Afuá and Breves to carry out entomologic studies of potential vectors of YF virus. From March 11 to March 25, 1999, captures of hematophagous insects were performed at ground level and in the forest canopy. A total of 2,164 insects were collected on human bait in 126 pools; 546 of these were *Hg. janthinomys*, which furnished 23 pools for inoculation in attempts at virus isolation. Eleven strains of YF virus were obtained from pools of *Hg. janthinomys*, for an MIR of 2.01%. No virus was isolated from the other mosquito pools. During this trip, three howler monkeys (*Alouatta belzebul*) were euthanized. The specimens from the monkeys produced three

YF virus isolates, two from the blood and liver of the same monkey and another from the liver of a second monkey. These monkeys were found within 200 m to 500 m of human dwellings. They had been showing abnormal behavior, i.e., moving slowly and not trying to escape from people.

Discussion

In 1998, in Afuá municipality, the first YF human case (based on epidemiologic information) had onset of symptoms on February 3. As the YF medium incubation period ranges from 3 to 6 days, infection probably occurred at the end of January. Our trip to the municipality was in May, >3 months after the index case. Despite the long interval between the index case and our expedition, we recovered YF virus from pools of *Hg. janthinomys* mosquitoes and from howler monkeys. These findings strongly suggest an elevated natural average infection rate of the vector mosquitoes in the area. This was the first detection of YF virus in the municipality of Afuá. YF virus has not been reported on Marajó Island since 1988, when a sporadic case occurred near Breves in a man who cut down a tree.

The study in Altamira in 1998 shows clearly how YF virus outbreaks happen. The rainy season, in the first months of the year, has the highest rainfall indexes in the Amazon forest region. This facilitates breeding of mosquitoes, including the potential vector *Hg. janthinomys*, in the forest. When the rains decrease, the amount of mosquitoes in the forest gradually decreases (Table). As the population of mosquito vectors decreases, YF virus disappears.

In Breves and Afuá in 1999, reported cases clearly resulted from a failure of the vaccination campaign, since after the outbreak in 1998, the inhabitants of Afuá and Breves were vaccinated. However, some people from Afuá were not immunized, and they migrated to areas near places where cases had been previously reported. The occurrence of these cases and the isolation of YF virus from *Hg. janthinomys* and monkeys indicate intense circulation of the virus. Circulation of the virus for 2 years in the same limited area probably occurred because eggs of infected mosquitoes remained in the region. When the rainy season began, the *Hg. janthinomys* eggs hatched, and probably many of them were born infected by vertical transmission. Although little evidence of this possibility has been obtained in nature, it is plausible because the monkey population is not thought to be large enough to maintain the sylvan cycle (17). Effectively, monkeys who are acutely infected either die or become immune, aborting further infections (8,18). On the other hand, evidence for smaller mammals (especially rodents and marsupials) playing a role in the maintenance cycle has been only rarely established (18-20). In Afuá and Breves municipalities, no evidence was found to incriminate hosts other than primates in the epidemic.

Table. Comparison of number of mosquitoes collected on human bait, number of YF strains isolated by place of capture, and minimum infection rate (MIR) for *Haemagogus janthinomys*, Pará State, Brazil, 1998–1999

Place	Year	Strains isolated	*Hg. janthinomys* baited	MIR	Season
Afuá	1998	4	296 (14)	1.35%	Rainfall
Altamira	1998	13	819 (54)	0.96%-2.01%	Rainfall
Altamira	1999	-	129 (8)	-	Dry
Breves	1999	11	546 (23)	2.01%	Rainfall

( )= No. of lots of pooled mosquitoes in which virus isolation was attempted.

The current theory for YF is that transmission occurs in cyclic waves of 7 to 10 years that result in epidemics (8,18). Our results, especially in Altamira, do not confirm that observation. Based on our results, we speculate that the occurrence of epidemics in the same limited geographic region of two neighboring municipalities was only possible because mosquitoes were born infected by vertical transmission. Infections in monkeys in 1998 should have made a large number of them immune, and the short interval between the outbreaks was not enough to renew the monkey population. We hypothesize that the persistence of YF virus in a region occurs by passing through several generations of mosquitoes and that this is the main mechanism responsible for maintenance of the virus and not the epidemic wave as has been suggested (11,18-20). The occurrence of YF cases or outbreaks, therefore, is a direct function of migration of nonimmune persons. In places such as Altamira, where people show high YF vaccination rates, it is quite difficult to find a human case, despite the municipality's situation in the endemic area with virus circulation.

The absence of human YF, however, is not enough to avert virus circulation because humans acquire infection accidentally and are a deadend host, playing an unimportant role in the maintenance of the virus in nature. Continued entomologic and epidemiologic studies must be conducted in different sites where YF outbreaks or cases have been reported to prove that *Haemagogus* mosquitoes maintain YF virus vertically.

The occurrence of YF cases in other areas at the same time or within a short time period also supports our hypothesis. Since some municipalities are located >1,500 km from Afuá and Breves municipalities (Figure 1), YF virus must be present there; when nonimmune people enter the forest, they become infected. Therefore, appearance of cases is the result of a silent restricted circulation of the virus in an area's forest.

On the other hand, YF cases in South America have thus far only been transmitted by sylvatic vectors, especially *Hg. janthinomys* (3,11,21). The susceptibility of the *Ae. aegypti* population in South America to YF virus must be established, in the face of the increased risk of reemergence of urban transmission (22-24). The annual occurrence of several cases in Brazil and hundreds of them in Peru and Bolivia may have permitted contact of YF virus with *Ae. aegypti*. Surprisingly, urban transmission has not yet been reported, except for six cases in Santa Cruz, Bolivia (25). Thus, it is necessary to establish the level of infectivity and susceptibility of South American *Ae. aegypti* with YF virus.

The most important step in the control of YF would be a continent-wide resolution to improve YF vaccination (6,26,27). Protection acquired after 17D vaccine lasts up to 10 years; after that period, the Pan American Health Organization (PAHO) and World Health Organization (WHO) recommend a new dose, although some studies show protection for 17 to 35 years with a single dose (28,29). Another important measure would be creation of a network (probably Internet based) to keep all countries in the continent quickly informed of YF cases or outbreaks, especially in major risk areas.

To date, financing YF vaccine has been a major problem for disease-endemic countries, but studies developed in Africa have suggested that low costs are possible (26). Moreover, Brazil has been producing YF vaccine with the 17D strain on a large scale for a long time. We believe that combined efforts under PAHO/WHO support to supply other countries in the region with vaccine for a massive vaccination campaign would save thousands of lives.

Acknowledgments

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Tuberculosis in North Carolina: Trends Across Two Decades, 1980–1999

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In North Carolina, we analyzed cumulative data for tuberculosis (TB) from 1980 through 1999 to determine trends in incidence, population subgroups at risk, and implications for health policy-makers. The overall incidence rates declined significantly over the study period ($p = 0.0001$). This decline correlates strongly with an increase in TB patients receiving directly observed therapy. Males have approximately twice the risk for disease, and persons ≥65 years of age are at the highest risk. For every Caucasian with TB, six blacks, six Hispanics, and eight Asians have the disease. TB incidence rates are declining in all other population subgroups but increasing in foreign-born and Hispanic persons.

According to estimates of the World Health Organization (WHO), one third of the world's population is infected with tuberculosis (TB) (1). During the 1990s, approximately 90 million new cases have developed worldwide (1). In the United States, from 1953 through 1984, the incidence of TB declined an average of 5% per year, but increased by 20% during 1985 through 1992 (2). The nationwide peak in incidence of TB in 1992 led to renewed public commitment and investment of resources. Simultaneously, scientific investigations, especially at the national level, have resulted in interesting and useful findings for policy-making. However, such strategies, based on evidence from pooled data from all U.S. states and territories, may not necessarily be effective in certain regions of the United States, where characteristics of TB patients may differ substantially from the national pattern. Additionally, state and local health departments involved directly with TB prevention programs may require evidence-based information derived from locally available TB records, which more accurately represent the realities of TB disease in that locale. In this study, we describe the trends in TB incidence in North Carolina and the sociodemographic characteristics associated with elevated risk for the disease.

Materials and Methods

Suspected or confirmed cases of TB are reportable by law in North Carolina. Cases reported to the state health department are verified according to Centers for Disease Control and Prevention (CDC) criteria (3). Confirmed cases are then registered, investigated, and managed according to a standard protocol. The complete information, including follow-up status, is recorded in the CDC Report of Verified Case of Tuberculosis (RVCT) form and forwarded to CDC electronically. The state health department also maintains all TB case records in its own database, which is the source of our data for this study. All confirmed incident cases of TB reported in North Carolina from 1980 to 1999 were considered in our analysis.

TB incidence rates (cases per 100,000) for North Carolina were computed from 1980 through 1999, allowing comparison of TB trends between the 2 decades (1980 to 1989 vs. 1990 to 1999). For comparison with national TB incidence rates over time as well as the ranking of North Carolina among other U.S. states, territories, and the District of Columbia, we included national TB cases and incidence rates from 1980 to 1999. In 1993, the state TB surveillance system was restructured and funding was increased after a nationwide peak in TB incident cases was observed. In determining whether the number of TB cases in the state has decreased substantially as a result of this investment of resources, a comparison of incident TB cases from 1980 to 1989 versus 1990 to 1999 can only underestimate any improvement. Consequently, any observed decline must be considerably important to be captured by this conservative approach. For the rest of the analysis, only information on TB cases from 1982 to 1999 was used because data were incomplete for most of the demographic variables before that time. The variables coding for country of origin and directly observed therapy (DOT) were added to the CDC RVCT form in 1993, so analysis involving these factors covers only 1993 to 1999.

The yearly rate of TB cases in the state was calculated by dividing the total incident TB cases by the 1990 population census or estimate for that year and multiplying by 10^5. The denominators were obtained from the state census bureau. The national TB rates for the whole United States for the period of study were extracted from yearly reports in MMWR, which provide TB cases and rates by state (4-8). We then computed the average national rates after subtracting the contribution from North Carolina for each year before comparing the two.

In estimating the rate of TB for a given sociodemographic factor, data were adjusted for age by the direct method of standardization. For each variable, the incidence density rate was computed by dividing TB cases by the expected

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person-years and multiplying by 10^5. Using the PROC GENMOD in SAS, we generated incidence-density ratios and their 95% confidence intervals by maximum likelihood estimation, assuming a Poisson distribution for our data, namely, that the probability (Pr) of TB cases (y) per 10^5 person-years is equal to some number r is given by

$$Pr(y=r)= \lambda e^{-\lambda}/r!$$

Where λ is the expected value (mean) of y and r!=r(r-1)(r-2)…(2) (1).

Statistical Analysis

Data were analyzed with SAS software (version 6.12). Incidence rates of TB (expressed as TB cases per 100,000) in the United States over the study period were compared with those of North Carolina by two-sample t-test. Paired sample t-testing was used to assess differences in TB incidence rates in North Carolina between the two decades (1980 to 1989 vs. 1990 to 1999), as well as the national ranking of the state during the two periods. The underlying assumption is that, since data for the two periods were obtained from the same population source, they are correlated. An overall regression equation linking year and TB incidence rates in the state was modeled to determine the amount and direction of change in rates across the 2 decades, and the best-fitting slope was plotted. In determining the best predicted trajectory, a quadratic in addition to the linear term (of the independent variable of year), was added to the model, yielding a curvilinear figure. A similar procedure was performed for the selected sociodemographic factors using the linear model only. A trend in the proportion of TB patients receiving directly observed therapy (DOT) was compared with the corresponding yearly incidence rates of TB, as well as rates of therapy completion, estimated by Pearson's correlation coefficient. All tests of hypothesis were two-tailed, with a type 1 error rate fixed at 5%.

Results

From January 1980 to December 1999, 13,564 incident cases of TB were recorded in North Carolina, for a yearly average of 678 new cases. From a peak of 1,066 cases in 1980, TB cases have steadily declined, with new TB cases for 1999 reported at 488, a decline of 54.2%. Comparison of trends over time in North Carolina TB rates with the U.S. national rates shows that both have declined continuously since 1992, the year the national TB incidence rates peaked after a continuous decline in the 1980s. The rate of decrease in the two populations was approximately the same (t = 0.98, p = 0.34). Analysis of the data for the national ranking of North Carolina across the 2 decades shows a significant improvement (p = 0.003) in the state's ranking in the second decade (1990 to 1999) compared with the first (1980 to 1989). From being third worst nationwide in 1980, the state now is in 17th position. A similar significant improvement was observed when rates were the comparative indices between the 2 decades (p = 0.0001). The regression coefficient for the trend in incidence rates over time shows that for each additional year since 1980, the rate of TB has declined by approximately 0.5 case per 100,000 population (p = 0.0001) (Figure 1).

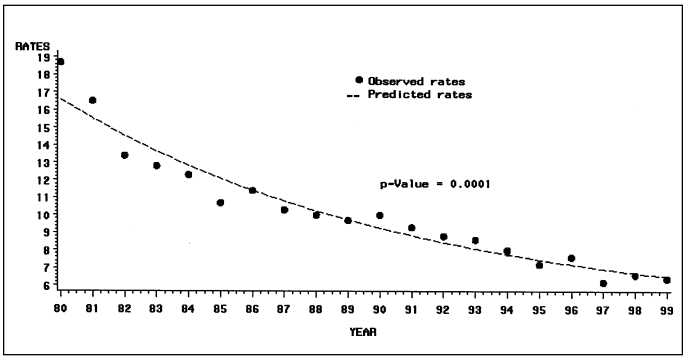

Figure 1. Incidence rates of tuberculosis, North Carolina, 1980-1999. (Rates are per 100,000)

Sociodemographic Distribution of TB Disease (Table 1)

In general, males are twice as likely to have TB than females (Table 1); this 2:1 sex ratio has been constant over both study periods. A test of equality of variance over the study period for the two groups strongly supported equal deviations independent of general trends in incidence rates (F = 1.01, p = 0.99). However, the distribution of male:female ratio differs by race, ethnicity, and age group. Among Asians, males and females have the same risk for TB disease (1:1), whereas among Hispanics and Native Americans the proportion of males with the disease is three times that of females. Blacks, whites, and non-Hispanics have a 2:1 sex ratio. In both U.S.-born and foreign-born persons, males have twice the risk of females. At birth and up to the age of 24, there is no difference in risk by sex. Thereafter, more men contract TB than women, reaching a 2:1 ratio in the age group 25 to 44.

Table 1. Tuberculosis incidence density rates and ratios in North Carolina, by sociodemographic characteristics (1982 to 1999)

Variable	Incidence Rate[a] (per 10^5 person-years)	Rate Ratio	95% Confidence Interval
Sex			
Female	5.88	1.00	2.07-2.24
Male	12.70	2.16	
Age Group[b]			
<15	1.51	1.00	
15-24	2.54	1.76	1.53-2.0
25-44	7.30	5.00	4.53-5.60
45-64	13.45	9.20	8.24-10.22
≥65	26.37	18.30	16.41-20.32
Race			
White	4.14	1.0	
Black	25.53	6.20	5.93-6.42
Asian	35.95	8.52	7.75-9.38
Native American	4.90	1.20	1.07-1.48
Ethnicity			
Hispanic	26.80	2.90	2.66-3.25
Other	9.10	1.0	
Country of birth[c]			
Foreign-born	10.70	1.0	
US-born, all	6.9	0.64	0.58-0.70
US-born, blacks	19.80	1.83	1.66-2.03

[a]Age-adjusted.
[b]Chi-square for trend = 7.66 (p = 0.006).
[c]1993-1999 only.

This trend reaches a peak in the age group 45 to 64, when for every woman contracting the disease, three men are affected. After the age of 65, the sex ratio returns to the 1:1 proportion observed at birth and childhood (<15 years).

The age distribution of patients with TB disease in North Carolina is negatively skewed, with a median age of onset of 51 years and a preponderance of cases in the older age groups. The highest risk for TB in the state is among the elderly (≥65 years of age). This group has 18 times the risk of children <15 years of age, the age group with the lowest risk (Table 1). With respect to trend over time, the incidence rates of TB have decreased in all age groups (Table 2). The most impressive decrease is in the elderly population (≥65) to approximately 3 cases per 10^5 person-years. The rate of decline in those <25 years of age is insignificant.

Blacks, Hispanics, and Asians have the highest TB rates in North Carolina (Table 1). For every white person affected by TB disease, six blacks, six Hispanics, and eight Asians have the disease. Among blacks, TB incidence rates have steadily decreased since 1982, from 37.3 cases per 10^5 person-years in 1982 to 16.2 cases per 10^5 person-years in 1999. This decline is highly significant, corresponding to 1 case per 10^5 person-years ($p < 0.0001$) (Table 2). The incidence rates among Asians have fluctuated since 1982. The highest rate, 59.6 cases per 10^5 person-years, was recorded in 1982, and the nadir, 27.0 cases per 10^5 person-years, occurred in 1990. The latest TB incidence rate (1999) for Asians is 47.4 TB cases per 10^5 person-years. Despite these fluctuations, the overall trend in the regression slope depicts a moderately significant decline over time among Asians (parameter estimate = -0.99, p = 0.03) (Table 2). Hispanics are the only population group with an increasing trend in TB incidence rate, equivalent to 3.2 cases of TB per 10^5 person-years. From a low of 4.2 cases per 10^5 person-years in 1982, the incidence rate in the Hispanic population reached a peak of 68.6 cases per 10^5 person-years in 1999. The only other group showing an increasing trend is foreign-born persons, among whom TB incidence increased at the rate of 1.2 cases per 10^5 person-years (Table 2). Compared with U.S.-born persons, foreign-born persons have a 36% higher risk for TB (Table 1). Those who have lived in the United States for <5 years constitute most of TB cases in foreign-born persons (62.2%). However, blacks still have about twice the risk of foreign-born persons (relative risk [RR] = 1.83; 95% confidence interval [95% CI] = 1.66-2.03).

Information about mode of administration of TB treatment was available in 91% of all cases. The proportion of TB patients receiving DOT (both exclusively and in combination with self-administered therapy) increased substantially, from 44.4% in 1993 to 94.7% by 1999 ($p < 0.0001$) (Figure 2). A high correlation was observed between declining rates of TB in the state and the increase in the proportion of TB patients receiving DOT (r = -0.95, p = 0.0008) (Figure 3). DOT was also found to correlate with completion of therapy over time (r = 0.64, p = 0.1).

Table 2. Trends in incidence rates of TB by selected variables, North Carolina, 1982–1999

Variable	Parameter estimate[a]	p-value
Sex		
Female	-0.24	0.0001
Male	-0.55	0.0001
Age group		
<15	-0.033	0.19
15-24	-0.027	0.59
25-44	-0.113	0.014
45-64	-0.60	0.0001
≥65	-1.41	0.0001
Race		
White	-0.21	0.0001
Black	-1.11	0.0001
Asian	-0.99	0.03
Native American	-0.024	0.19
Ethnicity		
Non-Hispanic	-0.47	0.0001
Hispanic	3.2	0.0001
Country of origin[a]		
Foreign-born	1.2	0.0084
US-born	-0.53	0.0011
US-born (blacks only)	-1.61	0.0033

[a]This denotes the slope that reflects an increasing (if positive) or decreasing (if negative) trend.
[a]Data from 1993 to 1999.

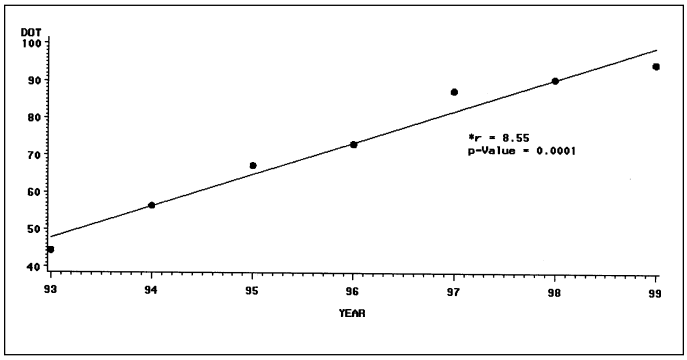

Figure 2. Proportion of tuberculosis patients receiving directly observed therapy (DOT) over time

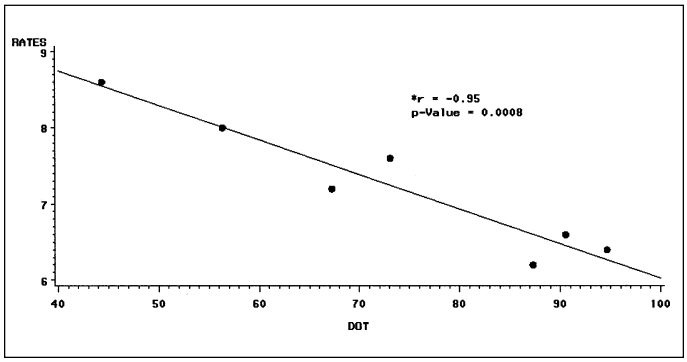

Figure 3. Correlation between incidence rates of tuberculosis (TB) and proportion of TB patients on directly observed therapy (DOT) over time

Conclusions

Two decades ago North Carolina had among the worst TB records nationwide, ranking third in number of cases in 1980. Since that time, however, the incidence rate of the disease has decreased threefold, from 18.7 in 1980 to 6.2 per 100,000 in 1999. Two factors that may account for this significant decline are improved supervised therapy of patients and the related increase in the proportion of TB patients completing therapy. The proportion of TB patients receiving DOT in North Carolina has increased significantly over the study period and correlates strongly with the concomitant decline in the rate of TB over time ($r = -0.95$, $p = 0.0008$). In addition, completion rates, defined as the proportion of TB patients completing a prescribed anti-TB regimen, have also improved, correlating, although nonsignificantly, with increased reliance on DOT.

Our study is consistent with other reports that have demonstrated this direct link between improvement in DOT and decline in incident cases of TB (9-11). Other authors have also found DOT to be superior to unsupervised therapy, in addition to its role in curbing TB relapses (12). In addition, in persons predicted to be noncompliant because of psychiatric disease, alcoholism, drug addiction, substance abuse, and homelessness (13,14), DOT might be more successful than unsupervised therapy (15). Despite this evidence in support of DOT, it would be erroneous to conclude that DOT alone could have accounted for the observed significant reduction in TB cases in North Carolina. Factors such as adequate training and commitment of care providers and those involved in TB control programs, as well as overall administrative efficiency are important factors. Inadequacies in such a system or its functional collapse could render TB control and prevention ineffective regardless of improvement in DOT as was observed elsewhere (16). Another explanation could be the steady improvement in the living conditions of the population in general, leading to better housing, less overcrowding, and improved nutrition, and these factors are known to be inversely correlated with levels of TB disease.

We also found that men are two to three times more vulnerable to TB disease, in accordance with other reports (11,17,18). Given that the sex difference exists only between the ages of 25 to 64, in other words, the working age group, differential exposure to TB-associated risk factors, such as HIV infection, injecting drug use, alcohol abuse, and probably occupation, may be responsible for the consistent increased male risk for TB disease. Our data confirm that alcohol (odds ratio [OR] = 2.9, 95% CI = 2.27-3.719) and HIV infection (OR = 1.76, 95% CI = 1.295-2.392) account independently and significantly for the preponderance of TB disease among men. Therefore, in the ongoing battle to eliminate TB the presence of either condition, especially in men, should be considered as a potential marker for the possible concomitant presence of TB disease.

Age is an established risk factor for TB disease acquisition. Nationwide, the elderly (≥65 years old) are the age group at the greatest risk, with incidence rates of 15 to 18 cases per 100,000, corresponding to a risk 4 to 9 times that of children (<15 years), the age group with the lowest risk (4-8,11,19). In North Carolina, TB risk among the elderly is 18 times that of children <15 years old. This age cohort represents people who must have been exposed to TB bacilli at a much younger age in the era of high TB disease rates in the United States. The disease remained latent as long as they enjoyed the enhanced immune status associated with younger age. Deterioration of the immune system with aging allows reactivation of dormant TB bacilli, leading to symptomatic disease.

The groups most affected by TB disease in North Carolina are racial and ethnic minorities: blacks, Hispanics, and Asians. Among Asians, the contribution of externally acquired TB infection may be the main source of TB disease. From 1993 to 1999, our data show that of 193 incident cases of TB reported in Asians, 181 cases (94%) are among foreigners. The main countries of origin are Vietnam (24%), the Philippines (15%), India (13%), Korea (9%), and Laos (7%). The same pattern is also observed among Hispanics, with 82% of cases in foreign-born persons, mostly from Mexico (78%). The high incidence of TB in these two minority communities is therefore externally acquired. A further source of concern is the sharp increase in incident cases of TB in the Hispanic population in the past 18 years, from 4.2 in 1982 to 68.6 per 10^5 person-years in 1999, a phenomenon caused by the persistent increase in rates of TB in the Hispanic population, averaging 3.2 cases per 10^5 person-years. In the state's black population, in contrast, only 85 TB cases (3.77%) of 2,252 recorded from 1993 to 1999 are accounted for by TB in foreign-born persons.

Nationwide, the proportion of U.S. TB cases attributable to foreign persons rose from 22% of the national total in 1986 to 37% a decade later (6). However, such a comparison based on shift in proportions may not be an appropriate scale for measuring the magnitude of TB disease in foreign-born persons, since a downward trend at one end of the balance (U.S.-born) implies an increase at the other end (foreign-born). Nevertheless, other studies have also found a two- to fourfold elevated risk for TB disease among immigrant residents compared with U.S.-born persons, tallying with similar reports of high TB incidence rates among immigrants (20-23). Although in North Carolina foreign-born persons have a 36% higher risk for TB disease than the U.S.-born, this elevation in risk is moderate compared with the national average risk for foreign-born persons (24). Indeed, our data show that U.S.-born blacks have an 83% higher risk for TB disease than foreign residents in the study area (RR 1.83; 95% CI 1.66-2.03).

The overall incidence of TB disease has significantly decreased over the past decade in North Carolina. The level of TB disease, however, is still very high among ethnic minorities (blacks, Hispanics, and Asians). Although rates of TB are declining in all other population subgroups, rates among foreign-born and Hispanic persons are increasing. The elimination of TB in the state may only be achieved by addressing the elevated rates in these groups, as well as the increase in incident cases among foreigners and Hispanics.

Dr. Salihu is a physician with special interest in population-based epidemiologic research. He also holds a PhD in epidemiology and biostatistics.

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Waterborne Outbreak of Tularemia Associated with Crayfish Fishing

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In 1997, an outbreak of human tularemia associated with hare-hunting in central Spain affected 585 patients. We describe the identification of *Francisella tularensis* biovar palaearctica in a second outbreak of ulceroglandular tularemia associated with crayfish (*Procambarus clarkii*) fishing in a contaminated freshwater stream distant from the hare-associated outbreak. The second outbreak occurred 1 year after the first.

Human tularemia is a rare but highly virulent bacterial zoonosis with endemic foci in the Northern Hemisphere (1). Its clinical manifestations depend on the route of infection. The ulceroglandular form, the most common, occurs after handling contaminated sources. Ingestion of contaminated food or water can cause an oropharyngeal form. Pulmonary, typhoidal, glandular, and ocular forms are less frequent. The disease occurs in outbreaks, usually associated with direct contact with infected game or contaminated water, or in a seasonal pattern in arthropodborne tularemia (2). *Francisella tularensis* is the causative agent (3). Two main biovars are included in this species: the most virulent (Jellison type A or *F. tularensis* biovar tularensis), described mainly in North America and recently reported from central Europe (4), and a less virulent (Jellison type B or *F. tularensis* biovar palaearctica), mainly found in Eurasia and to a lesser extent in North America (5). The current third biovar, type C (*F. tularensis* biovar novicida) (6), was formerly considered one of the three species of the genus (3). Types A and B are related to human disease as the cause of severe and mild tularemia, respectively. Type C has been isolated from water and is an infrequent cause of disease in humans (6).

This microorganism is perpetuated in nature in an enzootic cycle involving wild mammals (mainly rodents and lagomorphs) and invertebrates (ixodid ticks, mosquitoes, tabanids, and other bloodsucking arthropods). The reservoir has not been clearly assessed, although the disease can be passed in nature by tick bite (2) and both transtadial and transovarial transmission have been described in ticks (7). While *F. tularensis* can survive for months in cold water, it is adversely affected by direct sunlight and hot temperatures.

Several enzootic cycles have been described in the Old World. Direct contact with infected hares accounts for most human cases in Western and Central Europe and the former Soviet Union, where water-related cases have also been described. Mosquitoes are the main vector for infection of humans and hares in northern Europe, and tick- and airborne cases have also been reported (2).

This disease is uncommon in southern Europe, but cases have occurred in Italy and France (8-10). In Spain, apart from the retrospective identification in 1999 of one ulceroglandular case acquired in 1996 (11), the first human cases were identified in 1997 (12,13), when a hare-associated outbreak affected 585 patients (14; and references thereafter in same issue). Since then, a few sporadic cases have been diagnosed in the same area.

We describe the identification of *F. tularensis* biovar palaearctica in a second outbreak of ulceroglandular tularemia associated with crayfish (*Procambarus clarkii*) fishing in a contaminated freshwater stream distant from the hare-associated outbreak.

Methods

Subjects

A confirmed patient was defined as a person with compatible signs and symptoms (ulcerated lesions in the hands and regional lymphadenopathies with or without fever and general discomfort) and an accompanying positive laboratory result (seroconversion or single antibody titer ≥1:128 to *F. tularensis* as measured by microagglutination or a positive polymerase chain reaction [PCR] result).

Epidemiologic Study

Several visits were made to the epidemic site. Fishing areas were identified by photographs that were subsequently shown to patients and controls. Interviews were conducted with local persons to obtain relevant information and with experts in red swamp crayfish ecology. Mean monthly dam water levels for 1998 were obtained.

An active search was made for compatible cases and for persons reporting possible contact with any potential reservoir of the disease. Health-care centers were alerted. We

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searched reports of emergency visits (for January 1 to August 31, 1998) and discharges (for January 1 to September 30, 1998) at the provincial hospital for patients who met the case definition or had tularemia-compatible symptoms.

Patients were interviewed to evaluate the probability of any contact with other tularemia reservoirs, pinpoint the fishing site, and define risk factors linked to crayfish fishing. We selected 20 controls who did not meet the case definition, had been in contact with crayfish caught on the same dates and in the same places, and generally resided in the same towns as patients. All patients and controls were interviewed from October 8 through December 7, 1998.

The crude odds ratio (OR), its 95% confidence intervals (CI), as well as adjusted OR, were determined by logistic regression.

Bacterial Strains and Culture

The live vaccine strain of *F. tularensis* type B (NCTC 10857) was used for serologic diagnosis and as a positive control for PCR. The strain was grown in modified Thayer-Martin (Difco Laboratories, Detroit, MI) chocolate, supplemented with Isovitalex (10 mg/L; BBL Microbiology Systems, Cockeysville, MD) and 1% L-Cysteine (Sigma-Aldrich Co., Alcobendas, Madrid, Spain) at 37°C in 5% CO_2 (3,13).

Environmental Samples

Eight water samples were collected at several points on the river where all patients had fished. Three water samples from a sewage plant located 15 km upstream were also collected. Water sampling was repeated 1 month later. Three crayfish captured by one patient had been frozen and were available for study (batch A). Twenty more crayfish were collected from two stretches of the river where the outbreak had started (batch B). Sixteen hares (*Lepus* spp.) (12 alive; 4 dead), 7 common shrews (*Sorex araneus*), 26 field mice (*Apodemus sylvaticus*), 2 gudgeons (*Gobio gobio*), 3 rats (*Rattus rattus*), 1 badger (*Meles meles*), and 1 common vole (*Microtus arvalis*), as well as sera from 60 sheep from the epidemic area were also studied. Wild mammals were euthanized in a CO_2 chamber and both liver and spleen cultured (13).

Intestines of batch A crayfish were not available for study as they were removed in situ and arrived at the laboratory after being extensively washed in tap water. Consequently, branchial tissue from three crayfish, one stomach, two gonads, and one hepatopancreas were aseptically collected and thinly ground with a scalpel. The carcasses were immersed in autoclaved distilled H_2O, shaken at 33°C for 30 min, centrifuged at 4,000 x *g* for 15 min at 4ºC, and the pellet (named wash) resuspended in 100 µL of autoclaved distilled H_2O. The rest of the process was as described above. For batch B crayfish, intestines were also dissected and studied. All fractions were plated in the same media as above.

Water samples (10 mL) were concentrated by centrifugation at 8,000 x *g* for 15 min and the pellet resuspended in 1 mL of the original sample and subjected to acidic shock, as described for *Legionella pneumophila* (15). Briefly, 0.1 mL of each concentrated sample was mixed with 0.9 mL of buffer HCl/KCl pH 2.2, and after an incubation of 5 min at room temperature, the samples were plated. This method was based on the assumption that the survival capacities of these microorganisms in the intracellular environment could account for resistance to acidic solutions and reduce the level of contaminants. Individual colonies that looked similar to *F. tularensis* live vaccine strains were pooled in groups of 10 in 100 µL of autoclaved distilled H_2O, carefully mixed, and half the volume was reserved for PCR. The rest of these samples were kept at 4°C.

Microaglutination Test

Agglutinating antibodies against whole *F. tularensis* live vaccine strain were determined as described (16,17). Titers $\geq$1:64 were considered positive.

PCR Detection of *F. tularensis*

A lymph node aspirate from one of the patients was subjected to guanidine thyocianate lysis for DNA extraction as described (18). All reagents were purchased from Sigma-Aldrich, except for glycogen and proteinase K (Boehringer Mannheim, Roche Molecular Systems, Inc., Branchburg, NJ).

For the water samples, 1 mL of each was centrifuged at 15,000 x *g* for 10 min, and the pellet was resuspended in 100µL of the same sample and clarified by a 5-min centrifugation at 3,000 x *g* to eliminate insoluble material. The supernatant was then collected, lysed as above, and DNA precipitated and resuspended with 10 µL of autoclaved distilled H_2O.

For the crayfish, all fractions studied by culture were also digested as above and subjected to PCR by using the 16S ribosomal DNA-based universal F1-R13 and *Francisella*-specific F5-F11 sets of primers described by Forsman et al. (19) in a nested PCR (Table 1).

The F5-F11 amplicons obtained from water, lymph node aspirates, and crayfish samples were sequenced by PCR with

Table 1. Polymerase chain reaction results of crayfish and water samples tested for *Francisella tularensis*[a]

Crayfish, Batch A					Crayfish, Batch B						Water SP			Water R	Patient
B	S	H	G	W	B	S	H	G	W	I	1	2	3	1-8	LNA
-	+	+	-	-	-	-	-	-	-	-	+	-	3	-	+

Batch A = crayfish collected from a patient's house; Batch B = crayfish collected from the river; SP = sewage plant; R = river; LNA = lymph node aspirate; B = branchia; S = stomach; G = gonads; H = hepatopancreas; W = washed pellet; I = intestines.

[a]Several annealing temperatures were tested, from 60°C to 68°C for the 25 cycles of the first round. The second round consisted of 45 cycles with an annealing temperature of 60°C. The rest of the parameters were as described (21). All reagents were from Perkin Elmer (Foster City, CA); the cycling was done in a PCT-100 thermocycler (MJ Research Inc., Watertown, MA). The PCR products, with a size of 1550 bp and 950 bp for the first and second rounds of amplification, respectively, were visualized in 1% low-melt agarose gels (Pronadisa, Alcobendas, Madrid, Spain) stained with ethidium bromide (Sigma-Aldrich). Cross-contamination was avoided by using standard methods. Half the samples studied were kept unprocessed and frozen in a different area. A positive result was confirmed by processing the rest of the sample. To assess the sensitivity of our system, 10 µL of serial twofold dilutions of an aqueous suspension of bacteria, ranging from 10^4 CFU to 10^2 mL was added to 1-mL volumes of distilled water. These samples were subjected to DNA extraction and PCR as above. Negative controls (autoclaved distilled H_2O) were included in all extractions at a ratio of a negative control for each five samples. The specificity was checked against DNA from *Salmonella* Typhimurium, *Escherichia coli, Legionella pneumophila, Yersinia enterocolitica,* and *Proteus vulgaris*.

the BigDye Terminator Cycle Sequencing kit on an ABI 377 automated DNA sequencer (Perkin-Elmer Corp., Foster City, CA) following the manufacturer's instructions. Sequence homology was searched by using the BLAST 2.0 queueing system (20).

Results

Patients and Serology

Nineteen patients who fulfilled laboratory criteria for tularemia were identified. All had had contact with river-caught crayfish in the period July 13-31. Overall, 11 (58%) required hospitalization, with a mean stay of 9.4 days (Table 2). Symptoms included adenopathies on the elbow, armpit, or both (100%); cutaneous lesions (89%); fever (80%); discomfort at the injury site (42%); vomiting (32%); diarrhea (5%); and general symptoms (84%) such as headache, arthralgia-myalgia, malaise, anorexia, dysuria, and bad taste in the mouth.

All cutaneous lesions were on hands, generally the fingers; no specific finger or hand predominated. In six cases, ulcers were present. The remaining patients reported inflammation of the finger or phalanx to which they had received the crayfish-related injury. All ulcers were at the injury site.

Forty-one serial serum samples from all the patients were collected (Table 2). All 19 patients reached *F. tularensis* specific microagglutination titers ≥1:256. Increases in serum antibody titers were observed in five cases (Table 2). For the remaining patients, high antibody titers in the presence of compatible clinical symptoms and full recovery after antibiotic treatment were interpreted as confirmatory criteria.

Serum samples from wild animals and sheep were negative.

Table 2. Serologic results and date of onset of tularemia, 19 patients

Patient no.	Days since onset	MA titer	Sex	Age (years)	Exposure date/ onset date	Hospitalization/ other laboratory tests
1	40	4,096	F	71	7-14/7-16	15 d
	69	4,096				
2	38	2,048	F	63	7-13/7-18	11 d
	69	2,048				
3	52	256	M	72	7-15/7-17	NO
	69	1,024			(+) PCR	
4	44	2,048	F	71	7-16/7-18	15 d
	66	2,048				
5	38	512	F	62	7-14/7-18	14 d
	58	512				
6	38	256	F	53	7-14/7-18	NO
	54	256				
	82	256				
7	36	512	M	39	7-17/7-20	NO
	53	512				
8	22	16	M	54	7-19/7-21	7 d
	42	256				
	64	256				
9	17	2,048	M	38	7-15,22/7-24	NO
	94					
10	74	512	F	60	7-20/7-23	NO
	95	512				
11	47	512	M	51	7-22/7-24	5 d
	52	2,048				
12	47	2,048	M	Unknown	7-22/7-25	9 d
	61	2,048				
13	30	1,024	M	48	7-19/7-26	7 d
	50	1,024				
14	Unknown	256	F	65	7-15/Unknown	NO
15	16	16	M	75	7-25/7-27	9 d
	52	256				
	73	256				
16	42	256	F	60	7-27/7-30	NO
	55	256				
	70	256				
17	18	2,048	M	49	7-30/8-2	6 d
	38	2,048				
18	22	512	M	67	7-30/8-3	6 d
	53	512				
19	103	8,192	M	65	7-26/8-5	NO
	178	2,048				

MA = microagglutination; d = day; PCR = polymerase chain reaction; NO = no hospitalization required.

Case Survey and Risk Factors

Patients included 11 men and 8 women, mean age 59.1 years (range 38-75); the occupations of housewife (7 patients) and pensioner (5) predominated. None had professions entailing risk practices for tularemia. Most had been catching and cleaning crayfish for many years.

Cases were concentrated (by date of symptom onset) within a 3-week period (July 16-August 5; Table 2 and Figure 1); the greatest concentration of cases occurred during July 17-26. All patients had been in contact with crayfish caught during July 13-30. Fishing had been officially permitted on June 1 but was prohibited on August 6 because of the outbreak. The estimated incubation period was 3.5 days (range 1-8 days).

Patients resided in six towns situated around the Mayor River fishing area. Two patients had been in contact with other tularemia reservoirs (hares). No insect bites were reported for any patients.

All crayfish caught were of the red swamp species (*P. clarkii)*. The section of the river authorized for fishing is 14 km, from the town of Huete to the beginning of the Buendía

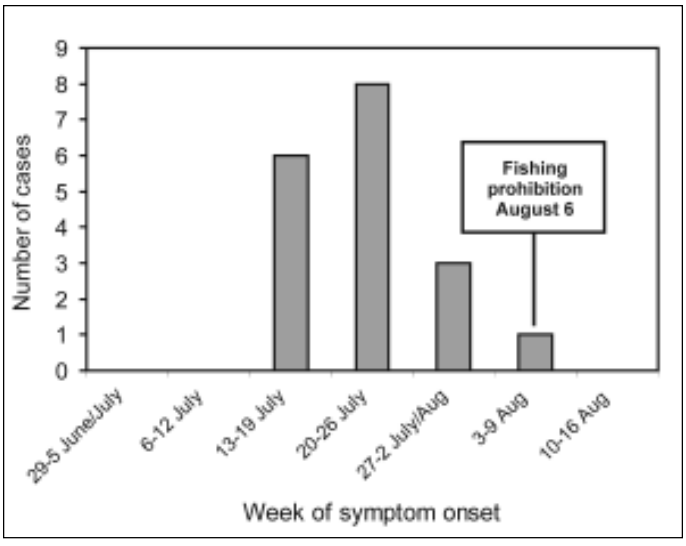

Figure 1. Epidemic curve of tularemia cases, central Spain, by week. One case had unknown symptom-onset date.

Dam into which the river flows. There are designated hunting (including hare-hunting) areas on both river banks. Patients had been fishing a ±5-km stretch, particularly the area at the neck of the dam, where access to the river was simplified by the proximity of the road and two footbridges (Figure 2). This terrain is alluvial, so crayfish can easily dig their underground burrows (22); it is prone to periodic variations in the water level and marked by reeds. The abundance of crayfish favored fishing by hand (an outlawed practice) rather than with a crayfish net.

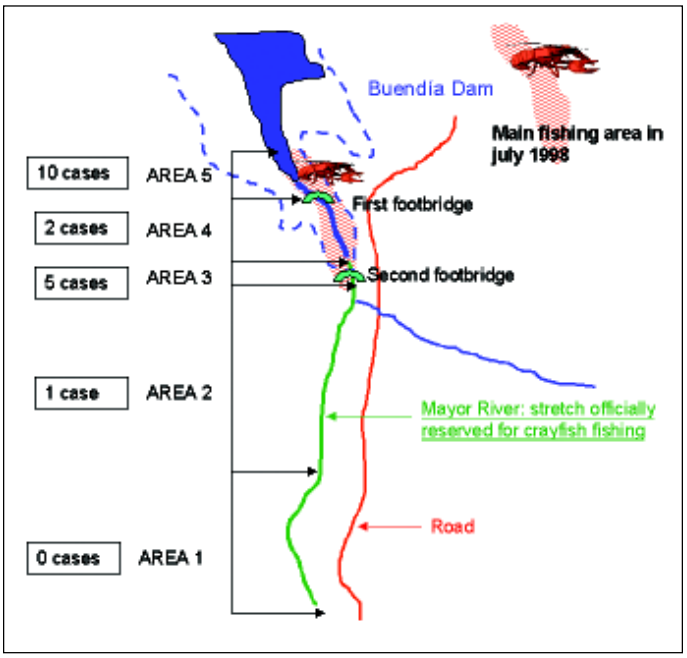

Figure 2. Fishing areas where crayfish handled by patients were caught. (Data were unavailable for one patient.)

Our case survey showed that 113 patients had caught crayfish; at least 9 of these patients had also cleaned them. Six patients had cleaned crayfish but had neither caught them nor been in the fishing area; five of these six had cleaned crayfish the day after the catch, and one had done so 5 days afterwards. In all, 15 patients had cleaned crayfish, and at least 17 of the total 19 had also eaten crayfish. Cooking methods used were all low risk.

At least 17 patients had incurred crayfish-related scratches, cuts, and abrasions while fishing, emptying the net, or cleaning the catch; 8 had only one injury. One patient, who had been injured when fishing and cleaning, also grazed against a riverside reed; the cutaneous lesion subsequently appeared at the site of the scratch.

Water used for washing crayfish was from the residential drinking water supply; chlorine levels were checked and shown to be acceptable. In 9 (75%) of 12 respondents who cleaned crayfish, the washing method consisted of leaving the crayfish to soak and changing the water several times. During this operation, a considerable quantity of mud and dirt was released, which the cleaner touched. In the remaining three cases, crayfish were rinsed in running tap water. These two washing operations were performed by 58% (7/12) of patients before they gutted the crayfish. All but one of the 11 respondents reported washing their hands after handling the crayfish, but 5 of those 10 used no soap or disinfectant.

Case-Control Study

Demographic information on patients was obtained (Table 3). Although there was a higher proportion of women and a lower proportion of crayfish catchers among controls, these differences were not statistically significant.

The most important result (Table 4) was that those persons who were injured during the handling (catching or cleaning) of crayfish had a significantly higher risk for tularemia (94% of exposed subjects among cases versus 30% among controls; OR = 39.7; 95% CI 4.3-369.7). Furthermore, once data were reciprocally adjusted for the catching- and cleaning-related injury variables, merely incurring an injury (regardless of whether it was received while catching crayfish [OR adjusted for cleaning-related-injury = 29.1; 95% CI 2.6-330.9] or cleaning crayfish [OR adjusted for catching-related-injury = 38.8; 95% CI 3.5-427.6]) led to a statistically significant rise in the risk for tularemia.

Environmental Study

Local environmental variations detected over the previous 2 years were higher rainfall, with correspondingly higher river levels; abundant crayfish; and increased murkiness of the river water. In July 1998, release of water through the sluice gates led to an estimated 1.8-m drop in the overall dam level, leading in turn to a 200- to 400-m narrowing in the shoreline at the neck of the dam, i.e., the fishing area (Figure 3). This coincided with a mass movement of crayfish to the surface.

Culture and PCR

Attempts to culture the organism from water, patients, crayfish, and wild animals were unsuccessful. A positive PCR result was obtained from one of the pools of 10 individual colonies taken from the culture of a water sample from the sewage plant, but we were unable to recover any *F. tularensis* in culture from the rest of the same sample kept at 4°C.

The PCR conditions best adapted to our system were an annealing temperature of 62°C for the first round of cycles and 60°C for the second. These conditions allowed us to

Table 3. Comparison of characteristics, tularemia cases versus controls

Variable	Cases [a]	Controls	p
Age: mean (range) in years	59.1 (38-75)	56.2 (25-100)	0.7
Sex:			
Women	42% (8/19)	55% (11/20)	0.4
Men	58% (11/19)	45% (9/20)	
Town of residence:			
No.1	3	3	
No.2	6	7	
No.3	4	4	
No.4	4	3	
No.5	1	2	
No.6	1	0	
No.7	0	1	
Type of contact with crayfish:			
Catching	68% (13/19)	82% (15/18)	0.6
Cleaning	94% (17/18)	85% (17/20)	0.9
Eating	60% (12/20)	95% (19/20)	0.9

[a]The denominator indicates the number of persons who answered each question.

Table 4. Results of case-control study of tularemia in crayfish

Risk factors considered	Prevalence of exposure[a] in Cases	Controls	Crude OR	95% CI	Adjusted OR	95% CI
Injury on handling crayfish	17/18	6/20	39.7	4.3-369.7		
Injury on catching [b] crayfish	8/17	3/20	5.0	1.1-23.8	29.1[c]	2.6-330.9
Injury on cleaning crayfish	10/17	3/20	8.1	1.7-38.6	38.8[d]	3.5-427.6
Not washing crayfish before cleaning	5/12	4/17	2.3	0.4-15.7		
Washing crayfish by soaking	9/12	10/17	2.1	0.4-10.7		
Washing hands with soap after cleaning	5/11	11/15	0.3	0.06-1.6		

[a] Denominator indicates number of persons who answered each question.
[b] Injury on catching = injury while fishing or emptying net.
[c] Adjusted for cleaning-related injury.
[d] Adjusted for catching-related injury.
OR = odds ratio; 95% CI = 95% confidence interval.

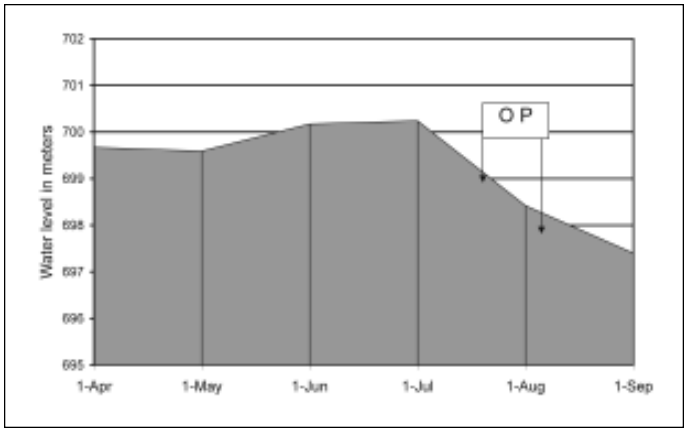

Figure 3. Buendía Dam water levels, April-September 1998. OP = outbreak period.

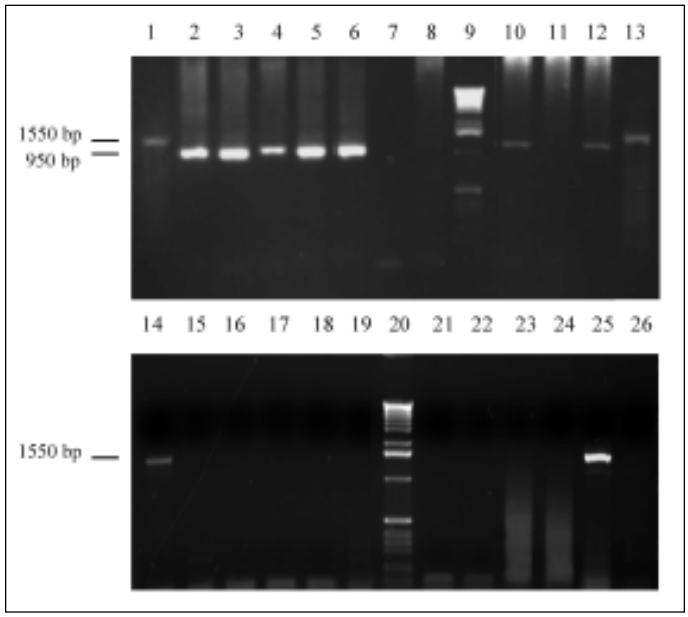

Figure 4. Polymerase chain reaction results in selected samples from water, crayfish, patients, and controls. Lanes 1 and 2: positive control (first [1550-bp] and second [950-bp] rounds of amplification); lanes 3 and 4: water samples 1 and 3, respectively, from the sewage plant, second round; lanes 5 and 6: stomach and hepatopancreas, respectively, from batch A of crayfish, second round; lane 7: negative control; lanes 8 and 10: control of amplification of 1 CFU for the first and second rounds of amplification, respectively; lanes 11 and 12: human lymph node aspirate, first and second amplifications, respectively; lanes 13 and 14: positive control, first round; lanes 15-19, 21, and 22: water samples from the river; lanes 23 and 24: stomach and hepatopancreas, respectively, from batch B of crayfish; lanes 25 and 26: pooled DNA from *Salmonella* Typhimurium, *Escherichia coli, Legionella pneumophila, Yersinia enterocolitica,* and *Proteus vulgaris* (first and second rounds of amplification, respectively); lanes 9 and 20, 1-kb DNA ladder size standards.

amplify DNA from 1 CFU of spiked distilled water, which yielded a faint band only in the second amplification run (Figure 4), as did the lymph node aspirate from a patient (Figure 4 and Table 1).

Two of three samples from the sewage plant yielded amplicons, although those samples were negative in culture. Stomach and hepatopancreas samples from the crayfish specimens collected at a patient's house were positive, and branchial tissue, gonad, and the washed pellet were negative. All water samples collected from different parts of the river were negative. PCR results of three additional water samples collected 1 month later from the same locations yielded the same results. Intestinal, stomach, and hepatopancreas samples from crayfish collected from the river after the outbreak started were negative (Figure 4, Table 1).

Sequencing of 16S rDNA

An identical fragment of the 16S rDNA gene (nucleotide position 145-1290 as for *Escherichia coli*) was sequenced from the water, crayfish, and lymph node aspirate amplicons and analyzed. The homology in this region between the lymph node aspirate amplicon and the published sequences for *F. tularensis* tularensis, *F. tularensis* palaearctica, *F. novicida, F. phylomiragia, Salmonella* Typhimurium*, Legionella pneumophila,* and *E. coli* was determined (Table 5). The lymph node aspirate sequence shared 100% of homology with the sequence L26086 from *F. tularensis* palaearctica (99.2% with *F. tularensis* tularensis and *F. novicida*, and 97.7% with *F. philomiragia*). The homology with *S.* Typhimurium*, E. coli,* and *L. pneumophila* was 81.0%, 81.1%, and 80.8%, respectively. When a shorter fragment was analyzed, between nucleotide positions 1113 and 1188, the sequence of the PCR products obtained had 100% homology with *F. tularensis* palaearctica, 98.6% with *F. tularensis* tularensis, 99.3% with *F. novicida*, and 98.9% with *F. philomiragia* (data not shown). In this region, we found 15 of 75 nt positions specific for *Francisella* spp., which implies a divergence of 20% compared with *S.* Typhimurium*, L. pneumophila*, and *E. coli* (Figure 5A). All sequences generated shared the nucleotide signature that differentiates *F. tularensis* palaearctica from other

Table 5. 16S rRNA gene sequence similarity matrix (from position 145 to 1290 as for *Escherichia coli*)

	LNA	Ftp	Ftt	Fn	Fph	Lp	Ec	St
LNA	100							
Ftp	100	100						
Ftt	99.2	99.2	100					
Fn	99.2	99.2	98.6	100				
Fph	97.7	97.7	97.2	100				
Lp	80.8	80.8	80.3	80.2	80.8	100		
Ec	81.1	81.1	80.5	80.5	80.3	80	100	
St	81	81	80.8	80.4	80	79.7	95.9	100

LNA: human lymph node aspirate amplicon, this study (GenBank Accession No. AF227314); Ftp: *F. tularensis* palaearctica (L26086); Ftt: *Francisella tularensis* tularensis (Z21932); Fn: *F. novicida* (L26084); Fph: *F. philomiragia* (Z21933); Lp: *Legionella pneumophila*; (M36023); Ec: *Escherichia coli* (AE000406); St: *Salmonella* Typhimurium (X80681).

members of the genus (an A at nucleotide position 1153, as described for *F. tularensis* palaearctica, compared with a G, as described for *F. tularensis* type A, *F. novicida,* and *F. phylomiragia)* (23) (Figure 5A).

Conclusion

We describe the identification by PCR of *F. tularensis* biovar palaearctica as the agent of a waterborne outbreak of tularemia. The fragments of the 16S rRNA gene sequenced were specific for *Francisella* spp. The homology of these fragments with the members of the *Francisella* genus and the nucleotide signature at position 1153 confirmed that this organism caused this outbreak. The results obtained are compatible with a transient contamination of the river and crayfish. A sewage plant, which intermittently pours water into the river 15 km upstream, could be the source of the organisms, as the samples taken 1 month after the outbreak began remained positive by PCR. Alternatively, crayfish could have acquired the organism during a previous contamination of the river and maintained the bacteria in their internal organs, as water samples taken from the river a few days after the outbreak started were negative by PCR. That the crayfish captured 4 weeks after the outbreak started were negative by PCR and that the only positive ones were those recovered from the house of a patient indicated that contamination was transient and that the organism cleared from the digestive tract of the crayfish.

Waterborne human tularemia is usually oropharyngeal. We have described an unusual waterborne outbreak of ulceroglandular tularemia. As the patients' skin lesions were granuloma-like, a *Mycobacterium marinum* infection (24) was at first suspected, thus delaying extraction of the first serum samples. In spite of this, we were able to identify the cause of the illness by serology and PCR.

Data indicate that the *F. tularensis* complex is highly variable in Eurasia (4,8,9,25-27). Whether this variability could account for the microorganism's ability to survive and multiply in different ecosystems and lead to different forms of transmission to humans deserves further study.

The presence of *F. tularensis*, both in the sewage-plant water and in the river crayfish, along with the association between the disease and hand injuries incurred on coming into contact with river water and mud at the fishing site or during crayfish cleaning, led us to conclude that the river is contaminated with *F. tularensis*. The beginning of the official open fishing season and the emergence of large number of crayfish to the surface (28) brought on by the drop in river levels in July enhanced the possibility of contact with the crayfish and thus the outbreak.

The temporal-spatial features of the outbreak suggest that contamination of the river must have been limited in time and to a single stretch of the river. Favoring this hypothesis is the fact that bacteria were not found in specimens of potential animal reservoirs in the area.

Evidently, the crayfish played the role of mechanical transmission agents of disease to humans, through contact

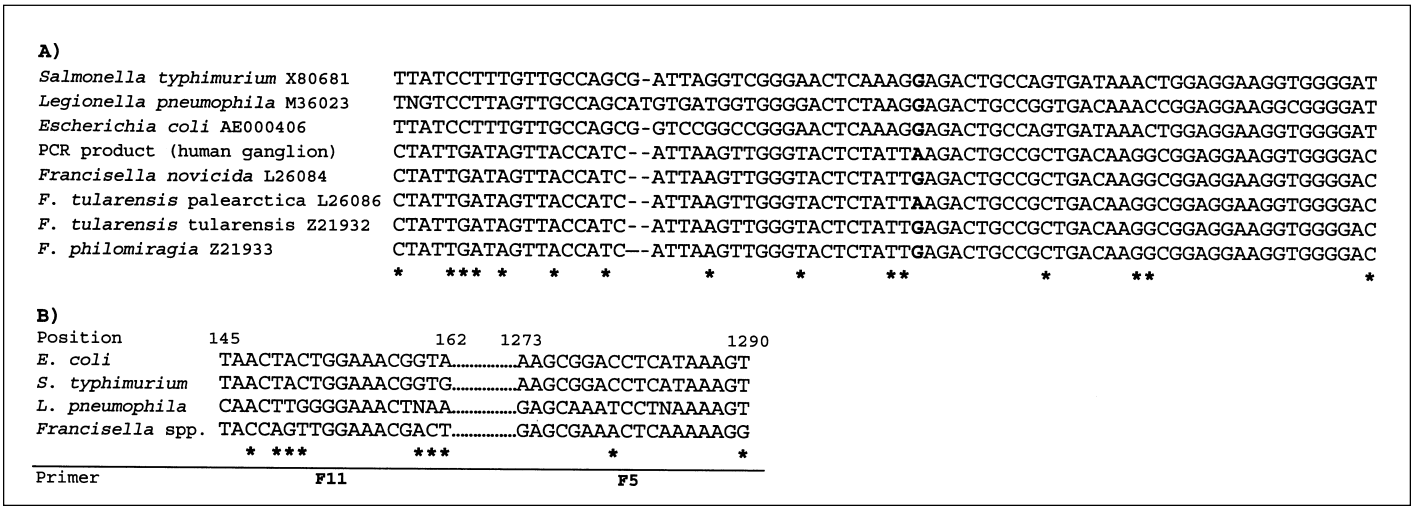

Figure 5. A) Sequence alignment of a 75-nt fragment of the 16S rRNA gene of different bacterial species (nucleotide position 1113 to 1188 as for *Escherichia coli*). The signature nucleotide (nt 1153), which allows differentiation between *F. tularensis* palaearctica and the other members of the *Francisella* genus, is set in bold face. B) Sequence comparison of the regions corresponding to F11- and F5-specific *Francisella tularensis* primers between *F. tularensis* and other bacterial species. Asterisks depict *Francisella* spp.-specific nucleotides. Sequences generated in this study were deposited in GenBank with accession numbers of AF227312 for the amplicon from water, AF227313 for the fragment amplified from crayfish stomach, and AF227314 for the human lymph node aspirate. Sequences generated in this study were compared with those of *Salmonella* Typhimurium (X80681), *Escherichia coli* (AE000406), *Legionella pneumophila* (M36023), *F. tularensis* biovar tularensis (Z21932), *F. tularensis* biovar palaearctica (L26086), *F. novicida* (L26084), and *F. philomiragia* (L26085).

with carapace-contaminated mud and water; skin injuries acted as the portals of entry for infection. The presence of *F. tularensis* in the crayfish stomach and hepatopancreas, coupled with this type of crayfish's known capacity for bioaccumulation of toxins, poses the possibility of transmission through contact with the intestinal contents during the gutting process.

Commercial farming of this crayfish species is expanding internationally because of its high profitability (28). Thus, the potential role of this species as a vector for tularemia needs further study.

F. tularensis has been classified as one of the microorganisms that could be used as a biological warfare agent (29-31). The waterborne route for infection that we have described here supports the possibility that intentional contamination of water could be the source of a rare bacterial disease in the future. Thus, it is important to consider these factors at the clinical and public health levels.

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Cholera Outbreak in Southern Tanzania: Risk Factors and Patterns of Transmission

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To identify risk factors and describe the pattern of spread of the 1997 cholera epidemic in a rural area (Ifakara) in southern Tanzania, we conducted a prospective hospital-based, matched case-control study, with analysis based on the first 180 cases and 360 matched controls. Bathing in the river, long distance to water source, and eating dried fish were significantly associated with risk for cholera. Toxigenic *Vibrio cholerae* O1, biotype El Tor, serotype Ogawa, was isolated in samples from Ifakara's main water source and patients' stools. DNA molecular analyses showed identical patterns for all isolates.

The reemergence of cholera is presenting unprecedented challenges (1,2). Since the seventh pandemic caused by *Vibrio cholerae* biotype El Tor began in Indonesia in 1961, most regions of the world continue to report cholera. 1997 was marked by a cholera epidemic affecting most countries in East Africa, with spread toward central and southern parts of the continent. Africa reported 118,349 cases to the World Health Organization in 1997, for 80% of cases worldwide. Africa also had the highest overall case-fatality rate (4.9%), compared with 1.3% in the Americas and 1.7% in Asia (3). Tanzania has consistently reported cholera cases; annual reports ranged from 1,671 cases in 1977 to 18,526 in 1992 (4,5). During the last 2 decades, three major cholera epidemics have occurred: 1977-78, 1992, and 1997 (3-5). In 1997, Tanzania had one of the highest case-fatality rates in East Africa (5.6%), with 2,268 deaths in 40,226 cases (3). We describe risk factors and pattern of spread of the 1997 cholera epidemic in a rural area in southern Tanzania.

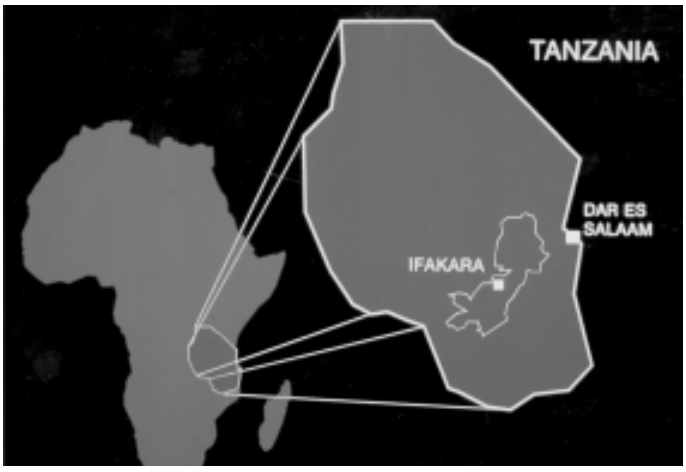

Figure 1. Map of Tanzania showing Ifakara.

Methods

Ifakara is 270 m above sea level in the Kilombero District river valley in southeastern Tanzania (08ºS; 32ºE) (Figure 1). It is a rural area (estimated population 57,000), and most inhabitants are subsistence farmers growing rice and maize. Fishing is also common. The town is crossed by the Lumemo River, a tributary of the Kilombero River. There are two rainy seasons: March through May, and December through January (1997 rainfall 1,439 mm). A short, cool dry season follows the long rains in June and July.

In Ifakara, the last cholera epidemic was in 1992. Most outpatient and admissions occur at Ifakara's Saint Francis Designated District Hospital (SFDDH), a 375-bed, district referral hospital. Demand-driven research takes place in an adequately equipped, district-based international research center, the Ifakara Health Research and Development Centre (IHRDC).

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The Outbreak

An increase in acute severe diarrhea cases in adults was noted in early June 1997 at SFDDH. On June 23, the first cholera case was confirmed by culture. At that time, a cholera control campaign was launched, following standard recommendations (6,7). As part of control activities, an outbreak investigation was begun, and two cholera treatment sites were established. One was near the area where the outbreak was first noticed, the Lumemo River; the other site was in a ward at the district hospital.

The outbreak investigation consisted of a prospective hospital-based, case-control study designed to identify cholera risk factors. A case was defined as illness in a patient

>5 years of age, visiting either of the cholera sites from June 23 to December 31, 1997, and hospitalized with acute onset of watery diarrhea. For each case, two age- and sex-matched controls were selected and interviewed on the same day as the case interview. Controls were selected from patients admitted to the district hospital within 2 days of the date of case admission. Data on possible risk factors were documented by a standardized questionnaire (Table 1). After preliminary analysis of the first 60 cases and 120 controls, access to the Lumemo River was restricted. The final analysis was based on 180 cholera cases and 360 controls. We evaluated possible sources of bias 1 year later by interviewing a random sample of 100 community controls matched by age, sex, and neighborhood. This sampling procedure was a two-stage cluster (10 families per cell unit) intended to assess prevalence of cholera risk factors at the community level.

The distribution of cholera cases in relation to the main water source was determined by mapping the distance from ill persons' houses to the Lumemo River. Mapping was facilitated by use of a GeoExplorer II Mapping system and two Trimble Global Positioning System receivers (Trimble, Liverpool, United Kingdom). Differentially corrected positions were then processed with PFINDER software. To assess whether cases were equally distributed outwards from the river, areas were mapped at 250-m intervals. Four major zones were used, two on each side of the river; these zones were used for sampling rather than streets, which are not well defined in Ifakara. Zone-specific attack rates were calculated as cases per 1,000 residents for the duration of the outbreak, after a house-to-house census of all residents living within 500 m of the river was conducted.

Fecal specimens and rectal swabs were obtained from all patients admitted to the treatment sites until 60 positive cultures were obtained. Samples were obtained at the beginning of the epidemic and 1 month later from two water sources (the Lumemo River and the water pump closest to the river) and were sent to the IHRDC laboratory. Each specimen was immediately processed and plated onto thiosulfate-citrate-bile salt-sucrose agar. Sucrose-positive colonies were further tested on the basis of standard biochemical reactions (8). *V. cholerae* isolates were agglutinated with polyvalent 01 and monospecific Ogawa, Inaba, and Hikojima antisera (Difco Laboratories, Madrid, Spain). The isolates were tested for susceptibility to seven antibacterial drugs by disk diffusion (9). Susceptibility testing was done for the following antibiotics: ampicillin, 30 mg per disk; chloramphenicol, 60 mg; ciprofloxacin, 10 mg; gentamicin, 40 mg; nalidixic acid, 130 mg; tetracycline, 10 mg; and trimethoprim-sulfamethoxazole, 5.2 mg and 240 mg, respectively.

Polymerase chain reaction (PCR) was used to detect the *ctx* gene for all isolates. The primers used were specific for the *V. cholerae* enterotoxin (Takara Shuzo Co. Ltd., Otsu, Shiga, Japan). The analysis of chromosomal DNA by digestion with low-frequency-of-cleavage restriction enzymes and separation by pulsed-field gel electrophoresis (PFGE) was done with the *Not* I enzyme on a CHEF-DR IIII system (Bio-Rad Laboratories, Richmond, CA).

Data were double-entered into FoxPro databases (Microsoft Corp.) and checked for range, internal consistency, and referential integrity. Statistical analysis was performed with STATA statistical software (Stata Corp., 1997). McNemar's chi-square test was used for the univariate analysis. Variables significantly associated with cholera at or below the 5% level in the univariate analysis were included in the modeling. Multivariate conditional logistic regression was performed, the final model was fitted, and attributable risk percentages were calculated (Table 2).

Table 1. Univariate analysis results of potential risk factors during the 5 days before onset of illness in cases and matched controls

Risk factors	Cases, % (n=180)	Controls exposed % (n=360)	Matched odds ratio	95% CI	p-value
Social activities					
Attended funeral recently	6.2	2.2	2.7	1.1-6.8	0.03
Attended party recently	2.8	4.7	0.6	0.2-1.7	0.33
Travel in the past 2 weeks	19.0	13.6	1.5	0.9-2.5	0.10
Standard of living					
Mud housing[a]	48.0	36.0	1.7	1.2-2.5	0.01
Non-iron sheet roof	55.6	39.2	2.0	1.4-2.8	0.00
No latrine at home	56.6	9.8	11.4	6.3-20.5	0.00
Simple pit latrine[b]	91.4	81.2	2.7	1.4-5.1	0.001
Water exposure					
>10 minutes to water source	26.1	11.4	2.8	1.7-4.6	0.00
Unboiled drinking water	86.7	79.2	1.9	1.1-3.2	0.02
Unfiltered drinking water	89.4	84.2	1.7	0.9-3.0	0.07
River bathing	56.6	9.8	11.4	6.3-20.5	0.00
Inside tap water[c]	18.9	9.4	3.0	1.7-5.1	0.00
Other source water[c]	18.3	7.5	3.7	2.0-6.7	0.00
Food Exposures					
Dried fish	52.5	7.2	13.0	7.3-23.3	0.00
Prawns	13.9	5.0	3.1	1.6-5.9	0.00
Uncooked vegetables	25.1	20.1	1.3	0.9-2.0	0.20
Fruits	32.4	30.4	1.1	0.7-1.6	0.60
Religion[d]	41.2	23.1	2.3	1.1-3.4	0.00

[a]Compared with brick houses.
[b]Compared with ventilated improved pit latrines.
[c]Compared with hand-pumped drinking water.
[d]Muslims (compared with other religions, including Catholics, Protestants, and others).

Results

From June 23 through December 31, a total of 785 patients were admitted to the Ifakara cholera treatment sites. The number of cases peaked between June 30 and July 4 (Figure 2); 369 (47%) were males; 376 (48%) of the cholera patients

Table 2. Multivariate analysis of risk factors for cholera in 1997 epidemic, Ifakara, Tanzania

Risk factor	Odds ratio	p-value	95% CI[a]	Attributable risk	(%)
>10 minutes to water source	2.7	0.00	1.7-4.4	63[b]	17[c]
River bathing	14.4	0.00	8.8-23.5	93[b]	49[c]
Eating dried fish	12.1	0.00	7.7-19.1	92[b]	52[c]

[a]CI = confidence interval.
[b]The attributable fraction among the exposed population, an estimate of the proportion of exposed cases attributable to exposure.
[c]The population-attributable fraction, which is the net proportion of all cases attributable to exposure.

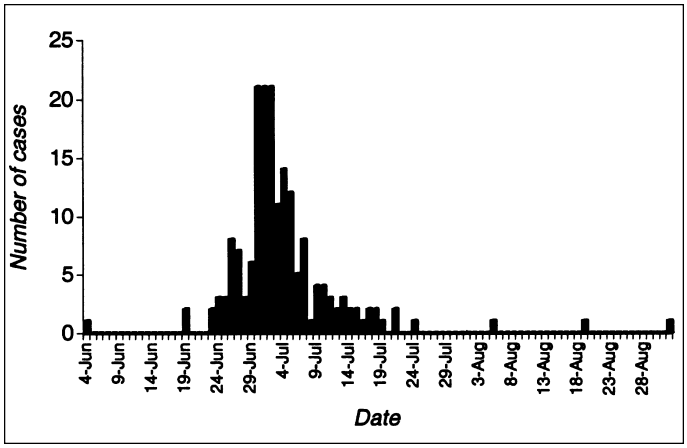

Figure 2. Distribution of disease onset by dates, in patients admitted for acute watery diarrhea to cholera treatment sites in Ifakara in 1997.

Table 3. Prevalence of cholera risk factors in Ifakara, Tanzania

Risk factors (exposures)	Cholera cases n=180	Hospital controls n=360	Community controls n=100
Activities			
Attended funeral recently	6.2	2.2	60.6
Attended party recently	2.8	4.7	88.5
Travel in the past 2 weeks	19.0	13.6	14.4
Fishing	54.0	51.0	53.8
Water			
>10 minutes to water source	26.1	11.4	14.4
Unboiled drinking water	86.7	79.2	90.4
Unfiltered drinking water	89.4	84.2	92.3
River bathing	56.6	9.8	33.7
Food			
Dried fish	52.5	7.2	76.9
Prawns	13.9	5.0	14.4
Uncooked vegetables	25.1	20.1	29.8
Fruits	32.4	30.4	73.1

were <25 years of age. Seventeen deaths were reported (case-fatality rate 2.1%). Zone-specific attack rates were from 11 (west zone) to 41 cases per 1,000 residents (east zone), showing an increase in the densely populated zones of the town.

In univariate analysis of recent exposure to potential risk factors, low standards of living (indicated by a mud house, having a thatched roof, and having simple pit latrine or none) affected significantly the likelihood of disease (Table 1). Risk factors for exposure to water, including long distance to water source (>10 minutes walk), use of unboiled water, bathing in the river, and the use of tap water inside the house instead of pumped water, were all significantly associated with cholera, as well as having recently eaten dried fish and prawns. Multivariate analyses showed that distance to the water source (odds ratio [OR] 2.7; 95% confidence interval [CI] 1.7-4.4), having eaten dried fish recently (OR = 12.1; 95% CI 7.7-19.1), and bathing in the river (OR = 14.4; 95% CI 8.8-23.5) were independently significantly associated with risk for cholera (Table 2). Interaction terms neither changed nor improved the fit of the model.

The attributable risk percentages (Table 2) provide an approximate summary of the importance of each risk factor, taking into consideration both the strength of the association and its prevalence; these estimates assume that all confounding variables were measured and no sources of bias exist. For example, river bathing accounted for 93% of all cases among the population with this exposure, and 49% of all cases could have been averted by preventing this practice.

Cholera patients were more likely to be exposed to bathing in the river than community controls ($p<0.01$) but hospital controls were less likely to be exposed than 100 community controls ($p<0.01$) (Table 3). Cholera cases were more likely to walk >10 minutes to water sources than community controls ($p<0.05$).

V. cholerae was isolated from stool specimens and water samples from the Lumemo River, but not from any other water source. All isolates were toxigenic *V. cholerae* O1, biotype El Tor, serotype Ogawa; DNA genotyped by PCR-PFGE in samples isolated from the river and patient stools had identical patterns (Figure 3). All isolates were susceptible to tetracycline, ampicillin, amoxycillin + clavulanic acid, nitrofurantoines, and quinolones and were resistant to cotrimoxazole.

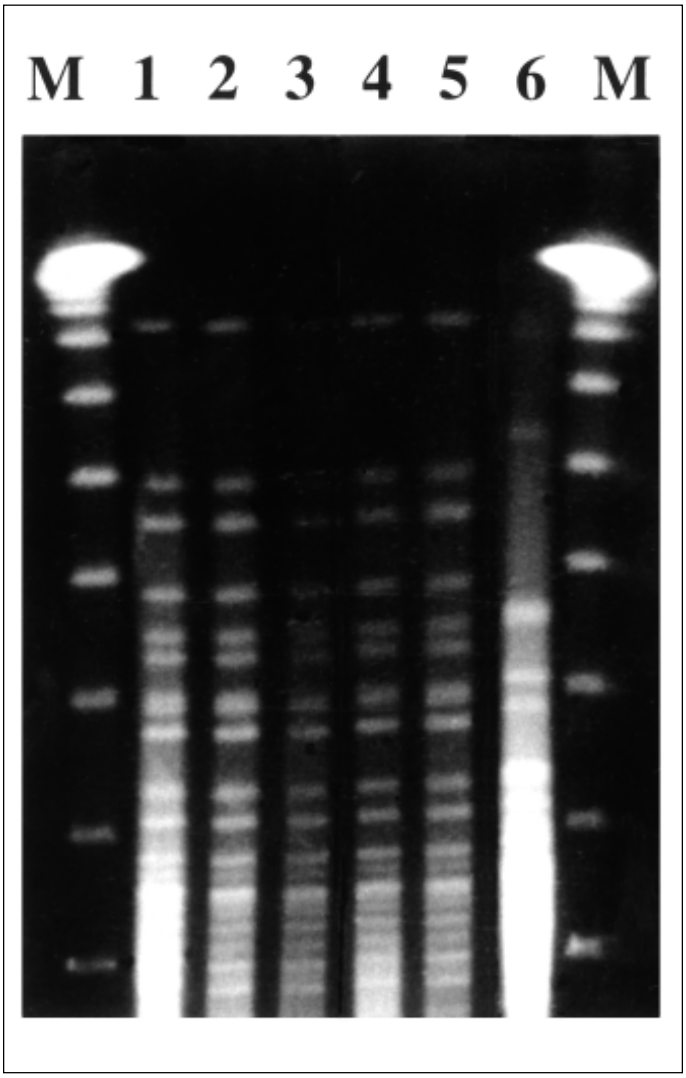

Figure 3. Pulsed-field gel electrophoresis patterns of *Vibrio cholerae* isolates. DNA molecular weight markers (M lanes); *V. cholerae* strain isolated from water (Lane 1); *V. cholerae* showing isolates from patients (Lanes 2-5); *V. cholerae* strain 01 El Tor from the Spanish type culture collection (Lane 6).

Conclusion

This hospital-based, case-control study identified three risk factors independently associated with the spread of the 1997 cholera outbreak in Ifakara, rural Tanzania. These factors were bathing in the river, eating dried fish, and living >10 minutes walking distance from the closest water source. Data from zone-specific attack rates also suggest an increase in cholera cases in the densely populated zones of the town.

Bathing in Ifakara's principal water source, the Lumemo River, had an independent, statistically significant OR of 14.4. More than 90% of river bathers were ill, and approximately 50% of these cases could have been prevented if access to the river had been closed. Moreover, identical strains, based on the DNA typing, were isolated both from the patient stools and river water, as previously reported in Bangladesh and the 1992 epidemic in Burundi (10,11). After the 1997 outbreak in Ifakara, the largest town in Kilombero District, most areas of the district were affected by the epidemic. Cases in other areas in the district began to be reported on September 2, reaching 134 by December 1997 and >1,000 by September 1998 (Ministry of Health, unpub. data). In the town of Itete, we were able to follow the beginning of the outbreak, which appeared to have been due to contamination of the Itete River with clothes of a cholera adult patient who came from Ifakara town and died in Itete. The epidemic in Ifakara was preceded by a funeral near the Lumemo River, which travelers from Dar es Salaam had attended (data not shown). Indeed, the last three cholera epidemics in Ifakara have been preceded by epidemics in Dar es Salaam (5).

Long distance to water source, defined as >10 minutes walk, was also independently associated with risk for cholera. Twenty-six percent of cases and 11% of controls had to walk >10 minutes to collect water. Long distance may be an indirect measure of poor hygienic habits caused by scarcity of water. In addition, this link may be indirectly associated with drinking water from the river: 23% of cholera patients living >10 minutes walking distance from a water source and 9% of controls actually collected water from the river. The other 77% and 91%, respectively, collected water from a pump or tap. Persons living farther away from the river may store water for longer periods, thus facilitating *V. cholerae* growth; risk for cholera is dependent on inoculum size (12,13).

Eating dried fish in Ifakara also seemed to be a cholera risk factor. Although to our knowledge, this is the first time a foodborne exposure has been identified as a cholera risk factor in East Africa, this association should be interpreted with caution. We were unable to isolate *V. cholerae* in dried fish samples, and hospital controls ate less dry fish than community controls (Table 3). However, hospital controls may have been more likely to recall food served at the hospital, where fish is rarely offered.

These associations could also be explained by different sources of bias. At the design stage, we asked questions to identify possible sources of bias. First, we asked questions about activities such as recent travel or attendance at a party. We aimed to identify whether hospital controls, as a result of their cause of admission, were as capable of bathing in the river as patients. We then assumed that this increased risk could not have resulted from selecting hospital controls who were physically incapable of bathing in the river, since they were equally capable of having attended a party or traveled recently. Additionally, we recruited only recently admitted patients (within 2 days of admission). Second, we included prawns in the questionnaire. Since prawns are never offered at the hospital, if food availability at the hospital were the underlying reason for the fish-cholera association, prawns should be associated as well. However, multivariate analysis showed only fish to be associated. The extent to which this fish-cholera association may be due to bias remains unclear.

The hospital-based case-control study, which offers the advantage of lower cost compared with a community-based design, seemed to be a reasonable approach for identifying risk factors such as bathing in the river and distance to water source. However, it may lead to over-estimation of the strength of the association, and its role in identifying food as risk factor is debatable.

In conclusion, in Ifakara, as reported previously in Tanzania (14), cholera transmission seems to have been predominantly waterborne. The 1997 Ifakara cholera outbreak could have been initiated by river contamination and facilitated by poor sanitation and lack of safe water. Control measures such as blocking access to the river and surveillance of travelers to avoid contamination of water sources seem to be impractical. Besides other control measures described elsewhere for African settings (15), trying to control cholera in densely populated cities such as Dar es Salaam may help control its spread. In the absence of adequate sanitation and water supply in less developed countries, health education campaigns stressing the need for safe water, food handling, and prompt medical care remain feasible options to reduce disease and death from cholera in rural East Africa.

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A One-Year Study of Foodborne Illnesses in the Municipality of Uppsala, Sweden

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Surveillance was enhanced and a retrospective interview study performed in 1998–99 to determine incidence, causes, and costs of foodborne illnesses in Uppsala, Sweden. Sixty-eight percent of the detected foodborne illness incidents were single cases, and 32% were outbreaks. Most (85%) of the incidents came to the attention of the municipal authorities through telephone calls from affected persons. Calicivirus, *Campylobacter* spp., and *Staphylococcus aureus* were the most common etiological agents; meat, meat products, and mixed dishes were the most implicated food categories. The incidence of foodborne illness was estimated to be 38 cases per 1,000 inhabitants per year. The estimated average costs per illness were 2,164 Swedish Krona (SEK) ($246) to society and 500 SEK ($57) to the patient. The annual cost of foodborne illnesses in Sweden was estimated to be 1,082 million SEK ($123 million).

Foodborne illnesses are a widespread global problem (1). In most cases, the clinical picture is mild and self-limiting, with few deaths. However, the socioeconomic impact may be high (2-4). Possible chronic sequelae, which have been estimated to occur in 2% to 3% of cases (5,6), may add to the suffering and costs associated with foodborne illnesses. For several reasons, foodborne illnesses are seriously underreported (7), but investigation and surveillance remain essential in efforts to understand and prevent them (8,9).

In Sweden, 794 to 2,965 cases of foodborne illness were reported yearly from 1992 to 1997 (10). In contrast, findings from a 1994 interview study in Sweden indicated that 500,000 persons per year experienced foodborne illnesses (11). This discrepancy illustrates both our lack of knowledge of the true extent of the problem as well as difficulties in reporting.

The aim of this study was to improve our understanding of foodborne illnesses. The specific objectives were 1) to detect and investigate as many outbreaks and single cases as possible in the municipality of Uppsala and determine the specific causes behind these illnesses and 2) to estimate the incidence and costs associated with foodborne illnesses.

Material and Methods

Surveillance of Foodborne Illnesses in Sweden

In Sweden, the municipal public health authorities are responsible for preventing the spread of foodborne illnesses, whereas the County Medical Officer (CMO) at the County Council has coordinating responsibility for communicable diseases and other foodborne diseases. Physicians are responsible for epidemiologic investigations in relation to their patients and should report communicable diseases to the CMO and the Swedish Institute for Infectious Disease Control. In addition, physicians should report communicable diseases that may be contracted from food, water, and the environment, as well as other illnesses suspected to result from commercially served or produced food to the municipal public health authority. Physician reporting and direct contacts with patients are the main ways in which foodborne illnesses come to the attention of municipal public health authorities. These authorities, in turn, are encouraged, but not required, to report the results of their investigations to the National Food Administration on a standardized form.

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Overview of the Study

The municipality of Uppsala has 186,000 inhabitants and includes the city of Uppsala, which is a center for research and education with two universities and a university hospital. In preparation for our study, we enhanced surveillance for foodborne illness in the municipality by adding analytical capabilities and staff to the municipal public health office and by providing information to the general public and the medical staff through media reports and at meetings. This information encouraged them to contact municipal public health inspectors by calling a dedicated telephone number to report cases of diarrhea, vomiting, or both, if they suspected food as the source of illness, and the affected person was a resident of the Uppsala municipality. Two types of incidents were distinguished: a) an outbreak, an incident in which two or more persons experienced a similar illness after ingestion of a common food and epidemiologic analysis implicated food as the source of the illness; and b) a single case, an incident in which one person became ill, with food as the suspected cause.

Investigation of Outbreaks and Single Cases

Ill persons were interviewed by using a standardized questionnaire designed for this study (12); it contained questions about personal details (age, health, income), illness (symptoms, duration), animal contacts, and eating habits (what, where, with whom) the week before the illness.

When possible, food and fecal samples were collected and the implicated premises were inspected. Because resources were limited, criteria were developed for when to collect fecal samples. The goal was to collect feces from at least one ill person per incident, single or outbreak-associated. Samples were not collected if >2 days had passed since the diarrhea ended or if the person had been treated with antibiotics the week before sampling. Microbiologic evidence was not necessarily available from multiple cases to determine etiology in outbreaks. Fecal samples were analyzed for the following bacteria: *Aeromonas* spp., *Plesiomonas* spp., *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., and *Yersinia enterocolitica*. Polymerase chain reaction (PCR) analysis (13) was performed for enterohemorrhagic *Escherichia coli*, enterotoxigenic *E. coli*, enteropathogenic *E. coli*, and enteroinvasive *E. coli*. The following viruses were analyzed: caliciviruses (PCR method [14], and electron microscopy [15]), rotaviruses, and astroviruses (electron microscopy [15]). In a few incidents, samples were analyzed for *Vibrio* spp.

Relevant microbiologic analyses were performed on food remains if available. In their absence, other food samples were analyzed only if they could be presumed to have the same microbiologic properties as the suspected food.

Recorded incidents were classified as verified, probable, or possible foodborne incidents based on the following criteria. An incident was considered verified if the agent was isolated from both an ill person and the food. If the agent was isolated from either the food or an ill person, the incident was considered verified if a link between them could be determined on the basis of the information in the questionnaire but probable if the available information linking them was inconclusive. An outbreak could be classified as a probable incident entirely on the basis of information collected during the interview if the association was strong enough, e.g., several cases with similar symptoms and with only one meal in common. The remaining incidents that did not meet the exclusion criteria were considered possible foodborne incidents. An incident was excluded from the study if the ill person had traveled outside the country less than 1 week before onset of disease, if the investigation showed that the illness was not foodborne, or if the responses given during the interview of the affected person were insufficient. In total, 28 incidents were excluded.

Retrospective Interview Study

To estimate the degree of reporting and incidence of foodborne illnesses in Uppsala, a retrospective interview study was performed 2 months after the study had ended. By random selection, 400 names were chosen from the telephone book. Persons answering the calls were interviewed by using a separate standardized questionnaire (12). If the person answering the phone was <15 years old, the interviewer asked to speak with an adult. In total, 266 persons were interviewed.

Results

From February 1998 to January 1999, 268 incidents were recorded, compared with 31 to 44 incidents reported yearly between 1993 and 1997. Of the 268 incidents, 183 (68%) were single cases and 85 (32%) were outbreaks (Table 1). Collectively, 515 cases of foodborne illness were documented; interviews were conducted for 354 of these; 61% were women. Each week during the study period, 1 to 21 incidents were reported, but no obvious trend over the year was apparent (data not shown).

For 101 (38%) of the 268 incidents, 123 fecal samples were collected. A microbiologic agent was detected in 47 (38%) of the 123 fecal samples and in 45 (45%) of the 101 sampled incidents (Table 2).

In most incidents, no relevant food samples were available for analysis. In about one third of the 66 incidents in which food was sampled, potentially pathogenic microorganisms such as *Bacillus cereus*, *E. coli,* and *Staphylococcus aureus* were detected at levels between 1.3-5.9 log CFU g^{-1} (Table 2).

In 213 (79%) of the 268 incidents, the etiologic agent was unknown because of insufficient information (Table 2). These incidents involved 334 (65%) of the 515 documented, single and outbreak-associated, cases. Bacteria caused 26 (10%) of the 268 incidents and 128 (25%) of the 515 documented cases; viruses caused 24 (9%) of the 268 incidents and 45 (9%) of the 515 documented cases (Table 2). The most common etiologic agent was calicivirus (20 incidents, 41 cases), followed by *Campylobacter* spp. (12 incidents, 16 cases) and *S. aureus* (5 incidents, 99 cases, Table 2).

Of the 268 incidents, 76 (8 single cases and 68 outbreaks) were classified as verified or probable (Table 3). These incidents resulted from ingestion of food prepared in a) restaurants or similar establishments (46%); b) homes (8%); c) grocery stores (4%); d) other places (2%); and e) unknown places (40%). The source of contamination was unknown in 27 incidents involving 58 ill persons. In 10 incidents (25 ill persons), only the meal, not the specific food item, was implicated. Meat and meat products (13 incidents, 34 ill persons) and mixed dishes (12 incidents, 128 ill persons) were the two most implicated food categories; poultry (5 incidents, 10 ill persons), beef (4 incidents, 7 ill persons), and sandwiches (4 incidents, 112 ill persons) were the most implicated subcategories.

One or more contributing factors could be identified in 18 (24%) of the 76 verified and probable incidents. These factors were a) lack of hygiene in processing, preparing, storing, and

Table 1. Number of single and outbreak-associated cases in incidents of foodborne illness detected in Uppsala municipality, Sweden, February 1998 through January 1999

No. of cases in each incident	No. of incidents of indicated size (% of total incidents)	Total no. of cases in incidents of indicated size
1	183 (68)	183
2	59 (22)	118
3	11 (4)	33
4	5 (2)	20
5	3 (1)	15
6	2 (<1)	12
7	2 (<1)	14
13	1 (<1)	13
14	1 (<1)	14
93	1 (<1)	93
Total	268	515

Table 2. Disease agents detected in feces and food samples and implicated[a] as etiologic agents in the investigated illnesses

Agents	Detected in (no. of samples/incidents) Feces	Food	Implicated in (no.) Incidents	Illnesses
Bacteria				
Bacillus cereus	na[b]	12/9	3	5
Campylobacter spp.	12/12	0	12	16
EHEC[b]	4/4[c]	5/5[d]	3	4
EIEC[b]	1/1	na	1	1
EPEC[b]	1/1	na	1	2
ETEC[b]	1/1[c]	na	0	0
Salmonella spp.[e]	1/1	0	1	1
Staphylococcus aureus	na	10/9	5	99[f]
Total	20/20	25/21[c]	26	128
Viruses				
Astroviruses	2/2	na	2	2
Caliciviruses	25/23[c]	na	20	41
Rotaviruses	3/3[c]	na	2	2
Total	29/27[c]		24	45
Histamine	na	na	2	3
Several agents	[c]		3	5
Unknown			213	334
Negative	76/56	133/45		
Total agents	123/101[g]	158/66	268	515

[a]The agent was implicated as a cause of an illness incident on the basis of laboratory evidence, the interview, and assuming foodborne transmission.
[b]na = not analyzed; EHEC = enterohemorrhagic *Escherichia coli*; EIEC = enteroinvasive *E. coli*; EPEC = enteropathogenic *E. coli*; ETEC = enterotoxigenic *E. coli*.
[c]In two of the incidents, two (caliciviruses and EHEC) and three agents (calicivirus, ETEC, and rotaviruses), respectively, were detected in feces samples, and in two other incidents, two agents (*E. coli* and *B. cereus*, and *S. aureus* and *B. cereus*, respectively) were detected in food samples.
[d]Refers to generic *E. coli*. No further characterization was done.
[e]*Salmonella* Enteritidis (phage type 21).
[f]In the largest incident (93 cases), disease agents other than *S. aureus* may have been involved since atypically long incubation times were recorded for some of the cases.
[g]Sum minus negative does not equal the number of positive samples since two or more agents were detected in some samples. See footnote c.

Table 3. Causes of verified, probable, and possible foodborne incidents (single cases and outbreaks)

Etiologic agent	No. single cases	No. outbreaks[a] (no. cases)	Total no. of single and outbreak-associated cases, by agent
Verified			
Bacillus cereus	1	1 (3)	4
Calicivirus	0	5 (23)	23
Campylobacter spp.	0	2 (6)	6
Histamine	1	1 (2)	3
Staphylococcus aureus	1	3 (97)	98
Multiple agents[b]	0	1 (2)	2
Subtotal	3	13 (133)	136
Probable			
B. cereus	1	0 (0)	1
Calicivirus	0	2 (4)	4
Campylobacter spp.	3	0 (0)	3
EHEC[c]	0	1 (2)	2
EPEC[c]	0	1 (2)	2
S. aureus	1	0 (0)	1
Unknown	0	51 (148)	148
Subtotal	5	55 (156)	161
Possible			
Astroviruses	2	0 (0)	2
Caliciviruses	12	1 (2)	14
Campylobacter spp.	7	0 (0)	7
EHEC	2	0 (0)	2
EIEC[c]	1	0 (0)	1
Rotavirus	2	0 (0)	2
Salmonella Enteritidis	1	0 (0)	1
Multiple agents[d]	1	1 (2)	3
Unknown	147	15 (39)	186
Subtotal	175	17 (43)	218
Total	183	85 (332)	515

[a]An incident in which two or more persons experienced a similar illness after ingestion of a common food, and epidemiologic analysis implicated food as the source of the illness.
[b]An outbreak in which caliciviruses were detected in feces samples, and high levels of *B. cereus* and *S. aureus* were detected in suspected food samples.
[c]EHEC = enterohemorrhagic *Escherichia coli*; EPEC = enteropathogenic *E. coli*; EIEC = enteroinvasive *E. coli*.
[d]One incident in which rotaviruses, caliciviruses, and EHEC were detected in the feces sample from a single case, and one outbreak in which EHEC and caliciviruses were detected in the same feces sample.

handling food (11 incidents); b) temperature errors, i.e., inadequate refrigeration, cooking, or cooling (8 incidents); c) contamination by an infected person or equipment (5 incidents); d) cross-contamination from other products, ingredients, or the environment (4 incidents); e) contaminated raw food (2); and f) other factors (6).

Most (85%) of the 268 incidents were detected by a telephone call from an affected person, 13% (36/268) were detected through medical authorities, and 2% (5/268) were detected through other sources. However, in several incidents the caller had been in previous contact with the medical authorities.

The illness forced 122 (79%) of the 154 employed or self-employed patients to miss work (Table 4). Most patients never contacted medical authorities. In total, 45 (14%) of the 312 respondents included in this analysis had visited a doctor and 19 (6%) were hospitalized (Table 4). The average cost for a case of foodborne illness was estimated to be 2,164 Swedish Krona (SEK) ($246), of which 1,027 SEK ($116) were direct costs including doctor visits, hospitalization, and medicine; the rest were indirect costs (i.e., loss of production) (Table 4). The average expenses to a patient were estimated at 500 SEK ($57), mostly from loss of income. For a hospitalized patient, the average cost was 18,652 SEK ($2,117), time spent in the hospital was 3.1 days, and loss of production was 5.6 days.

Of the 266 respondents in the retrospective interview study, 10 persons (3.8%) suspected they had had a foodborne illness during the study period. This translates to an incidence of 38 illnesses per 1,000 inhabitants per year. Only 1 of the 10 affected respondents (10%) had called the municipal authority. Based on this degree of reporting, the actual number of foodborne incidents was estimated to be 2,700 (268/0.1) When the average number of illnesses per incident (1.9) was used, the number of illnesses per year was calculated to be 5,100 (1.9 x 2,700). This translates to an incidence of 28 illnesses per 1,000 inhabitants per year (5,100 illnesses / 186,000 inhabitants), which is in the same range as the first estimate.

Discussion

Enhanced surveillance in combination with a telephone interview study was used to improve our understanding of foodborne illnesses and to address three limitations

Table 4. Estimated costs per case of foodborne illness

Costs included	No. of persons[a]	Average[b] no. of visits or days	Average cost per illness[b] SEK[c] ($)	Min. SEK	Max. SEK
Direct costs					
Doctor visits	45	0.2 visits	173	0	3,714
Hospitalization	19	0.2 days	809	0	43,150
Medicine	21	na[d]	5.3	0	200
Other costs	23	na[d]	40	0	4,200
Total direct costs	76[a]		1,027 (117)	0	43,265
Indirect costs					
Loss of production	122	1.3 days	1,137	0	17,934
Total	157[a]		2,164 (246)	0	55,221

[a]Number of persons who reported a cost for each of the items in the questionnaire. Several persons reported more than one direct costs.
[b]Averages based on 312 persons answering the questions in the standardized questionnaire.
[c]$1 = 8.81 Swedish Krona (SEK) (May 2, 2000).
[d]na = not applicable.

commonly named in foodborne research (16): 1) underreporting; 2) lack of data on the incidence and severity of foodborne illnesses; and 3) lack of medical cost data on foodborne illness episodes, including those for which no medical care is sought. Surveillance was enhanced by improving some of its preconceived weaknesses, i.e., the awareness of foodborne illnesses and motivation to report them on the part of consumers and physicians and surveillance activities of the health authorities (8). This approach has both strengths and weaknesses. The advantages include the theoretical size of the study population (186,000 people), which makes the detection of rare disease agents possible, and the detection and investigation of single (17) and milder cases (3), not only outbreak-associated cases or cases occurring in persons who seek medical attention. Study limitations include the difficulty of defining the actual size and composition of the study population and establishing a case definition, which is partly based on suspicion. These limitations were addressed by conducting the retrospective telephone interview study and by classifying incidents on the basis of available evidence (Table 3).

Public attention raised by the study could have led to a shift from the normal underreporting to overreporting. However, the telephone interview study indicated that a bias towards underreporting still existed. Further, the use of this degree of reporting and the number of illnesses per incident yielded an annual incidence estimate that was in reasonable agreement with the first estimate based on the telephone interview study (28 and 38 illnesses, respectively, per 1000 inhabitants). The uncertainty of the second estimate is probably greater than the first since it is based on only 10 suspected cases, and the average number of illnesses per incident is probably an underestimate because of the large proportion of single cases.

The annual incidence estimates obtained from our telephone interview study and from a national interview study (11) were also in reasonable agreement, 38 compared to 79 illnesses per 1,000 inhabitants. In comparison, 6.5 to 33 million cases of food-related illness per year have been estimated to occur each year in the United States (18). This translates to an incidence (25 to 130 cases per 1,000 inhabitants) similar to that in our study. A more recent report estimated the annual U.S. incidence of foodborne illnesses to be 278 cases per 1,000 inhabitants (4). However, caution should be exercised when comparing incidence estimates from different studies since they may partly reflect differences in the surveillance systems used and the assumptions behind the estimations.

Based on the number of foodborne illnesses reported after this study (130 and 100 incidents reported in 1999 and 2000, respectively), improved detection appears to be persisting. The average annual number of incidents in 1993-97 was 40, which (by using the estimated actual number of incidents, 2,700) indicates underreporting by a factor of 67 (2,700/40).

Based on data from 1987 (19), the cost per case of salmonellosis in Sweden can be estimated at $1,322 (converted from United Kingdom Pounds), which is much higher than our estimate for the cost per case of foodborne illness (Table 4). Our lower estimate is not unexpected since it is based on illnesses caused by a variety of agents and a spectrum of symptoms, from mild to more severe. Comparing costs between countries is difficult since the methods, types of illnesses, and health-care systems may differ. Razem and Katusin-Razem (20) estimated the cost per case of salmonellosis in Croatia to be $284 by adjusting estimates from different countries based on the ratio of their gross national products. In New Zealand (21), the estimated cost per case of foodborne infectious disease, $200, was in the same range as our estimate, whereas a considerably higher cost, $1,250, was estimated for a case of foodborne illness in the United States (22). The New Zealand estimate, however, was based on infectious diseases only, and the second estimate included costs for business losses, deaths, legal settlements, and investigation (22). Our estimate did not include the latter costs nor costs resulting from potential medical sequelae (5,6) and personal consequences not usually estimated in monetary terms (3). By combining the present cost per illness with the previously estimated 500,000 cases of foodborne illness per year in Sweden (11), the costs to society can be estimated at 1,082 million SEK ($123 million).

Both the present data and those from voluntary reports from the local authorities to the National Food Administration (10) indicate that a substantial proportion of foodborne illnesses occur because of mistakes in or a lack of knowledge of food-handling procedures at commercial food establishments. Another similarity is the relatively large proportion of incidents with unknown causes. These comparisons indicate that the surveillance system gives useful information but also has several limitations (2). A lower proportion of incidents in which *Salmonella* spp. was implicated was found in this study (Table 2), compared to other reports of foodborne illnesses both in North America (8) and in Europe (23). It is not likely that this result is due to a sampling bias since fecal samples were analyzed for salmonellae in the same frequency as for caliciviruses and *Campylobacter* spp. Instead, it may reflect the low prevalence in Sweden of salmonella in food, cattle, pigs, and poultry (<1%) because of an extensive control program (10).

The study failed to establish a rapid link between the physicians and the municipal public health inspectors as

indicated by a review of communicable diseases reported to the CMO during the study period. Indeed, at least 27 incidents, which according to the public health inspectors were possible foodborne illnesses, were not reported. These incidents were not part of the study. This means that serious incidents may go undetected and that valuable time is lost since the CMO generally receives notification more than a week after the incident occurs. Also, the number of incidents forwarded from medical staff in the different areas in the municipality varied. This may reflect a true difference in incidence but more likely is a reflection of their motivation to participate in the study. Guzewich et al. (9) stressed the importance of the motivation of those involved in the surveillance and suggested improved feedback to stimulate motivation.

On the basis of the results and experiences obtained during our study (12), several suggestions to improve detection of foodborne illness incidents can be proposed. First, information should be directed to the general public and the medical staff to motivate them to report suspected incidents to the local public health authorities. To meet the increased number of reported incidents, probably mostly involving persons not in need of medical care, and to optimize use of available resources, the municipal public health authorities should develop criteria for when and how to investigate incidents. The minimum requirement should be conducting an interview of the affected person according to a standardized questionnaire. Detection and investigation of incidents can be improved by arranging opportunities for medical and public health staff to meet on a regular basis and by establishing channels for rapid communication. This will facilitate both cooperation and coordination. These groups also need more training in food safety and in modern techniques to investigate incidents. Reporting of investigated incidents to the National Food Administration should be facilitated through a web-based system, and the feedback to those contributing should be improved. Foodhandlers in restaurants and similar establishments should be educated regarding food hygiene. Finally, to improve our knowledge, the existing passive surveillance should be supplemented with additional studies and approaches.

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Measles Outbreak in a Community with Very Low Vaccine Coverage, the Netherlands

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A 1999-2000 measles epidemic in the Netherlands started with an outbreak in an orthodox reformed elementary school with 7% vaccine coverage. The overall attack rate was 37%: 213 clinical cases among the 255 participating pupils (response 62%) and 327 household members. The attack rate ranged from 0% for the oldest groups of pupils to 88% for the youngest, who had not been exposed in previous measles epidemics. None of 25 vaccinated pupils had clinical symptoms. Among pupils with clinical symptoms, the self-reported complication rate was 25%. These data confirm that measles infection causes severe disease and that vaccination is the most effective means of preventing the disease and its complications. The data also show that clusters of persons refraining from vaccination interfere with measles elimination even in populations with very high overall vaccine coverage (96%).

In the Netherlands, measles vaccination started in 1976, with 14-month-old babies. A two-dose schedule, implemented in 1987, offered a combined measles, mumps, and rubella (MMR) vaccine to 14-month-old babies and 9-year-old children. The national vaccine coverage for both doses of MMR is 96% (1), but this rate is not uniform throughout the country. In 1999, 34 (6%) of the 539 municipalities had vaccine coverage of <90% for the first dose of MMR (1). These 34 municipalities, which are concentrated in a geographic belt from the southwest to the mideast of the country, contain clusters of orthodox reformed communities, most of whose members refrain from vaccination on religious grounds. The communities (estimated population 300,000, 2% of Dutch population) form a strongly coherent social group that has its own churches and schools and consists of large families (2). Notification data show that measles epidemics have mainly affected unvaccinated persons and have occurred every 5 to 7 years since the introduction of vaccination: in 1976, 1983, 1987-1988, 1992-1994, and 1999-2000 (3,4).

The most recent epidemic was first noticed on June 21, 1999. Five cases of measles were reported to a Public Health Service (PHS) in the Netherlands by a general practitioner (GP) from a municipality with low vaccine coverage (78% for the first dose of MMR)(1,5). The five patients all attended the same regional, orthodox reformed elementary school. Two days later, the headmaster informed PHS that 80 (19%) of the 412 pupils were ill at home.

After laboratory confirmation (specific serum immunoglobulin [Ig] M antibodies) of the first clinical cases, we started a study with a twofold aim: 1) to evaluate alternative methods for diagnosing measles (including detection of specific IgM antibodies in saliva and measles virus in oropharyngeal swabs and urine through reverse transcriptase-polymerase chain reaction [RT-PCR]) and 2) (on which this article reports) to assess the attack rates among pupils and their families and the severity of disease associated with measles infection.

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Methods

Study Population

We sought participation of all patients whose cases were reported to PHS between June 21 and July 2, 1999, and their household contacts, as well as all pupils from grade 1 (n = 48, 5 and 6 years of age) of the orthodox reformed elementary school. We requested two house calls, the first right before summer holidays (July 2), and the second right after the holidays (August 23). On the first visit, a questionnaire was completed and blood, saliva, oropharyngeal swab, and urine specimens were collected from all consenting household members, even those without symptoms. On the second visit, a questionnaire was completed, and blood and saliva specimens were obtained. On the first questionnaire, demographic variables, symptoms, and history of measles, measles vaccination status, travel abroad, and contact with measles were detailed. On the second visit, the section on symptoms was completed, if applicable, and two forms with additional questions were filled out. The first form inquired about complications, GP consultations, hospitalization, and medication. On the second form, limited information was gathered on all other household members (date of birth, sex, and recent measles infection).

Pupils from grades 2 to 8 were sent the same questionnaires and additional questions before and after summer vacation. We received the names of pupils in grade 0

(preschool, which is voluntary) after vacation; therefore, we sent them the questionnaires in August only. Measles vaccination history of all 412 pupils of the school (grades 0 to 8) was verified at the Provincial Vaccination Administrations (PVA).

Laboratory Tests

The presence of specific serum IgM antibodies was determined with a commercially available IgM-capture enzyme-linked immunosorbent assay (ELISA) according to procedures recommended by the manufacturer (Meddens mu-capture ELISA for measles, Biotest, Denville, NJ). IgG antibody concentrations were measured by an in-house ELISA (6).

Case Classification

We classified cases according to a modified Centers for Disease Control and Prevention case definition (7): Confirmed cases had ≥3 days of rash, fever ≥38.3°C, and either cough, conjunctivitis, or coryza; suspected cases had rash and fever according to questionnaire or recent measles according to form, with limited information on household members of pupils. We considered positive serologic results (positive IgM or a minimal fourfold rise in IgG titer) or virus isolation from blood or oropharyngeal swab to be laboratory evidence of measles infection.

Data Analyses

Attack rates for clinically confirmed and suspected measles cases were calculated by sex, year of birth, vaccination history, history of measles, and susceptibility, i.e., no vaccination, no history of measles, and birth in 1986 or later. Persons born before 1987 experienced measles epidemics in 1987-88 and in 1992-93. This was confirmed by the fact that we observed only one clinical case (the patient was born in 1986) among all persons born before 1987 (n = 226). Therefore, we considered all persons born before 1986 without information on history of measles to have had measles.

Vaccine efficacy estimates were based on the attack rate of measles among pupils who reported no history of measles in the questionnaire and by vaccination history as given by PVA. Symptoms and complications as reported in the questionnaires were described for those who had clinically confirmed or suspected measles and who had completed at least one questionnaire. We used the chi-square test to test differences in attack rates regarding categorical variables. A p value of <0.05 was considered statistically significant.

Results

Response

Responses to questionnaires, limited information, and collected biological samples (from pupils and household members) are shown in Table 1. All families with one or more reported measles patients from June 21 through July 2, 1999, had elementary school pupils in their households. We obtained questionnaires on 299 persons and limited information on 283 of their household members from 123 families, and we obtained biological samples from 100 persons in 26 families.

Table 1. Participation in measles outbreak investigation, the Netherlands, 1999–2000

	Pupils (n = 412)		Household members (n = 375)[a]		Total (n = 787)	
Sources	n	%	n	%	n	%
Questionnaire and biological samples[b]	50	(12)	36	(10)	86	(11)
Questionnaire	197	(48)	16	(4)	213	(27)
Limited information and biological samples	0	(0)	14	(4)	14	(2)
Limited information (August only)	8	(2)	261	(70)	269	(34)
No information	157	(38)	48	(13)	205	(26)

[a] Number of household members not attending the elementary school of the 255 participating pupils.
[b]Questionnaires for all 86 participants from whom questionnaires and biological sample(s) were collected both in July and August.

Description of the Outbreak

In total, 213 cases of measles (110 confirmed and 103 suspected) were identified (Table 2); 138 were in pupils. All suspected cases were epidemiologically linked to a confirmed case through school or family contacts. Therefore, we consider suspected cases true measles cases and describe our results for the confirmed and suspected cases together.

The epidemic curve is shown in Figure 1. Day 1 of rash was known in 137 of the 213 confirmed and suspected cases and occurred from June 15 to July 20, 1999. The number of

Table 2. Measles cases by clinical and laboratory case classification, the Netherlands, 1999–2000

Clinical case classification	Number	Number providing biological samples	Positive laboratory confirmation
Confirmed	110	41	34[a]
Suspected	103	10	5[a]
Noncase	369	48	5[b]
Total	582	99[c]	44

[a]All 12 clinically confirmed and suspected cases without laboratory confirmation had no rash until 3-20 days after sampling.
[b]Details on these 5 persons are in Table 4.
[c]Here, 99 persons with laboratory case classification are shown instead of 100 as in Table 1, since 1 person did not provide blood and throat swab specimens, but only saliva and urine.

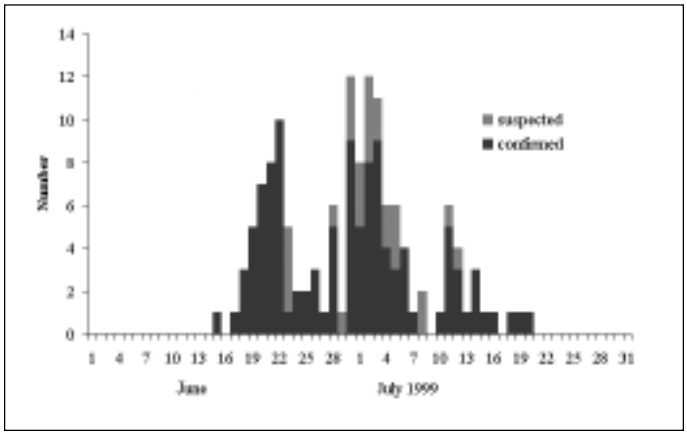

Figure 1. Distribution of clinically confirmed and suspected cases by date of onset of rash (n = 137).

persons per household was 3 to 18 (median 6). The number of reported cases per household was 0 to 9 (median 2): 37 (30%) households reported no cases, including 12 (10%) households with children vaccinated against measles.

Attack Rates

The overall attack rate among confirmed and suspected cases was 37% (Table 3), 0% for the oldest pupils to 88% for the youngest (Figure 2). Two (1%) of the 213 patients were born in 1999; 166 (78%) from 1992 to 1998; and 43 (20%) from 1988 to 1991. Two (1%) patients were born before 1988 (1986 and 1987). The distribution of cases and attack rate by sex, vaccination history, history of measles, and susceptibility (i.e., no vaccination, no history of measles, and born in or after 1986), is shown in Table 3. Except for sex, all variables were associated with the attack rate ($p < 0.05$).

The attack rate among susceptible pupils was 91% (133 of 146). Of the 28 nonpupils considered susceptible, 24 (86%) had clinical cases (Table 3). Three of the four who did not become ill were probably protected by maternal antibodies (date of birth from December 1998 to April 1999).

Among the 69 pupils considered not susceptible because of reported history of measles, one had clinical symptoms and laboratory confirmation of measles infection (Table 3). According to the questionnaire, this grade 1 pupil had measles in 1998. No vaccination was registered at PVA. This child probably had another rash disease in 1998. No cases were observed among the 195 nonpupils considered not susceptible (Table 3).

Laboratory Results

The diagnosis was laboratory confirmed for 39 of the 51 clinically confirmed and suspected cases with one or two biological samples, the first of which was collected at or just after Day 1 of rash (IgM postive or IgG titer rise). We had collected only one sample in each of the remaining 12 cases; measles rash did not develop in these patients until 3 to 20 days later. As expected, IgM antibodies could not be detected in these cases. Five of 48 asymptomatic persons who had provided biological samples had laboratory evidence of measles infection (Tables 2, 4).

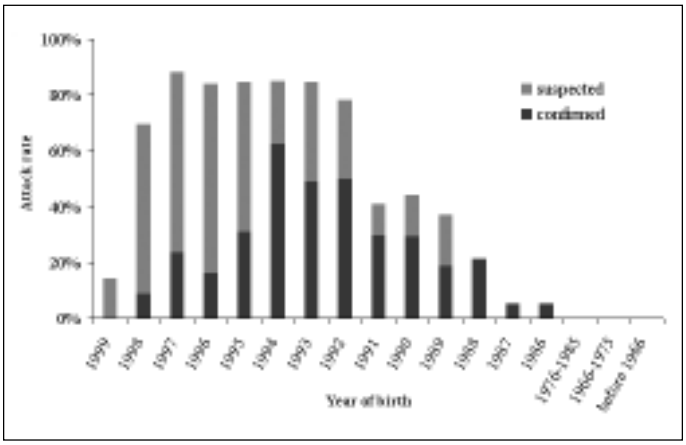

Figure 2. Attack rates by year of birth for clinically confirmed and suspected cases (n = 213).

Vaccination History and Vaccine Efficacy

Of all 412 pupils, 28 (7%) had been vaccinated, according to PVA records. Of the 255 participating pupils, 25 (10%) had been vaccinated: 20 had one dose of MMR vaccine, and 5 had had two doses. None of the 25 vaccinated pupils reported measles symptoms (Table 3). Four (one parent and three young children) (10%) of the 42 nonpupils with a questionnaire reported vaccination against measles. None reported symptoms.

Symptoms and Complications

The median number of days the rash lasted was 5 (10th-90th percentile, 3-9), the median number of days with fever was 6 (10th-90th percentile, 3-9). Of the 148 patients with confirmed or suspected measles who had given at least one answer about symptoms during measles disease, 54 (37%) reported Koplik's spots; 93 (63%) itching; 139 (94%) coughing; 136 (92%) conjunctivitis; 116 (78%) sore throat; 101 (68%) coryza; 86 (58%) diarrhea; 57 (39%) vomiting; 79 (53%) headache; and 28 (19%) aching joints.

Table 3. Attack rates (ARs) for clinically confirmed and suspected measles cases among pupils and their household contacts, by sex, vaccination history, history of measles, and susceptibility, the Netherlands, 1999–2000

	Pupils			Household members			Total			
	n	Cases	AR (%)	n	Cases	AR (%)	n	(%)	Cases	AR (%)
Sex										
Male	129	69	(53)	174	38	(22)	303	(52)	107	(35)
Female	126	69	(55)	153	37	(24)	279	(48)	106	(38)
Vaccination history[a]										
Vaccinated	25	0	(0)	4	0	0	29	(5)	0	(0)
Unvaccinated	230	138	(62)	48	24	(50)	278	(48)	162	(58)
Unknown	0	0	0	275	51	(18)	275	(47)	51	(19)
History of measles										
Yes	69	1	(2)	192	0	(0)	261	(54)	1	(0)
No	168	133	(79)	39	26	(67)	207	(36)	159	(77)
Unknown	18	4	(31)	96	49	(47)	114	(20)	53	(45)
Susceptibility[b]										
Yes	146	133	(91)	28	24	(86)	174	(30)	157	(90)
No	92	1	(1)	195	0	(0)	287	(49)	1	(0)
Unknown	17	4	(31)	104	51	(49)	121	(21)	54	(45)
Total	255	138	(54)	327	75	(23)	582	(100)	213	(37)

[a]Vaccination history for pupils according to Provincial Vaccine Administration records, for nonpupils according to questionnaire.
[b]Susceptibility of pupils: with no recorded measles-containing vaccination(s) and with no reported history of measles. Susceptibility of nonpupils: with no reported measles-containing vaccination(s) and with no reported history of measles for those born in 1986 or later.

Table 4. Asymptomatic persons with laboratory confirmation of measles virus infection, the Netherlands, 1999–2000

Participant	Year of birth	History of measles	Measles vaccination	Laboratory confirmation
1	1974	Unknown	No	IgM positive Data on IgG titer rise not available
2	1980	Yes	Data missing	IgM negative ≥fourfold IgG titer rise
3	1982	Yes	Data missing	IgM negative ≥fourfold IgG titer rise
4	1992	No	No	IgM negative ≥fourfold IgG titer rise
5	1994	No	Yes	IgM negative ≥fourfold IgG titer rise

IgM = immunoglobulin M; IgG = immunoglobulin G.

Of the 162 patients with confirmed or suspected measles who completed at least one questionnaire, 40 (25%) reported one or more complications; one of the 40 was hospitalized for delirium (Table 5). Of the 40 patients with complications, 27 (68%) consulted GPs, who prescribed medication for 22 (55%) children. Of the 22 children, 19 were given antibiotics: 9 for pneumonia, 9 for otitis media, and 1 for cystitis. Antipyretic and analgesic medications were also prescribed. The complication rate did not differ between confirmed and suspected cases (26% vs. 24%).

Table 5. Self-reported complications in clinically confirmed and suspected cases, from questionnaire data, the Netherlands, 1999–2000

Self-reported complications	Number	%
Hospitalization for delirium	1	0.6
Otitis media	18	11
Pneumonia	10	6
Earache	5	3
Stomachache	3	2
Cystitis	1	0.6
Laryngitis	1	0.6
Severe coughing	1	0.6
No complications	113	70
Data missing	9	6
Total	162	100

Conclusion

We have described an outbreak of measles in a mostly unvaccinated population. From this outbreak, measles spread and affected mainly (94%) unvaccinated persons from orthodox reformed communities. By May 2000, 3,292 cases of measles were reported to the national registry, including three measles-related deaths and 72 hospitalizations.

Attack Rates

The susceptibility levels and attack rates were closely related to the number of previous epidemics encountered; those persons born after 1992, when the last epidemic began, had the highest susceptibility levels and attack rates. The 1999 birth cohort and part of the 1998 birth cohort are exceptions because they were partially protected by maternal antibodies. Sex was not associated with the attack rate, which is in accordance with previous reports (8). The infectivity of the measles virus is shown by the high attack rate (90%) among those considered susceptible (i.e., those with no history of measles or vaccination).

Import and Export of Measles Virus

Measles viruses isolated from patients showed that the epidemic was caused by a D6 type measles virus, a genotype widely distributed throughout Europe (9). Genotype D6 had frequently been isolated from unrelated cases in the Netherlands between 1993 and 1999 (van Binnendijk et al., unpub. data). During this period, the number of measles cases reported in the Netherlands decreased to one of the lowest rates in Europe (<1 per million in 1998). However, because of low vaccine coverage in orthodox reformed communities, the number of susceptible persons increases. Consequently, measles epidemics still occur, despite high national vaccine coverage and population immunity (1,6). Previously, we showed that measles is not endemic in the Netherlands, not even in areas with low vaccine coverage (10). This was confirmed in this 1999-2000 epidemic; no more cases were reported within 1 year after the start of the outbreak. Therefore, we assume that the epidemic was initiated by import from another country. Until the measles virus is eradicated, circulation will continue worldwide and epidemics will occur. During this epidemic, visiting relatives exported measles to Canada. The outbreak was restricted to 17 cases within an orthodox reformed community in Canada as a result of stringent measures (e.g., closing the school) (11).

Laboratory Results

We observed five asymptomatic persons with serologic proof of measles infection. All had been in close contact with one or more measles patients. Two were children, one vaccinated (#5 in Table 4) and one without recorded measles vaccination or history of measles disease (#4). Incomplete immunity in the presence of residual maternal antibodies may have developed in the latter child during the 1992 measles epidemic (12). Two adults (#2 and #3) reported history of measles, the third (#1) reported no history of measles but might have had measles, on the basis of the year of birth. However, this person might also have had subclinical primary infection.

We assume that the increase in specific IgG (#2-#5) reflects secondary immune response in persons reexposed to measles virus, as has been demonstrated (13-15). We have not been able to detect virus, either by virus culture or RT-PCR from blood or oropharyngeal swab (data not shown), from any of these subclinically reinfected persons, as was recently shown for an immune mother of an adult measles patient (16). However, even if virus can be detected in blood, urine, or saliva, the critical issue is whether the virus load in these subclinically reinfected persons is high enough to transmit the measles virus.

Vaccination History and Vaccine Efficacy

We observed low vaccine coverage (7% to 10%), but excellent vaccine effectiveness (100%) for the measles component of the MMR vaccine; none of the vaccinated persons had measles symptoms. In the measles epidemic following this outbreak, 5% of the reported cases patients were vaccinated; almost all of them had received one dose (5).

The real percentage of vaccinated patients is probably smaller. We expect that more vaccinated than unvaccinated persons with measles symptoms are seen and reported by GPs.

Symptoms and Complications

Measles is sometimes thought of as a mild disease. However, we observed a self-reported complication rate of 25% for all patients, 68% of whom consulted a GP. We do not know whether children who did not complete a form on complications consulted a GP. The percentage of consultations for uncomplicated measles cases could be smaller than that for complicated cases. Therefore, the percentage of consultations for all cases may be overestimated.

The complication rate of 25% is based on self-reported complications, and the diagnosis was not always confirmed by a physician. This could explain why the complication rate is somewhat higher than expected for measles (8,12). Still, burden of disease was very high in the participating measles patients. During the following epidemic (1999-2000), three measles-related deaths and 72 hospitalizations were reported (5).

In this descriptive study of a measles outbreak with an attack rate of 90% among susceptible persons, we have shown that measles disease is severe, even in an industrialized country. Vaccination is the most effective means of preventing the disease and its complications. The national vaccine coverage of 96% for both doses of MMR is theoretically high enough to eliminate measles (17). However, despite this very high coverage, measles epidemics still occur as a result of areas with low vaccine coverage. In these sociodemographically clustered, mainly unvaccinated communities, the number of susceptible people increases, and consequently epidemics occur periodically. The clustering of unvaccinated persons is the critical factor for measles elimination in the Netherlands.

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Afterthoughts about Bovine Spongiform Encephalopathy and Variant Creutzfeldt-Jakob Disease

The recent review (1) of bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD) stimulated a large number of questions and comments about additional issues discussed in this commentary.

Advice for Travelers to Europe

The most frequently asked question concerned travel to Europe this summer: what is safe to eat, and what should be avoided. In principle, beef and beef products should no longer carry any risk because the European Community, as well as individual European countries, have taken measures to prevent the entry of potentially contaminated products into the human food chain. These measures include regulations to end the recycling of mammalian protein into ruminant feed (extended by some countries to feed used for all mammalian species); removal of the heads and vertebral columns of slaughtered cattle before further processing; and prohibition of certain nervous system and visceral tissues for human consumption. In the United Kingdom, mechanically removed meat, even after removal of the vertebral column, is prohibited for human use.

Nevertheless, because the extent of BSE outbreaks in most European countries is still not known—more and more cases are being discovered through active surveillance using sensitive immunologic tests—and because there is always an imprecise lead time before the implementation of precautionary measures, it would probably be wise to remain wary of beef products in continental European countries (and certain other countries) in which BSE already exists or is likely to appear, and in which safeguards may not yet be fully implemented. The European Commission of Food Safety has published a global country-by-country BSE risk analysis, with continuing web site updates as new information becomes available at http://europa.eu.int/comm/food/fs/sc/ssc/outcome_en.html.

It is also important to reemphasize that beef as such is not dangerous; the villains were almost certainly beef products that (in the past) contained mechanically recovered meat contaminated by nervous system tissue. These beef products were often added to such precooked items as sausages, hot dogs, bologna and other luncheon meats, canned beef products, beef stews and broths, and meat pies—in short, any product containing beef in a precooked and often unrecognizable form. Cooking cannot be guaranteed to sterilize BSE infectivity: experiments using different strains of spongiform encephalopathy agents have shown only partial inactivation at temperatures as high as 350°C (662°F) (2,3).

Dairy and Other Bovine-Derived Products

Many readers asked about the safety of milk and dairy products. Persuasive evidence indicates that these important consumables are risk free: milk from infected cows has been fed to, and injected into the brain of, susceptible RIII mice without transmitting disease. Even more convincingly, calves suckling gallons of milk from their infected mothers have not contracted the disease (4).

Questions have also arisen about the safety of other products derived from bovine sources, such as gelatin and tallow derivatives, which find their way into a dazzling array of items eaten or used by humans. These include products as varied as jellybeans, cosmetics, and vaccines. Scientific advisory committees in many countries, including the United States, have addressed these questions and have concluded that infectivity in the tissues used as sources of these products is either low or nonexistent, and that even if existent, infectivity would in most cases be destroyed by the processes used in product manufacturing. Nevertheless, depending on estimates of risk inherent in tissue sources and use of different products, industry has been either obliged or strongly urged to ensure BSE-free status of source cattle and to include processing steps known to destroy infectivity.

Nonbovine Products

Products derived from nonbovine species, including sheep, pigs, and chickens (both meat and eggs) should also be entirely safe, as there is no evidence that any of these species is naturally susceptible to BSE. Scrapie-infected sheep tissues have been eaten by humans for centuries without causing disease (5), and although pigs can be experimentally infected with BSE by direct brain inoculation, attempts to induce infection by feeding have not succeeded (6). Chickens are resistant to both natural and experimental infection.

The American Scene

Concerns have been raised about BSE status in the United States and about the safety of foods and other products from domestic bovine sources. BSE could occur in the United States only as a result of 1) cases arising de novo in domestic cattle, 2) cases arising from exposure to spongiform encephalopathies in other domestic species, or 3) cases arising from the importation of infected cattle or livestock feed. With respect to de novo cases, it is possible that cattle (and perhaps all mammals) are subject to the same rate of spontaneously occurring disease as that of CJD in humans, approximately one case per million animals per year. If true, this incidence has so far escaped detection, despite extensive recent search for cases.

One oft-repeated but misguided objection to this statement is that very few apparently healthy cattle have been examined. However, if we wish to know whether or not a given disease exists in a population, we do not concentrate on asymptomatic persons, but instead on population subsets at highest risk. For example, if we wished to know whether or not cases of Alzheimer's disease occurred in a given country, we would first examine older adults, not the general population. The same principle applies to BSE. Although asymptomatic cattle with BSE might be infected, it is far more probable that BSE will be diagnosed in animals with neurologic signs or at least some indication of illness (even if atypical), and it is precisely these animals that are being tested.

Microscopy examination of brain specimens from >12,000 cattle categorized as downers (abnormal recumbency from any cause) or with suspected neurologic disease has failed to reveal a single case of BSE. Brain specimens from >3,000 of these cattle have also been tested immunologically

for prion protein. By way of comparison, in Switzerland (a country in which BSE has been carefully researched), tests for the protein have been carried out in 40,000 animals, including 15,000 downer cattle, and yielded approximately one diseased cow for every 1,000 examined, half of these from the downer group. The comparison is statistically significant; that is, testing already completed in the United States would have detected BSE if it existed at the incidence level of Switzerland. Testing of cattle in the United States will continue, and many thousands more animals will be examined in the next few years, which will provide increasing assurance of BSE-free status.

With respect to cattle or cattle feed imported from the United Kingdom (or other countries in which BSE has occurred), the United States led the way in taking measures to prevent or correct any such occurrences. During the years before the importation of live ruminants and ruminant products was banned (in 1989), 500 cattle and a single shipment of 12 tons of meat and bone meal feed were imported from the United Kingdom. Almost all of the cattle were traced, and if still living, were slaughtered and destroyed. We can say today that any animal or animal feed that might have been contaminated did not transmit the infection because with an incubation period of approximately 5 years, BSE would already have spread through recycled carcass cattle feed to cause a recognizable outbreak of disease.

Import barriers have since been extended to include all countries in which BSE exists or which have not convincingly demonstrated its nonexistence. Thus, the argument that undetected cases of BSE might be present in the United States, although impossible to disprove, is not supported by current evidence. Because of the everpresent risk for human error, vigilance is still required to see that all established preventive measures are properly and continuously monitored.

Allied Diseases

Three varieties of spongiform encephalopathy present in the United States, scrapie in sheep, transmissible mink encephalopathy (TME), and chronic wasting disease (CWD) of deer and elk, under the right circumstances may be capable of infecting other animal species. Scrapie first appeared in the United States in 1947 in Michigan sheep of British origin that had been imported from Canada and has since spread to most regions of the USA. Scrapie has not been convincingly shown to cause disease in any other species (apart from goats), despite its certain inclusion in rendering mixes for livestock until the 1997 mammalian to ruminant feed ban. Exactly why species barriers have not been crossed is unclear but may be due to a relatively low flock incidence of scrapie and a relatively small proportion of sheep to cattle in rendering plants, such that the very low amount of infectivity entering the total rendering mix does not survive processing into livestock feed.

TME was also first reported in 1947 on a Wisconsin mink ranch and has occurred in several further outbreaks on mink ranches both in the United States and abroad. TME was originally thought to have resulted from feeding (scrapie-infected) sheep carcasses to the mink, but in one U.S. outbreak in 1985, epidemiologic study indicated that the dietary source might have been downer cattle carcasses (7). No experimental verification of this hypothesis was undertaken, no recurrence of TME has been identified in the United States during the past decade, and no outbreak of TME has occurred in any country (including the United Kingdom) that has BSE in native-born animals.

CWD was first recognized in 1967 in captive deer on a Colorado wildlife research facility. It occurs endemically in wild deer in contiguous sections of northcentral Colorado and southeastern Wyoming and episodically on elk farms along the eastern border states of the Rocky Mountains. No disease in humans or other animals has been attributed to CWD, but the potential for disease is very real: infected tissues could be eaten by predators or enjoyed by aficionados of wild game, and carcasses could be rendered for feed that (by error) could find its way to cattle. Regional hunters and elk farmers have been alerted to the risks, but more attention at the national level is urgently needed.

What if…?

What would result from the mistaken feeding of contaminated mammalian protein to a herd of cattle? During the 1980s, millions of cows in the United Kingdom were eating at least half pound of meat and bone meal dietary supplement each day, some of which was certainly contaminated, yet the incidence of BSE in affected herds never exceeded 2% to 3%. Today in the United States, the worst that could happen after a contaminated feed incident would be that a few cattle might be infected and come to slaughter unrecognized, but without a sequence of similar errors, no infectious tissue would be recycled in the livestock food chain, and a regulatory breakdown of this magnitude is virtually impossible.

Nevertheless, if even a single case of BSE were to be discovered in the United States, the economic and perceived public health consequences could be immense. How would the current package of preventive measures stand up to future judgment? With generally high marks, although a few points of vulnerability still exist (the elimination of which would substantially dislocate segments of the rendering and livestock industries):

- The 1997 ban on using mammalian protein in ruminant feed exempts plate waste from restaurants (which could contain bovine brain or paraspinal ganglia in the uneaten remains of some cuts of meat). They could be recycled to cattle in feed produced by rendering plants.
- Feed for ruminants and nonruminants can be processed in the same feed mills, creating the potential for cross-contamination. Rendered carcasses of deer and elk with chronic wasting disease could conceivably in this way be fed to cattle.
- If farmers or ranchers mistakenly or deliberately use nonruminant feed for ruminants, the mammalian to ruminant feed ban would be bypassed. Spontaneous TSE occurring in nonmammalian species, if it occurs, would also escape the mammalian to ruminant feed ban.
- Unlike the European Union, the United States does not mandate that the rendering process be capable of sterilizing BSE infectivity. (Most feed mills render carcasses at 134°C without the concomitant use of steam and pressure, and thus cannot be guaranteed to sterilize).

- Mechanically separated meat expressed from crushed carcasses can be added to cooked and uncooked meat products, up to a concentration of 30% by weight. Since 1997, spinal cord has been removed, but the vertebral column (including the paraspinal ganglia) can still be processed and used in many products: hot dogs, sausages, canned beef, luncheon meats, and soups and stews. A recently introduced and much safer process (advanced meat recovery) has not yet completely replaced the crushing method.
- Organs known to be infectious in cattle with BSE (including brain) are not prohibited from human consumption.
- Glandular dietary supplements containing various animal organ powders, including cattle brain, were often imported from the United Kingdom or countries in continental Europe until the U.S. Department of Agriculture import ban in 1989. The ban relies on proper labeling of the shipment and can be abused.

As an alternative to the entire issue of precautions against the occurrence and spread of BSE, we must finally ask, what would be the economic consequences of eliminating animal protein from livestock feed or replacing it with plant protein?

The rendering industry would disappear, and the incineration industry would expand in conjunction with the production of nutrition crops such as soybeans. Would plant protein be as effective as animal protein? Is the public prepared to pay more for meat or eat only as much as can be produced from range-fed animals? These are not trivial questions, and the answers will need to be weighed against the overarching issue of public health.

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An Infected Heart

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Lovers have always known that love can go bad and infect the metaphorical heart. And physicians have long known that love can infect the literal heart, too. My first palpable encounter with the literal heart, early in medical school, in gross anatomy lab, showed me just how resistant such infections can be.

The body on which we worked was that of a man, perhaps fifty years old. He was muscular, in life a laborer, I imagined. His body was like a textbook, perfect, flawless. As freshman medical students, we had no idea why such a perfect body might have died—until we opened the chest.

His heart, like his other muscles, was heavy with the weight of life. But the most striking abnormality within the cave of his chest was the enlargement of his aorta, the artery that distributes blood over the body. His aorta was ballooned out, swollen to three times its normal size. Such a weak, bulging aorta, an aneurysm, could have come only from syphilis, which he had contracted years—or, more likely, decades—before. This revelation completely transformed our daily encounters with our cadaver. This was now no longer a mere anatomy textbook, yielding up its slow secrets. No. This was a man, instantly humanized, who had walked and worked among us and died of love. And, in some metaphysical sense, the woman from whom he had contracted the disease was still there with him on the dissection table, after all those years. As Racine wrote in *Phèdre,* "It is no longer a passion hidden in my heart; it is Venus herself fastened to her prey."

I saw him first in April on cardiology consultation rounds. I was working with two emergency medicine residents and two cardiology fellows that month, swooping all over the hospital to see patients with suspected or clear-cut heart disease. The call over the beeper was from the burn unit.

We entered the sanctum of the unit through the swinging doors. Signs on the wall confronted us: DID YOU WASH YOUR HANDS? We did. And donned the gowns, masks, and puffy surgeon's caps that were required for the unit. We began to review the chart.

The man was young—maybe twenty-five years old—and had been burned—widely, severely. The surgeons had struggled for weeks to try to cover the burned areas with skin grafts before his wounds could be irreversibly infected. He'd been in the hospital since January—almost four months now. I thought of how much it hurts to burn a finger on a hot stove, then tried mentally to magnify it.

We listened as one of the fellows took the history: How had he been burned? The patient told us he'd been walking late one night in January when he spotted the distinctive orange glow and thick smoke of a major fire in a building he passed. He knocked loudly on the door and yelled for help. A young boy appeared at an upstairs window. The patient told us he broke in the door, roused the family out, then went back in for the boy. He'd saved the boy but had been badly burned in the process.

The man—his name was Robert—was sitting upright in a high-backed wheelchair. His limbs, abdomen, and back were swathed in bandages; he sat propped up on foam so as to put as little pressure as possible on the tender skin underneath. From a distance, his face reminded me of one painted by Rouault: dark, broad brushstrokes outlining it, another dark stroke lending prominence to the nose, and lighter tones for the rest of the face. Up close, the landscape of his face was made up of glistening caramel-colored islands of partially melted tissue; they were separated by—and seemed to be eroded by—the angry channeling red pigment of his blood that looked ready to well up at any moment from beneath the burned skin. The effect was that of a variably cooling recent lava flow. But that was not the most striking aspect of his countenance: his eyes had the look of pure terror, pupils dilated, lids widely separated—the eyes that everyone in medicine sees sooner or later. They lent to his face the unmistakable appearance of someone who's seen death, and been changed by it.

We examined Robert's heart. It was racing: 160 beats a minute. There was a soft heart murmur: "PfffFF . . . TT, PfffFF . . . TT, PfffFF ... TT." Not loud, but not normal either, we decided among ourselves.

Robert had done surprisingly well for several weeks as his own good skin was grafted over the burned areas. But then infection had set in. Antibiotics were started—first one, then two, then another set, as the bacteria became resistant to the drugs. The spiking daily fevers began—and drenching sweats. Cardiology was called to see him after bacteria were cultured from his blood: *Does he have endocarditis?* asked the consultation note.

We decided to do an echocardiogram. The machine was trundled up from the sixth floor of the hospital and set up in the room. An echocardiogram harks back to the sonar units used by submarines—the ones we all saw at the movies on Saturday afternoons while we were growing up, blithely

unaware of any hearts but our own. The echo machine uses ultrasound waves, as does sonar, bouncing the waves off the interior anatomy of the body. Using the echo, one can visualize the heart's perpetual energy and, within it, in the swirling sea of blood, the graceful heart valves keeping the blood flowing straight and full speed ahead.

Robert's valves all looked normal, thin but with great tensile strength, smoothly opening and closing, all except the mitral valve. Instead of smoothly dancing in place, the mitral valve's motions were weighed down by a mass of heavy echos that could mean only one thing: the blood- and bacteria-laden abnormal appendages of endocarditis. His heart was infected, all right, seeded from his infected burns and then itself constantly reseeding all parts of the body in its natural centrifugal energy. We were trapped and so was Robert. We might have had a better chance to cure the endocarditis had we not had the infected burns to contend with. As it was, the situation was a desperate one. We wrote out our orders and tried to explain the dilemma to Robert, being as optimistic as possible.

We saw Robert daily over the next two weeks. At first, by switching to yet another, more toxic, set of antibiotics, we seemed to be making some progress. His temperature came down—for four days it was almost normal. All the while the surgeons worked to cover his burns with grafts. They didn't take. Robert was given several transfusions of blood. But his kidneys were beginning to fail. We adjusted the medications, meeting incessantly to decide how to approach each new crisis. I could tell we were losing ground. Robert looked weaker and more resigned on each of our successive visits. The raging fever returned. He was going out in a blaze, an inferno of infection.

Several days later, as the group of us approached the door of Robert's room, we saw that he had a visitor. The visitor's back was toward us, but his gown was open. His suit was black, and above the tie-strings of the gown around his neck, we could see the white collar of the priesthood. We backed quietly out of the room and waited. In a few minutes the priest came out, closing his Bible as he approached us, a silver cross dangling from a broad white ribbon folded over his forearm. We nodded to his nod. We examined Robert. He looked exhausted. There was a rosary in his hand. He barely noticed that we were in the room.

That afternoon, the cardiology team got an emergency call to the unit. As we arrived, donning our apparel quickly, we found the entire surgical team gathered around Robert's bed, always a bad sign. Robert had had a seizure, then his heart had skipped, shuddered, and stopped. Despite our efforts, there was no way to save him. For my part, I wasn't at all sure that Robert *wanted* to be saved any longer. I thought about our sessions in ethics class on "quality of life." Robert had had little of that in the last few months.

As we left the room a deputy sheriff outside approached us. He asked about Robert. We told him what had happened, that we'd lost the battle. It was only then that we learned the truth about Robert. Robert, said the deputy, was an arsonist. He had set blazes in two parts of a building back in January. But he'd gotten trapped between the two fires, was badly burned, and had barely escaped with his life by jumping from a second-story window. Robert's tale about his heroism, the young boy he'd saved, was a total lie, something he'd wanted us to believe and, in the haze of morphine, may have begun to believe himself. Ordinarily, he would have been placed under an around-the-clock guard, said the deputy; but, as he told us, "In his condition, he wasn't goin' no place."

We shrugged out of our gowns, shaking our heads in disbelief. We had been had, all right. Robert must have figured that things would go better for him medically if his doctors thought he was a hero and not an arsonist. I like to think that knowing the truth about Robert wouldn't have affected our efforts to save him. But who can be sure of that? Robert couldn't.

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Visceral Leishmaniasis (Kala-Azar) Outbreak in Somali Refugees and Kenyan Shepherds, Kenya

To the Editor: A sharp increase in suspected visceral leishmaniasis (VL or kala-azar) cases was reported in April through May 2000 in three Kenyan refugee camps (Ifo, Dagahaley, and Hagadera). Located around Dadaab town in Northeastern Province, the three camps house an estimated 125,000 Somali refugees. VL outbreaks have been well documented in five distinct foci in Kenya (1,2), but until this outbreak, VL was only sporadically seen in the refugee camps or the province.

We investigated a possible outbreak in the refugee sites. Before April 2000, doctors would request a formol-gel test (FGT) in case of suspected VL and treat an FGT-positive case with antimonials. Although the FGT is of uncertain validity, it is still used in district hospitals in Kenya for lack of alternative diagnostic tests. We considered a clinician's request of an FGT as a proxy for "clinical VL suspicion" and assessed the number of FGTs done from January 1999 to March 31, 2000. The first suspected VL patient was traced back to August 1999; this 40-year-old male Somali refugee had been ill for 8 months and sought treatment at Dagahaley camp. He responded well to antimonial treatment. From that date to April 1, 2000, an FGT was requested for five more patients; results were positive for two.

Specific surveillance for VL was set up by the refugee health services in April 2000. Suspected patients or their caretakers were interviewed. Finger-prick blood was collected on filter paper and analyzed by direct agglutination test (DAT) (3). In August 2000, splenic aspirates were performed on eight patients for direct microscopic examination, and parasite culture was attempted for three specimens. In vitro isolation and gp63 polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) molecular typing was done at the Protozoology Unit of the Prince Leopold Institute of Tropical Medicine in Antwerp, Belgium. Serologically or parasitologically confirmed cases were given stibogluconate (Pentostam), 20 mg/kg/day, for 28 days.

We reviewed surveillance data for the period April 1–August 31, 2000, and interviewed the health staff. For case classification, a probable case of VL was illness in a patient with 1) a fever of >2 weeks' duration, 2) splenomegaly or wasting, and 3) positive DAT serology. A confirmed case had these clinical signs, as well as a positive parasitology smear or culture. From April 2000 to August 31, 2000, 26 probable (DAT-positive) VL cases were observed and 8 others were confirmed parasitologically. Gp63 PCR-RFLP molecular typing showed *Leishmania donovani* in one specimen. The case-fatality rate was 10 (29.4%) of 34 patients in the group of probable and confirmed VL cases. Six deaths occurred before treatment could be started, and one was a complication of the diagnostic procedure (spleen aspirate).

Thirty-two interviews were completed in the group of 34 probable or confirmed VL patients. Median age was 15 years, and 8 (25) of the 32 were female. Median delay between onset of symptoms and date of diagnosis was 8 months. Six were Kenyan citizens, five of them shepherds who were grazing their cattle in the area around Dadaab. Of the Somali refugees, seven had been living for >2 years in the refugee camp when their symptoms began. (Five had been born in the camps.) However, 16 (61.5%) of 26 patients arrived in the camps after the onset of their symptoms. Most of them were Ogadeni shepherds, who reportedly grazed their cattle in the Lower Juba region.

Other evidence points to a serious problem inside Somalia as well. Médecins sans Frontières reported 48 VL cases from July 28 to September 21, 2000, in Hudur, Bakol region. Other nongovernmental organizations reported cases from several towns in Gedo region. The distribution of VL in Somalia before the war is poorly documented, but the disease was known to be endemic in Giohar district, north of Mogadishu (4). In 1994, Woolhead reported VL in a woman from Baidoa and warned of potential outbreaks because of the war (5). Although several of the 34 patients reported here may have been infected in Somalia, local transmission in Kenya cannot be excluded, since some of the refugees denied having left camp and six were Kenyan citizens.

This outbreak is reason for concern in the context of the deteriorating nutritional situation in drought-affected northeastern Kenya. Malnutrition is a known risk factor for the development of clinical VL in infected persons (6). In southern Sudan, deaths caused by VL were attributed to malnutrition in a famine- and war-stricken population (7). The current nutritional status of the Somali refugees in the Dadaab camps is precarious. After the 1996 food scarcity problem (8), food rations for refugees were maintained at the recommended 2,100 kcal/person/day. In February 2000, however, the ration was again reduced below the vital minimum. A cross-sectional random cluster survey on August 29-31, 2000, showed rising malnutrition levels in <5-year-old refugee children (Médecins sans Frontières, unpub. data).

Immediate outbreak control measures have been taken by refugee camp health authorities, the surveillance system was strengthened (including initiation of active case-finding measures), and diagnostic and therapeutic facilities were upgraded. Six-month peridomestic spraying of the refugee shelters with lambda-cyhalothrin (ICON) is a routine vector control measure in the camps. Special attention needs to be paid, however, to the food security for the refugees in the light of the current outbreak. Our observations on 16 imported cases also raise concerns about VL transmission inside Somalia, where access to health care is virtually nonexistent in many areas and a VL outbreak might go undetected.

Acknowledgment

We thank the doctors and nurses in the Dadaab refugee health services for data collection and J.C. Dujardin for species identification.

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Doxycycline and Eradication of Microfilaremia in Patients with Loiasis

To the Editor: *Wolbachia* are intracellular symbionts found in 20% of insects and in several nematodes, including filarial worms. Because tetracycline eradicates *Wolbachia* in nematodes, this drug has been proposed for chemotherapy in filariasis (1). We report two patients with loiasis in whom no Wolbachial DNA was detected in microfilariae by polymerase chain reaction (PCR), and for whom 6 weeks of doxycycline failed to eradicate the microfilaremia. We conclude that doxycycline may not be an efficient therapy for loiasis.

Filariae are responsible for 150 million infections worldwide, some of them devastating diseases such as elephantiasis (caused by Bancroftian and Brugian filariasis) and blindness (caused by onchocerciasis). There is no satisfactory treatment for filariasis: although diethycarbamazine citrate has been used for 50 years to treat the disease, this drug is not efficient in most adults (2). Ivermectin has been reported to be efficient in treating microfilaremia and possibly for viability and fertility of adult *Oncocerca volvulus* worms if therapy is prolonged for 2 to 3 years (2). However, the microfilaricidal effect of ivermectin on *Wuchereria*, *Brugia*, and *Loa loa* is similar to diethycarbamazine: ultrasonography shows that it has no effect on adult Bancroftian worms, and microfilariae often reappear after a few months (2).

Intracellular bacteria have been observed in the lateral cords of adult female worms as well as in microfilariae of *W. bancrofti,* and these bacteria have recently been identified as belonging to the *Wolbachia* genogroup (3). *Wolbachia* have been detected by PCR in Brugian and Bancroftian filariae, dirofilariae, and most species of *Oncocerca* including *O. volvulus*, but have never been detected in *L. loa* worms (4). *Wolbachia* are causative agents of a variety of modifications in host development and reproduction, including cytoplasmic incompatibility and parthenogenesis. Consequently, it has been proposed that antibiotic eradication of *Wolbachia* from infected filarial worms would reduce microfilaremia. This has been demonstrated in an animal model with *Litomosoides sigmodontis* and recently confirmed in patients infected with *O. volvulus* (1-5). In experimental *L. sigmodontis* infection, after 41 days of therapy, microfilaremia in tetracycline-treated animals was one-tenth that in normally infected animals (5).

We report the failure of tetracycline to reduce microfilaremia in two patients with *L. loa* filariasis. Patient no. 1 was a 58-year-old man who worked in Gabon for many years and had cutaneous larva migrans. *L. loa* microfilaremia was detected ($4x10^3$/mL). Patient no. 2, a 15-year-old boy living in Cameroon, was diagnosed with Calabar swelling with *L. loa* microfilaremia ($1x10^3$/mL). After giving informed consent, both patients were treated with doxycycline 200 mg daily for 6 weeks as previously described in *O. volvulus*-infected patients (1). We observed patients for microfilaremia every week for 6 weeks and then every 2 weeks for 2 months. The presence of adult worms was detected by physical examinations.

Microfilaremia was detected in both patients at the completion of treatment and at day 120 of follow-up. In patient no. 1, the frequency of migrating adult worms seemed to diminish during therapy, but they never disappeared. For *Wolbachia* detection in worms, blood samples were collected both in Dupont-Isolator and EDTA-containing tubes. After centrifugation at 5000 x *g* for 30 min, the worm-enriched pellet was resuspended in 1 mL of sterile deionized water for erythrocyte lysis. DNA was extracted from the suspension by using the QIAmp-blood kit (Qiagen, Hilden, Germany) following manufacturer's recommendations. *W. pipientis* DNA was used as a positive control. Control of DNA extraction was performed by amplifying microfilarial DNA using the nematode-specific 18S r DNA-derived primers 18SF (5'-GAT-ACC-GCC-CTA-GTT-CTG-ACC-3') and 18SR (5'-ACC-AAC-TAA-GAA-CGG-CCA-TG-3'). *Wolbachia* detection was attempted with the FD1 (5'-AGA-GTT-TGA-TCC-TGG-CTC-AG-3') and Rp2 (5'-ACG-GCT-ACC-TTG-TTA-CGA-CTT-3') eubacterial primers, with the *Ehrlichia* genus-specific 16S rDNA primers EHR16SD (5'-GGT-ACC-YAC-AGA-AGA-AGT-CC-3') and EHR16SR (5'- TAG-CAC-TCA-TCG-TTT-ACA-GC-3'), and with primers specific for the 16S r DNA of *B. malayi* endosymbiont, Bsymbf (5'-ACG-AGT-TAT-AGT-ATA-ACT-3'), and Bsymbr (5'-CCT-TCG-AAT-AGG-AAT-AAT-3') (3-6). PCR reactions were performed on PTC-200 thermocycler (MJ-Research, USA) by using 45 cycles of denaturation at 94°C for 30 sec, hybridization for 45 sec, and elongation at 72°C for 1 min. Hybridization temperatures were 55°C for FD1/Rp2, 53°C for EHR16SD/ EHR16SR, 42°C for Bsymbf/Bsymbr, and 57°C for 18SF/18SR. Experiments were repeated three times.

We detected *Wolbachia* in the positive control and 18s rRNA of the nematode in the sample, but no signal compatible with Wolbachial DNA was obtained with the sets of primers used. In fact, four species of filariae *(Dipetalonema setariosum*, *Acanthocheilonema vitae*, *O. flexuosa*, and *L. loa*) tested for intracellular bacteria by electron microscopy, immunohistochemistry, or PCR had no bacteria (4). The absence of *Wolbachia* in *L. loa* microfilariae may explain the failure of tetracycline therapy in our patients.

More work is needed to determine the prevalence of *Wolbachia* in filariae, their impact on fertility in each species, and the use of antibacterial agents for eradicating these pathogens.

Acknowledgment

We thank MJ Taylor for providing *W. pipientis* DNA for controls.

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Bovine Spongiform Encephalopathy and Variant Creutzfeldt-Jakob Disease

To the Editor: The article by Brown et al. (1) contains the statement "it appears likely that changes in the rendering process that had taken place around 1980 allowed the etiologic agent in infected carcasses to survive." If that is the case, why not revert to the rendering methods used before 1980? That measure would seem more cost-effective than trade embargoes and mass killing of cattle. Meal made from meat and bone was used as a livestock feed additive in many countries without the apparent disastrous effect seen in the United Kingdom. Did the rendering methods remain unchanged in these other countries? Historically, rendering was viewed somewhat differently in continental Europe; the primary purpose was not to make animal feed but to destroy infectious agents, as indicated by the very name of the facility: destruction plant (Destruktionsanstalt). The plants were under governmental inspection and there were mandatory time-temperature requirements for processing meat, bones, and other offal. Temperature requirements varied from 120°C to 140°C, which presumably would reduce if not eliminate prions. It would be of interest to see a description and objective analysis of rendering methods and regulations in various countries before and after 1980. In these times when the concept of hazard analysis and critical control points (HACCP) is gaining popularity, it would seem natural to extend the principle of process control to rendering. One might easily get the impression that policies to control bovine spongiform encephalitis are dominated by some of the stakeholders: the researchers writing their next research grant proposals, the public agencies eager to show prompt response, and those who would like to see their competitors' beef kept off the market.

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Mad Cow Disease

To the Editor: In regards to "Bovine Spongiform Encephalopathy and Variant Creutzfeldt-Jakob Disease: Background, Evolution, and Current Concerns" (1), use of the name "variant Creutzfeldt-Jacob disease" (vCJD) for human cases of bovine spongiform encephalopathy (BSE) is regrettable. The disease that occurs in humans exposed to BSE is zoonotic and CJD is not; in addition, the human form of BSE has important clinical, pathologic, and epidemiologic differences from CJD. Continued use of this terminology perpetuates the error.

The fact that 12 years after the feed ban bovine cases continue to occur in the United Kingdom at a much higher rate than in any other country could have two possible causes: inefficient controls or additional routes of transmission. Data on alternative routes of transmission must be evaluated, and important gaps in our understanding of BSE in cattle must be addressed.

Extensive epidemiologic data on BSE in the United Kingdom seemed to clearly implicate the practice of feeding cattle bovine offal as the primary, if not the sole, cause of the spread of BSE. Alternative theories for the origin and spread of BSE, e.g., use of insecticides on bovines or the practice of artificial insemination, appear to have been ruled out quickly on the basis of early epidemiologic data. Confidence in the reliability of these data seems to have been so great as to unduly delay transmission experiments to assess the role of alternative pathways (e.g., artificial insemination) in the propagation of BSE. Is prion protein present in semen or is it not? What if it were present in semen? Would this route lead to shorter incubation than the alimentary route? There seems to be no experimental information on the effect of freezing mutated prion protein to -196°C and placing it, after thawing, directly on the stimulated uterine mucosa.

Although the United Kingdom has acknowledged that compliance with the feed ban improved after 1996, we should not be too eager to accept lack of compliance as the only possible reason for the persistence of BSE. Fifteen years after the BSE epidemic began, it cannot be disputed that the ban on offal feed has interrupted spread of the disease. However, if continued spread by the alimentary route can be excluded as the cause of more recent cases, each of these cases should be carefully evaluated to uncover heretofore unknown or underappreciated routes of transmission.

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Report Summary

Under the Weather: Climate, Ecosystems, and Infectious Disease

Committee on Climate, Ecosystems, Infectious Disease, and Human Health, National Research Council, Washington, DC, USA[1]

The observation that a change in weather can lead to the appearance of epidemic disease has been appreciated since the dawn of medical science. In modern times, our increasing abilities to detect and predict climate variations such as El Niño, coupled with mounting evidence for global warming, have fueled a growing interest in understanding the impact of climate on the transmission of infectious disease agents. Studying these linkages between climate and disease may yield insights into the factors that drive the emergence and seasonal or interannual variations in contemporary epidemic diseases and possibly into the potential future impacts of long-term climate change.

Understanding these linkages, however, can present a daunting research challenge. Climate-related impacts must be understood in the context of other influences on disease dynamics, such as rapid evolution of drug- and pesticide-resistant pathogens, swift global dissemination of microbes and vectors through expanding transportation networks, and deterioration of public health programs. In addition, the ecology and transmission dynamics of infectious diseases differ widely from one context to the next, making it difficult to draw general conclusions or compare results from individual studies. Finally, the interdisciplinary nature of this issue necessitates collaboration among scientists who may have little understanding of the capabilities and limitations of each other's fields. Such factors have contributed to vigorous debates on this topic in the scientific community. In response, a National Research Council committee was formed to address three tasks: to assess scientific understanding of the relationships between climate, ecosystems, and infectious diseases; to evaluate the potential for developing climate-based disease early warning systems; and to identify priorities for future research on this topic. The following is a summary of the committee's key findings and recommendations.

Linkages between Climate and Infectious Diseases

Disease Impacts of Weather and Climate Variability

The characteristic geographic distributions and seasonal variations of many infectious diseases are prima facie evidence that their occurrence is linked with weather and climate. Studies have shown that factors such as temperature, precipitation, and humidity affect the life cycle of many disease pathogens and vectors (both directly and indirectly, through ecologic changes) and thus can potentially affect the timing and intensity of disease outbreaks. However, the incidence of disease is also affected by factors such as sanitation and public health services, population density and demographics, land use changes, and travel patterns. The importance of climate relative to these other variables must be evaluated in the context of each situation.

Interpreting Observational and Modeling Studies

Numerous studies have shown an association between climatic variations and disease incidence, but such studies cannot fully account for the complex web of causation that underlies disease dynamics and thus may not be reliable indicators of future changes. Likewise, a variety of models have been developed to simulate the effects of climatic changes on incidence of diseases such as malaria, dengue, and cholera. These models are useful heuristic tools for testing hypotheses and carrying out sensitivity analyses, but they are not necessarily intended to serve as predictive tools and often do not take into account processes such as physical or biological feedback and human adaptation. Therefore, caution must be exercised in using these models to create scenarios of future disease incidence and to provide a basis for early warnings and policy decisions.

Potential Disease Impact of Global Climate Change

Changes in regional climate patterns caused by long-term global warming could affect the potential geographic range of many infectious diseases. However, if the climate of some regions becomes more suitable for transmission of disease agents, human behavioral adaptations and public health interventions could mitigate many adverse impacts. Basic public health protections such as adequate housing and sanitation, as well as new vaccines and drugs, may limit the future distribution and impact of some infectious diseases regardless of climate-associated changes. These protections, however, depend on maintaining strong public health programs and assuring vaccine and drug access in developing countries.

Climate Change and the Evolution and Emergence of Infectious Diseases

Another important but highly uncertain risk of climate change is its potential impact on the evolution and emergence of infectious disease agents. Ecosystem instabilities brought about by climate change and concurrent stresses such as land use changes, species dislocation, and increasing global travel could potentially influence the genetics of pathogenic microbes through mutation and horizontal gene transfer, giving rise to new interactions among hosts and disease agents. Such changes may foster the emergence of new infectious disease threats.

Extrapolating Climate and Disease Relationships

The relationships between climate and infectious disease are often highly dependent on local-scale parameters, and these relationships cannot always be extrapolated meaningfully to broader spatial scales. Likewise, the impact of seasonal to interannual climate variability on disease may not always provide a useful analog for the impact of long-term climate change. Ecologic responses on the timescale of an El Niño event, for example, may differ substantially from the ecologic responses and social adaptations expected under long-term climate change. In addition, long-term changes may influence regional climate

[1]Donald Burke (Chair), Ann Carmichael, Dana Focks, Darrell Jay Grimes, John Harte, Subhash Lele, Pim Martens, Jonathan Mayer, Linda Mearns, Roger Pulwarty, Leslie Real, Joan Rose, Chet Ropelewski, Robert Shope, Joanne Simpson, Mark Wilson, and Laurie Geller (study director)

variability patterns, hence limiting the predictive power of current observations.

Improved Modeling of Infectious Disease Epidemiology

Rapid advances in several disparate scientific disciplines may spawn radically new techniques for modeling infectious disease epidemiology. These innovations include satellite-based remote sensing of ecologic conditions, geographic information system (GIS) analytic techniques, inexpensive computational power, and molecular techniques to track the geographic distribution and transport of specific pathogens. Such technologies will make it possible to analyze the evolution and distribution of microbes and their relationship to different ecologic niches and may dramatically improve our ability to quantify the disease impacts of climatic and ecologic changes.

Early Warning Systems for Infectious Disease

Complementing "Surveillance and Response" with "Prediction and Prevention"

Current strategies for controlling infectious disease epidemics depend largely on surveillance for new outbreaks, followed by a rapid response to control the epidemic. In some contexts, however, climate forecasts and environmental observations could potentially be used to identify areas at high risk for disease outbreaks and thus aid efforts to prevent epidemics from occurring. Operational early warning systems are not yet generally feasible, due to our limited understanding of most relationships between climate and disease and limited climate forecasting capabilities, but establishing this goal will help foster the needed analytic, observational, and computational developments.

Effectiveness of Early Warning Systems for Disease

If relatively simple, low-cost strategies are available for mitigating risk for epidemics, it may be feasible to establish early warning systems based on a general understanding of associations between climate and disease. If the costs of mitigation actions are substantial, a precise and accurate prediction may be necessary, requiring more thorough mechanistic understanding of underlying relationships between climate and disease. In addition, the accuracy and value of climate forecasts will vary substantially, depending on the disease agent and the locale; and investment in sophisticated warning systems will be an effective use of resources only if a country has the capacity to take meaningful actions in response to such warnings and if the population is highly vulnerable to the hazards being forecast.

Components of an Effective Disease Early Warning System

Climate forecasts must be complemented by an appropriate set of indicators from ongoing meteorologic, ecologic, and epidemiologic surveillance systems. Together, this information could be used to issue a "watch" for regions at risk and subsequent "warnings" as surveillance data confirm earlier projections. Development of disease early warning systems should also include vulnerability and risk analysis, feasible response plans, and strategies for effective public communication.

Participants in Development of Early Warning Systems

The input of stakeholders such as public health officials and local policy makers is needed in the development of early warning systems to help ensure that useful forecast information is provided and that effective response measures are developed. The probabilistic nature of climate forecasts must be clearly explained to the communities using them, so that response plans can be developed with realistic expectations about the range of possible outcomes.

Recommendations for Future Research and Surveillance

Strengthen Research on Climate and Disease Linkages

Linkages between climate and infectious diseases are often poorly understood, and research to understand the causal relationships is in its infancy. Methodologically rigorous studies and analyses will likely improve our nascent understanding of these linkages and provide a stronger scientific foundation for predicting future changes. This research can best be accomplished through investigations that use a variety of analytic methods (including analysis of observational data, experimental manipulation studies, and computational modeling) and that examine the consistency of relationships between climate and disease in different societal contexts and across a variety of temporal and spatial scales.

Improve Disease Transmission Models

The most appropriate modeling tools for studying linkages between climate and disease depend on the scientific information available. For diseases in which there is limited understanding of the ecology and transmission biology but sufficient historical data on disease incidence and related factors, statistical-empirical models may be most useful. For diseases with insufficient surveillance data, first-principle mechanistic models that can integrate existing knowledge about linkages between climate and disease may have the most heuristic value. Models that have useful predictive value will likely need to incorporate elements of both approaches. Integrated assessment models can be especially useful for studying the relationships among the multiple variables that contribute to disease outbreaks, for looking at long-term trends, and for identifying gaps in our understanding.

Expand Epidemiologic Surveillance Programs

The lack of high-quality epidemiologic data for most diseases is a serious obstacle to improving our understanding of climate and disease linkages. These data are necessary to establish an empirical basis for assessing climate influences, establishing a baseline against which one can detect anomalous changes, and developing and validating models. A concerted effort should be made in the United States and internationally to collect long-term, spatially resolved disease surveillance data, along with the appropriate set of meteorologic and ecologic observations. Centralized, electronic databases should be developed to facilitate rapid, standardized reporting and sharing of epidemiologic data among researchers.

Coordinate Observational, Experimental, and Modeling Activities

Experimental and observational studies provide data necessary to develop and test models; and in turn, models can provide guidance on what types of data are most needed to further our understanding. The committee encourages the establishment of a climate and infectious disease research center dedicated to fostering meaningful interaction among the scientists involved in these different research activities through long-term collaborative studies, short-term information-sharing projects, and interdisciplinary training programs. The National Center for Ecological Analysis and Synthesis provides a useful model for the type of institution that would be most useful in this context.

Foster Interdisciplinary Research

Encouraging such efforts requires strengthening the infrastructure in universities and funding agencies for supporting interdisciplinary collaboration among climate modelers, meteorologists, ecologists, social scientists, and a wide array of medical and public health professionals, as well as developing educational programs in the medical and public health fields that explore environmental and socioeconomic factors underlying the incidence of infectious disease. A few programs have been established in recent years to foster the application of remote sensing and GIS technologies to epidemiologic investigations. The committee applauds these efforts and encourages all the relevant U.S. federal agencies to support interdisciplinary research programs on climate and infectious disease, along with an interagency working group to ensure effective coordination among programs. The U.S. Global Change Research Program could provide an appropriate forum for this type of coordinating body if the Centers for Disease Control and Prevention and the National Institute of Allergy and Infectious Disease were to become actively involved.

In closing, the committee emphasizes that there will always be an element of unpredictability in climate variations and in infectious disease outbreaks. Thus, a prudent strategy for all governments is to set a high priority on reducing overall vulnerability to infectious disease through strong public health programs.

The full report, Under the Weather: Climate, Ecosystems, and Infectious Disease, contains a detailed discussion of all the issues mentioned above. Copies of the report can be ordered from the National Academy Press, either through their website at http://www.nap.edu/catalog/10025.html or by calling 1-888-624-8373.

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Additional information is available at www.neglecteddiseases.org or by e-mailing access-nyc@newyork.msf.org or by calling 212-847-3153.

International Conference on Emerging Infectious Diseases, 2002

The National Center for Infectious Diseases, Centers for Disease Control and Prevention, has scheduled the third International Conference on Emerging Infectious Diseases (ICEID2002) for March 24 - 27, 2002, at the Hyatt Regency Hotel, Atlanta, Georgia, USA. More than 2,500 participants are expected, representing many nations and disciplines. They will discuss the latest information on many aspects of new and reemerging pathogens, such as West Nile virus and issues concerning bioterrorism.

More information about the conference will be posted soon at http://www.cdc.gov/ICEID/index.htm

Contact person is Charles Schable, cas1@cdc.gov

EMERGING INFECTIOUS DISEASES

In the next issue of
Emerging Infectious Diseases,
July-August 2001

West Nile virus

Crow Mortality as a Sentinel Surveillance System for West Nile Virus in the Northeast United States

Serologic Evidence for West Nile Virus Infection in Birds in the New York City Vicinity during an Outbreak in 1999

Dead Bird Surveillance as an Early Warning System for West Nile Virus

Equine West Nile Encephalitis in the United States

Widespread West Nile Virus Activity throughout the Eastern United States in 2000

West Nile Virus Surveillance in Connecticut in 2000: An Intense Epizootic without High Risk for Severe Human Disease

For a more complete list of articles included in the July–August issue, and for articles published online ahead of print publication, see
http://www.cdc.gov/ncidod/eid/upcoming.htm

The Cover

Georges Rouault, Les Trois Juges (The Three Judges), circa 1936

Georges Rouault (1871-1958), a French expressionist artist, started his career as an apprentice to a stained-glass maker, and after 1891, studied under Gustave Moreau. In 1905, he exhibited several paintings with the Fauves and Indépendants, two groups of artists not included in the official Salon of the French Royal Academy. Rouault received major recognition for his work in 1937, when his paintings were displayed in conjunction with the Paris World's Fair.

A devout Catholic, Rouault painted images of Christ, along with prostitutes, lawyers, judges, and clowns, as part of a commentary on the corruption of society. His sorrowful and bitter delineations of these human characters caused quite a stir in the Paris of his day. The suffering of Christ was his frequent subject. He believed in the teaching of the Gospel and stated that his only ambition was to paint a Christ so moving that those who saw the image would be converted. His thickly encrusted, powerfully colored images, outlined heavily in black, have the effect of icons and are suggestive of stained glass.

Around 1914, Rouault began more than a decade of work for the publisher Vollard. Using a variety of graphic techniques, he executed a series of prints called Miserere et Guerre to appear with text by the poet André Suarès. Rouault continued to work on the series through World War I and again from 1922 until 1927. He continued to paint the themes he had used earlier, but in a more tranquil style.

In much of his late work, Rouault addressed the theme of human suffering. In his paintings of judges he aimed to convey contempt for the system of human justice, in particular the presumption of the right of some to judge others, often less fortunate. The heavy, unsmiling features of the judges in The Three Judges underscore what Rouault saw as the dubious morality and potential cruelty of the judicial system, symbolized here by the judges' red ceremonial hats and robes. The artist once spoke of the anguish he felt at the sight of humans passing judgment on their fellow humans.

Examples of Rouault's work can be found in many European and American collections.

Abstracted from The Columbia Encyclopedia, Sixth Edition, 2001; http://www.marquette, edu/haggerty/exhibitions/past/rouault.html; and http://www.tate.org.uk/servlet/AText?id=12584&type=caption

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 404-371-5329 (tel), 404-371-5449 (fax), or eideditor@cdc.gov (e-mail).

Emerging Infectious Diseases is published in English and features the following types of articles: Perspectives, Synopses, Research Studies, Policy Reviews, 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.

Chinese, French, and Spanish 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 Intern Med 1997:126[1]36-47) (http://www.acponline.org/journals/annals/01jan97/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/mesh/meshhome.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 native format or convert to .TIF (Tagged Image File), 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.

Access the journal's style guide at http://www.cdc.gov/ncidod/EID/style_guide.htm

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 D 61, Atlanta, GA 30333, USA; e-mail eideditor@cdc.gov

Types of Articles

Perspectives, Synopses, Research Studies, and Policy Reviews: Articles 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. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch.

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. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

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. 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.

Research Studies: These articles report laboratory and epidemiologic results within a public health perspective. Although these reports may be written in the style of traditional research articles, they should explain the value of the research in public health terms and place the findings in a larger perspective (e.g., "Here is what we found, and here is what the findings mean").

Policy Reviews: Articles in this section report public health policies that are based on research and analysis of emerging disease issues.

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) 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.

Another Dimension: Thoughtful esays on philosophical issues related to science and human health.

Book Reviews: Short reviews (250 to 500 words) of recently published books on emerging disease issues are welcome.

Letters: This section includes letters that give preliminary data or comment on published articles. Letters (500 to 1,000 words) should not be divided into sections, nor should they contain figures or tables. References (not more than 10) may be included.

News and Notes: We welcome brief announcements (50 to 150 words) of timely events of interest to our readers. (Announcements can be posted on the journal web page only, depending on the event date.) In this section, we also include summaries (500 to 1,500 words) of conferences focusing on emerging infectious diseases. Summaries may provide references to a full report of conference activities and should focus on the meeting's content.