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# Emerging Infectious Diseases




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## Emerging Infectious Diseases

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# Editorial Policy and Call for Articles

The goals of *Emerging Infectious Diseases* (EID) are to promote the recognition of new and reemerging infectious diseases and to improve the understanding of factors involved in disease emergence, prevention, and elimination. EID 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 whose study elucidates the factors influencing the emergence of infectious diseases.

EID will be published in English and will feature three types of articles: *Perspectives*, *Synopses*, and *Dispatches*. The purpose and requirements of each type of article are described in detail below.

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# Instructions to Authors

***Editorial Material:*** Manuscripts should be prepared according to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (JAMA 1993:269[17]: 2282-6).

Begin each of the following sections on a new page and in this order: title page, abstract, text, acknowledgments, references, each table, figure legends, and figures. On the title page, give complete information about each author (full names and highest degree). Give current mailing address for correspondence (include fax number and e-mail address). Follow Uniform Requirements style for references. Consult *List of Journals Indexed in Index Medicus* for accepted journal abbreviations. Tables and figures should be numbered separately (each beginning with 1) in the order of mention in the text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Italicize scientific names of organisms from species name all the way up, except for vernacular names (viruses that have not really been speciated, such as coxsackievirus and hepatitis B; bacterial organisms, such as pseudomonads, salmonellae, and brucellae).

***Perspectives:*** Contributions to the Perspectives section should address factors known to influence the emergence of infectious diseases, including microbial adaption and change; human demographics and behavior; technology and industry; economic development and land use; international travel and commerce; and the breakdown of public health measures. Articles should be approximately 3,500 words and should include references, not to exceed 40. The section should begin with an introduction outlining the relationship of the issues discussed in the paper to the emergence of infectious diseases. Use of additional subheadings in the main body of the text is recommended. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text. Photographs and illustrations are optional. Provide a short abstract (150 words) and a brief biographical sketch.

***Synopses*:** Submit concise reviews of infectious diseases or closely related topics. Preference will be given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. Synopses should be approximately 3,500 words and should include references, not to exceed 40. The section should begin with an introduction outlining the relationship of the issues discussed in the paper to the emergence of infectious diseases. Use of additional subheadings in the main body of the text is recommended. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text. Photographs and illustrations are optional. Provide a short abstract (150 words) and a brief biographical sketch.

***Dispatches:*** Provide brief updates on trends in infectious diseases or infectious disease research. Dispatches (1,000 to 1,500 words of text) should not be divided into sections. Dispatches should begin with a brief introductory statement about the relationship of the topic to the emergence of infectious diseases. Provide references, not to exceed 10; and figures or illustrations, not to exceed two.

All articles will be reviewed by independent reviewers. The Editor reserves the right to edit articles for clarity and to modify the format to fit the publication style of *Emerging Infectious Diseases*.

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# Travel and the Emergence of Infectious Diseases

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*Travel is a potent force in the emergence of disease. Migration of humans has been the pathway for disseminating infectious diseases throughout recorded history and will continue to shape the emergence, frequency, and spread of infections in geographic areas and populations. The current volume, speed, and reach of travel are unprecedented. The consequences of travel extend beyond the traveler to the population visited and the ecosystem. When they travel, humans carry their genetic makeup, immunologic sequelae of past infections, cultural preferences, customs, and behavioral patterns. Microbes, animals, and other biologic life also accompany them. Today's massive movement of humans and materials sets the stage for mixing diverse genetic pools at rates and in combinations previously unknown. Concomitant changes in the environment, climate, technology, land use, human behavior, and demographics converge to favor the emergence of infectious diseases caused by a broad range of organisms in humans, as well as in plants and animals.*

Many factors contribute to the emergence of infectious diseases. Those frequently identified include microbial adaptation and change, human demographics and behavior, environmental changes, technology and economic development, breakdown in public health measures and surveillance, and international travel and commerce (1-4). This paper will examine the pivotal role of global travel and movement of biologic life in the emergence of infectious diseases. It will also examine the ways in which travel and movement are inextricably tied at multiple levels to other processes that influence the emergence of disease.

Travel is a potent force in disease emergence and spread (5). The current volume, speed, and reach of travel are unprecedented. The consequences of migration extend beyond the traveler to the population visited and the ecosystem (6). Travel and trade set the stage for mixing diverse genetic pools at rates and in combinations previously unknown. Massive movement and other concomitant changes in social, political, climatic, environmental, and technologic factors converge to favor the emergence of infectious diseases.

Disease emergence is complex. Often several events must occur simultaneously or sequentially for a disease to emerge or reemerge (Table 1) (6). Travel allows a potentially pathogenic microbe to be introduced into a new geographic area; however, to be established and cause disease a microbe must survive, proliferate, and find a way to enter a susceptible host. Any analysis of emergence must look at a dynamic process, a sequence of events, a milieu, or ecosystem.

Movement, changing patterns of resistance and vulnerability, and the emergence of infectious diseases also affect plants, animals, and insect vectors.

**Table 1. Basic concepts in disease emergence***

| |
|---|
| Emergence of infectious diseases is complex. |
| Infectious diseases are dynamic. |
| Most new infections are not caused by genuinely new pathogens. |
| Agents involved in new and reemergent infections cross taxonomic lines to include viruses, bacteria, fungi, protozoa, and helminths. |
| The concept of the microbe as *the* cause of disease is inadequate and incomplete. |
| Human activities are the most potent factors driving disease emergence. |
| Social, economic, political, climatic, technologic, and environmental factors shape disease patterns and influence emergence. |
| Understanding and responding to disease emergence require a global perspective, conceptually and geographically. |
| The current global situation favors disease emergence. |

*Adapted from Wilson ME (6).

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Analysis of these species can hold important lessons about the dynamics of human disease.

To assess the impact of travel on disease emergence, it is necessary to consider the receptivity of a geographic area and its population to microbial introduction. Most introductions do not lead to disease. Organisms that survive primarily or entirely in the human host and are spread through sexual contact, droplet nuclei, and close physical contact can be readily carried to any part of the world. For example, AIDS, tuberculosis, measles, pertussis, diphtheria, and hepatitis B are easily carried by travelers and can spread in a new geographic area; however, populations protected by vaccines resist introduction. Organisms that have animal hosts, environmental limitations, arthropod vectors, or complicated life cycles become successively more difficult to "transplant" to another geographic area or population. Epidemics of dengue fever and yellow fever cannot appear in a geographic area unless competent mosquito vectors are present. Schistosomiasis cannot spread in an environment unless a suitable snail intermediate host exists in that region. Organisms that survive only under carefully tuned local conditions are less likely to be successfully introduced. Even if an introduced parasite persists in a new geographic area, it does not necessarily cause human disease. In the United States, humans infected with *Taenia solium*, the parasite that causes cysticercosis, infrequently transmit the infection because sanitary disposal of feces, the source of the eggs, is generally available. In short, the likelihood of transmission involves many biological, social, and environmental variables.

## Historical Perspective

Human migration has been the main source of epidemics throughout recorded history. William McNeill (7), in his book *Plagues and Peoples*, describes the central role of infectious disease in the history of the world. Patterns of disease circulation have influenced the outcome of wars and have shaped the location, nature, and development of human societies.

Trade caravans, religious pilgrimages, and military maneuvers facilitated the spread of many diseases, including plague and smallpox. A map in Donald Hopkins' book, *Princes and Peasants: Smallpox in History* (8), traces the presumed spread of smallpox from Egypt or India, where it was first thought to have become adapted to humans sometime before 1000 B.C. Smallpox spread easily from person to person through close contact with respiratory discharges and, less commonly, through contact with skin lesions, linens, clothing, and other material in direct contact with the patient. Because patients remained infectious for about 3 weeks, many opportunities for transmission were available. Even in this century, until the 1970s, smallpox continued to cause epidemics. A pilgrim returning from Mecca was the source of a large outbreak in Yugoslavia in the early 1970s that resulted in 174 Yugoslav cases and 35 deaths (9). The pilgrim apparently contracted the infection in Baghdad while visiting a religious site. Because his symptoms were mild, he was never confined to bed and was able to continue his travels and return home.

For most of history, human populations were relatively isolated. Only in recent centuries has there been extensive contact between the flora and fauna of the Old and New Worlds. Schoolchildren hear the rhyme "Columbus sailed the ocean blue, in fourteen hundred ninety-two," but may learn little about the disaster brought upon the native populations of the Americas by the arriving explorers. By the end of the fifteenth century, measles, influenza, mumps, smallpox, tuberculosis, and other infections had become common in Europe. Explorers from the crowded urban centers of Europe brought infectious diseases to the New World (10), where isolated populations had evolved from a relatively small gene pool and had no previous experience with many infections (11). The first epidemics following the arrival of Europeans were often the most severe. By 1518 or 1519, smallpox appeared in Santo Domingo, where it killed one-third to half of the local population and spread to other areas of the Caribbean and the Americas (10). The population of central Mexico is estimated to have dropped by one-third in the single decade following contact with the Europeans.

Travel across the Atlantic Ocean transformed the flora and fauna of the New World as well. Some of the transported materials became important sources of food (plants), clothing, and transportation (animals). Other transfers were less welcome: Japanese beetles, Dutch elm disease, and chestnut tree fungus. A.W. Crosby, exploring these exchanges between the Old and the New Worlds, sounds a pessimistic note: "The Columbian exchange has left us with not a richer but a more impoverished genetic pool" (10).

The explorers also paid a price in loss of lives from disease. Philip Curtin (12) provides a quantitative study of "relocation costs," the excess illness and death among European soldiers in the nineteenth century when they lived or worked in the tropics. Until the most recent armed conflicts, infectious diseases claimed more lives than injuries during wars.

Plague holds a prominent place in history and remains with us today. A bacterial infection caused by *Yersinia pestis*, it is primarily an infection of rodents, spread by their fleas. Human infection is incidental to the maintenance of *Y. pestis* in animal

reservoirs. Yet plague periodically has erupted in human populations, wreaking great devastation, killing millions and causing infection that can be spread directly from person to person by the respiratory route. Human population movement has been essential in the spread of plague and the dispersal of rodents and their fleas to new areas. For centuries plague spread along trade routes. It reached California by boat around the turn of this century, caused epidemic infection in San Francisco, and then spread to wildlife, where it persists today in a large enzootic focus.

## Movement of People

Travel for business and pleasure constitutes a small fraction of total human movement (5,13). People migrating individually or in groups, may be immigrants, refugees, missionaries, merchant marines, students, temporary workers, pilgrims, or Peace Corps workers. Travel may involve short distances or the crossing of international borders. Its volume, however, is huge. In the early 1990s more than 500 million persons annually crossed international borders on commercial airplane flights (World Tourism Organization, Madrid, unpublished data). An estimated 70 million persons, mostly from developing countries, work either legally or illegally in other countries (14). Movement may be temporary or seasonal, as with nomadic populations and migrant workers who follow the crops. Military maneuvers worldwide employ and move huge populations. The consequences of armed conflict and political unrest displace millions. In the early 1990s, there were an estimated 20 million refugees and 30 million displaced persons worldwide (International Organization for Migration, personal communication).

Grubler and Nakicenovic estimated and plotted the average kilometers traveled daily for the French population over a 200-year period (1800-2000) and found that spatial mobility has increased more than 1000-fold (15). In the last 40 years, the size of Australia's population has doubled and the number of persons moving into and out of Australia has increased nearly 100-fold (16).

Although social, economic, and political factors push people from an area or draw them to another, environmental resources and their impact on food and water supplies are behind many conflicts leading to displacement of populations. Acute disasters, such as flooding, earthquakes, and hurricanes often force populations to seek shelter and sustenance in new lands. Chronic changes, such as drought, depletion of soil, and disappearance of fish from streams, lakes, and oceans, draw people to new territories, or, more frequently, to the fringes of large urban centers.

Another type of travel relevant to disease emergence is the shift of populations to urban areas. It is estimated that by the year 2010, 50% of the world's population will be living in urban areas. It is projected that by the year 2000, the world will comprise 24 "megacities"—sprawling metropolitan areas with populations exceeding 10 million (World Bank, UNDP, World Health Organization, unpublished data). These areas will have the population density to support persistence of some infections and contribute to the emergence of others. Many of these areas are located in tropical or subtropical regions, where the environment can support a diverse array of pathogens and vectors. Also developing are huge periurban slums, populated with persons from many geographic origins. Poor sanitation allows breeding of arthropod vectors, rodents, and other disease-carrying animals. Crowded conditions favor the spread of diseases that pass from person to person, including sexually transmitted infections. Travel between periurban slum areas and rural areas is common, paving the route for the transfer of microbes and disease. Transfer of resistance genes and genetic recombination may also occur in and spread from crowded environments of transients.

Acute disturbances, whether climatic or political, lead to interim living arrangements, such as refugee camps and temporary shelters, that provide ideal conditions for the emergence and spread of infections. Temporary living quarters often share similarities with periurban slums: crowding, inadequate sanitation, limited access to medical care, lack of clean water and food, dislocation, multiethnic composition, and inadequate barriers from vectors and animals. An example is the movement of 500,000-800,000 Rwandan refugees into Zaire in 1994. Almost 50,000 refugees died during the first month as epidemics of cholera and *Shigella dysenteriae* type 1 swept through the refugee camps (17).

Movement into a rural environment poses different risks and often places new rural populations in contact with pathogens that are in the soil and water or are carried by animals or arthropods (18). Some of these pathogens such as Guanarito (19) and Sabià viruses (20) in South America, were only recently recognized as capable of infecting humans.

## Consequences of Movement

Human migration favors the emergence of infectious diseases through many mechanisms. When people migrate, they carry their genetic makeup, their accumulated immunologic experience, and much more (Table 2). They may carry pathogens in or on their bodies and may also transport disease vectors, such as lice. Their technology (agricultural and industrial), methods for treating disease, cultural traditions, and behavioral patterns may influ-

ence their risk for infection in a new environment and their capacity to introduce disease into the new region. Their social standing and resources may affect their exposure to local infections and their access to adequate nutrition and treatment. People also change the environment in many ways when they travel or migrate—they plant, clear land, build, and consume. Travel is relevant in the emergence of disease if it changes an ecosystem. The following examples show the many ways in which migration can influence the emergence of disease in a new area.

1. Humans may carry a pathogen in a form that can be transmitted, then or later, directly or indirectly to another person. The pathogen may be silent (during the incubation period, chronic carriage, or latent infection) or clinically evident. Examples include hepatitis B virus, human immunodeficiency virus (HIV), *Mycobacterium tuberculosis*, *M. leprae*, *Salmonella typhi*, and other salmonella. Disease may be especially severe when a pathogen is introduced into a population that has no previous exposure to the infection. How long the consequences of migration persist varies with the specific infection. The two most critical characteristics are the duration of survival of the pathogen in a potentially infective form and its means of transmission.

2. Epidemic cholera in Africa spread along the West African coast and, when the disease moved inland, followed fishing and trading routes. Markets, funerals, refugee camps—events that involved migration of persons and large gatherings with close contact—helped spread the infection. With El Tor cholera, asymptomatic and mild infections can outnumber severe disease by 100 to 1 (21), thus permitting those infected to continue to move and work.

3. Pilgrims carried an epidemic strain of group A *Neisseria meningitidis* from southern Asia to Mecca in 1987. Other pilgrims who became colonized with the epidemic strain introduced it into sub-Saharan Africa, where it caused a wave of epidemics in 1988 and 1989 (22).

4. Humans may carry a pathogen that can be transmitted only if conditions are permissive. This permissiveness can pertain to human behavior, the environment, or the presence of appropriate vectors or intermediate hosts. For example, the ease with which HIV spreads in a population depends on sexual practices, condom use, the number of sex partners, and intravenous drug use, among other factors. Malaria requires specific mosquito vectors (with access to susceptible humans) to spread to new geographic regions. Schistosomiasis can be introduced into a new region only if the appropriate snail host is present and if the eggs excreted (in urine or feces, from an infected person), reach the snails in an appropriate environment.

**Table 2. What is carried by humans into new regions?**

| |
|---|
| Pathogens in or on body |
| Microbiologic flora |
| Vectors on body |
| Immunologic sequelae of past infections |
| Vulnerability to infections |
| Genetic makeup |
| Cultural preferences, customs, behavioral patterns, technology |
| Luggage and whatever it contains |

5. Humans may carry a strain of microbe that has an unusual resistance pattern or virulence genes. A multiple-drug-resistant strain of *Klebsiella pneumoniae* appears to have been transferred by an asymptomatic woman from a hospital in Bahrain to Oxford, where it caused outbreaks in two British hospitals (23). People also carry their background flora, in the intestinal tract, for example, which may contain plasmids and resistance genes that can interact with microbes in a new area. It is not just the classic pathogens that may be relevant to the emergence of a new disease but the individual traveler's total microbiologic "baggage."

6. Visitors to a region may lack immunity to locally endemic infections, such as hepatitis A and sand-fly fever. Visitors may suffer severe or different manifestations of infection or disease at an age when the local population is immune to it. Resettlement of populations into malaria-endemic regions can lead to a high death rate from falciparum malaria.

7. Kala-azar caused a deadly outbreak in remote villages in southern Sudan in 1994. The origin was thought to be the villagers' exposure to the sand-fly vector during migration to a food distribution center that had been established by a relief organization (24). The migration took a malnourished population from a nonendemic zone into the southern part of the kala-azar–endemic zone. Unfamiliarity with the disease and the poor nutritional status of the population probably contributed to a high death rate (24).

8. Behavioral patterns in a new region may place visitors at risk for infection, while the local population, possibly because of their knowledge of disease risks, may not be at risk. Behavior patterns may involve food preparation (such as eating some foods raw), clothing (or lack of it), (for example, going barefooted), sleeping arrangements (sleeping on the ground or out of doors in an unscreened area), and contact with animals.

9. Susceptibility of a population may vary because of genetic differences. A microbe introduced into a new region may have a greater or lesser impact, depending on the host population. Genetic factors influence susceptibility to and expression of several infectious diseases. Although these interac-

tions are not yet well defined for most infections, genetic factors influence infections caused by different classes of organisms, including cholera (25,26), parvovirus infection (27), malaria, and *Helicobacter pylori* infection (28).

To determine the consequences of travel both the traveler and the population visited must be considered. Migration may be in only one direction, though travel often involves returning to the point of origin, perhaps after the traveler has made many stops along the way. The changes in the various ecosystems as a consequence of the migration guide the emergence of diseases; any study that simply focuses on the traveler is too narrow.

The distance traversed is less important than the differences in biological life in different areas and differences in receptivity and vulnerability. In thinking about disease emergence, what matters is the potential of a disease to appear in a place, population, or extent not previously reported.

What is the long-term impact of migration and travel on human disease? Carriage of pathogens is only part of the influence on disease emergence. Introduced technology, farming methods, treatment and drugs, chemicals, and pesticides may have a far greater and longer impact on disease patterns in a region than the life of a person. Deforestation, building of dams, and opening of roads into previously inaccessible areas have all been associated with population movements and changes in distribution and frequency of a variety of infections in humans (such as malaria, schistosomiasis, Rift Valley fever, and sexually transmitted diseases).

Increasingly the vehicle of transportation is the site or even the source of outbreaks. During travel, people from diverse origins are enclosed in close proximity for a hours or days and then discharged to move on to many distant places. These temporary new habitats, jumbo jets or huge ocean liners, can be the sites for dissemination of the microbes (as happens, for example with *Legionella pneumophila* infections (29), foodborne infections, and cholera) or provide a milieu for person-to-person transmission (influenza, tuberculosis (30,31)).

## Shipping and Commerce

The biomass of humans constitutes only a fraction of the matter moved about the earth. Humans carry and send a huge volume of plants, animals and other materials all over the face of the globe. Much of this movement results from the planned transport of goods from one place to another, but some is an unintended consequence of shipping and travel. All has an impact on the juxtaposition of various species in different ecosystems. “Hitchhikers” include all manner of biologic life, both microscopic and macroscopic. Animals can carry potential human pathogens and vectors. The globalization of markets brings fresh fruits and vegetables to dinner tables thousands of miles from where they were grown, fertilized, and picked. Tunnels, bridges, and ferries form means to traverse natural barriers to species spread. The roads built to transport people often speed the movement of diseases from one area to another. Mass processing and wide distribution networks allow for the amplification and wide dissemination of potential human microbes.

Examples of introduced species include plants and animals—insects, microbes, and marine organisms.

1. Ships convey marine organisms on their hulls and in their ballast water. For example, 367 different species were identified in ballast water of ships traveling between Japan and Coos Bay, Oregon (32). Introductions have had devastating effects in some areas, for example such as the Black and Azov seas, where newly introduced jellyfishlike creatures called ctenophores have ruined local fishing (33).

2. *Vibrio cholerae* may have been introduced to South America by shipping (34). Researchers isolated the organism in samples of ballast, bilge, and sewage from 3 of 14 cargo ships docked at Gulf of Mexico ports. The ships had last ports of call in Brazil, Colombia, and Chile (35). *V. cholerae* O1, serotype Inaba, biotype El Tor, indistinguishable from the Latin American epidemic strain, was found in oysters and oyster-eating fish from closed oyster beds in Mobile Bay, Alabama (36). *V. cholerae* O139 has spread along waterways in Asia, although the people carried on the boats doubtless played a role (37,38).

3. *Aedes albopictus* was introduced into the United States inside used tires shipped from Asia (39,40). The mosquito's introduction causes concern because it is an aggressive biter, survives in both forest and suburban habitats, and appears to be a competent vector for several human pathogens. It has been associated with epidemic dengue fever transmission in Asia and is a competent laboratory vector of La Crosse, yellow fever, and other viruses (41). In Florida, 14 strains of eastern equine encephalitis virus have been isolated from *A. albopictus* (42). The mosquito is now established in at least 21 of the contiguous states in United States and in Hawaii.

4. The African anopheles mosquitoes arrived in Brazil in about 1929. This vector could breed under conditions other New World mosquitoes could not. Although the malaria parasite was already found in Brazil, this new vector expanded the range of transmission. An estimated 20,000 persons died of malaria before the introduced anopheles mosquitoes were eliminated.

5. It has been repeatedly demonstrated that mosquitoes are present—and survive—on international

flights. In random searches of airplanes in London, mosquitoes were found on 12 of 67 airplanes from tropical countries (43). Arthropods can survive even more extreme environments. In one study, mosquitoes, house flies, and beetles placed in wheel bays of Boeing 747B aircraft survived flights of 6-9 hours with external temperatures of -42° C (43). Airplanes have also carried infective mosquitoes that caused human infection outside malaria-endemic areas (in Europe, for example).

6. Vehicles can transport vectors over land. *Glossina palpalis*, a vector for African trypanosomiasis (sleeping sickness), can fly up to 21 km but can be transported much longer distances on animals and in land vehicles.

7. Seven persons in Marburg, Germany, died after handling blood and tissues from African green monkeys from Uganda. The tissues contained an organism later named Marburg virus (44).

8. Exotic animals transported from their usual habitats are clustered in zoos; others are used in research laboratories where they have occasionally caused severe disease in humans. Two examples are B virus from primates (45) and hemorrhagic fever with renal syndrome from rodents (46).

9. The world trade and globalization of organs, tissues, blood, and blood products is growing. Researchers are considering animals as sources for tissues and organs for transplantation (47).

10. Plants may not directly cause human disease. But they can alter an ecosystem and facilitate the breeding of a vector for human disease. This can also displace traditional crops that provide essential nutrition. Vertical transmission of plant pathogens (and spread of plant diseases) can result from seed movement (48). Carriage of seeds into new areas can introduce plant pathogens.

11. Migration and altered environments have increased the so-called weedy species. These species migrate easily and have high rates of reproduction. If they lack local predators, they can displace other species and often upset local ecology.

## Introduction of Species into New Areas

Introducing species into new geographic areas is not new, but the current volume and frequency of introductions are unprecedented. A pathogen's survival and spread in a new environment are determined by its basic reproductive rate, which is the average number of successful offspring a parasite can produce (49). To invade and establish itself in a host population, a parasitic species must have a basic reproductive rate exceeding one (49). The simplicity of this statement belies the complexity of circumstances that influence invasion and persistence. These circumstances encompass biological, social, and environmental factors.

As noted already, factors that can influence receptivity include climate and environmental conditions, sanitation, socioeconomic conditions (50), behavior, nutrition, and genetics. *V. cholerae* persists in an aquatic reservoir off the Gulf Coast of the United States, yet epidemic cholera has not been a problem in the United States. Where poverty and poor sanitation prevail, the presence of *V. cholerae* can be a source of endemic disease and periodic epidemics.

Disease emergence is often complex. An outbreak of malaria in San Diego, California, occurred when parasitemic migrant workers were employed in an area where mosquitoes capable of transmitting malaria had access to the workers and to a susceptible human population (51). Many conditions had to be met to allow transmission.

Migration may introduce parasites into an area where a different intermediate host or vector could change the incidence of disease. Cycling through a different host can lead to different transmission rates, different infectivity, and even different clinical expression. A parasite may be more successful in a new site because of a larger susceptible population or the absence of predators.

## Confluence of Events

Massive global travel is taking place simultaneously with many other processes that favor the emergence of disease. For example, the human population is more vulnerable because of aging, immunosuppression from medical treatment and disease (such as AIDS), the presence of prostheses (e.g., artificial heart valves and joints), exposure to chemicals and environmental pollutants that may act synergistically with microbes to increase the risk of diseases, increased poverty, crowding and stress, and increased exposure to UV radiation. Technologic changes, while providing many benefits, can also promote disease dissemination. Resistance of microbes and insects to antimicrobial drugs and pesticides interferes with the control of infections and allows transmission to continue. Changes in land use can alter the presence and abundance of vectors and intermediate hosts.

Microbes are enormously resilient and adaptable. They have short life spans, which allow rapid genetic change. Humans, by comparison, are slow to change genetically but can change their behavior. People move and construct barriers to prevent contact with microparasites, macroparasites, and the extremes of the environment. Technology fosters a perception of human invincibility but actually creates new vulnerabilities, as it enables us to go deeper, higher, and into more remote and hostile environments. Studies show that no place on earth is devoid of microbes. Their range and resiliency are truly phenomenal. Only a fraction of the existing microbes have been

characterized. Travel and exploration provide a greater opportunity for humans to come into unsampled regions with these uncharacterized microbes.

## Summary and Conclusions

Global travel and the evolution of microbes will continue. New infections will continue to emerge, and known infections will change in distribution, severity and frequency. Travel will continue to be a potent factor in disease emergence. The current world circumstances juxtapose people, parasites, plants, animals, and chemicals in a way that precludes timely adaptation. The combination of movement at many levels and profound change in the physical environment can lead to unanticipated diseases spread by multiple channels. In many instances, the use of containment or quarantine is not feasible. Research and surveillance can map the global movement and evolution of microbes and guide interventions. Integration of knowledge and skills from many disciplines—the social, biological, and physical sciences—is needed. The focus should be system analysis and the ecosystem rather than a disease, microbe, or host.

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# *Escherichia coli* Serotype O157:H7: Novel Vehicles of Infection and Emergence of Phenotypic Variants

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Escherichia coli *serotype O157:H7 was only recognized as a human pathogen a little more than a decade ago, yet it has become a major foodborne pathogen. In the United States, the severity of serotype O157:H7 infections in the young and the elderly has had a tremendous impact on human health, the food industry, and federal regulations regarding food safety. The implication of acidic foods as vehicles of infection has dispelled the concept that low-pH foods are safe. Further, the association of nonbovine products with outbreaks suggests that other vehicles of transmission may exist for this pathogen. In laboratory diagnosis, most microbiologic assays rely on a single phenotype to selectively isolate this pathogen. However, the increasing evidence that phenotypic variations exist among isolates in this serogroup may eventually necessitate modifications in assay procedures to detect them.*

Enterohemorrhagic *Escherichia coli* (EHEC) has emerged in recent years as the predominant cause of hemorrhagic colitis in humans. This illness, with characteristic symptoms of bloody diarrhea and abdominal cramps, can progress into a more severe, life-threatening complication known as hemolytic uremic syndrome (HUS). The pathogenicity of EHEC appears to be associated with a number of virulence factors, including the production of several cytotoxins (1,2). These toxins are collectively referred to as verotoxins or Shiga-like toxins (SLTs) because the SLT-I of *E. coli* closely resembles the Shiga toxin of *Shigella dysenteriae* type 1 (2). Although more than 60 *E. coli* serotypes produce SLTs (2) and more are being identified as capable of producing SLT, serotype O157:H7 is the predominant pathogen in the EHEC group and the one associated most frequently with human infections worldwide.

Isolates of the serotype O157:H7 were first implicated in foodborne illness in 1982; in the subsequent 10 years, approximately 30 outbreaks were recorded in the United States (1). In early 1993, however, serotype O157:H7 received considerable attention after a large foodborne disease outbreak, traced to the consumption of undercooked, contaminated hamburgers served at a regional fast-food restaurant (3). More than 700 persons in four states were infected; there were 51 cases of HUS and four deaths. Since that outbreak, the reported incidence of serotype O157:H7 infections has risen, partly because better surveillance systems have been implemented and awareness has increased amongphysicians, clinical microbiologists, and consumers. Fifteen additional outbreaks were recorded in 1993 and 20 in the first half of 1994.

Because serotype O157:H7 has only recently been recognized as a foodborne pathogen, our knowledge is limited. However, the notoriety of recent outbreaks and the severity of serotype O157:H7 infections have stimulated research on the organism, its ecology, antibiotic resistance properties, and virulence factors. Much has already been learned from the epidemiologic investigations of past outbreaks. For instance, foodborne infections of serotype O157:H7 have most often been associated with the consumption of bovine products; however, several recent outbreaks have implicated other less likely vehicles of infection and showed that the organism may have some unsuspected characteristics. Although genotypic studies show serotype O157:H7 to be a unique clone only distantly related to other *E. coli* serotypes (4,5), phenotypic diversity within the serogroup (6) may complicate existing laboratory diagnosis procedures. The introduction of various molecular diagnostic techniques may facilitate the detection of this serotype and its phenotypic variants.

This review examines unexpected and seemingly unlikely vehicles implicated in recent serotype O157:H7 outbreaks and the impact of emerging phenotypic variants and their effect on diagnostic assays used to detect this pathogen in clinical specimens or in the food supply.

## Novel Vehicles of Transmission

So far, serotype O157:H7 has caused a total of 60 outbreaks of foodborne illness in the United States. Consumption of contaminated, undercooked

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ground-beef products has accounted for most outbreaks; however, raw milk was also implicated in several outbreaks in the United States and Canada. Improper hygiene with secondary spread from person-to-person contact is another well-documented route of infection (1,2). In the last few years, however, several foodborne outbreaks of serotype O157:H7 have implicated unique and seemingly unlikely vehicles of infection: among them are acidic foods, fruits, salad vegetables, yogurt, and water.

## Acidic foods

In the *Retail Food Store Sanitation Code* of the U.S. Food and Drug Administration, foods with a pH value of less than 4.6 are generally regarded as low risk in terms of food safety. However, several recent disease outbreaks attributable to serotype O157:H7 have shown that this pathogen can persist in foods with low pH.

In the fall of 1991, an outbreak of serotype O157:H7 that affected 23 persons was traced to the consumption of fresh-pressed apple cider (7). The implicated cider, made from unwashed "dropped" apples at a farm, had a pH value of 3.7 to 3.9, was not pasteurized, and contained no preservatives. Although apple cider had been implicated in a previous outbreak of *Salmonella typhimurium*, it is not a common vehicle of enteric infection because of its high acidity. Several laboratory studies have subsequently demonstrated that isolates of serotype O157:H7 can tolerate acidic conditions. Some strains persist in media with pH values as low as 2.0 (8), and in cold (8°C) apple cider for 10 to 31 days (7,9). Although the source of serotype O157:H7 in the cider that caused illness was never fully established, it was suspected that the dropped apples had been contaminated by cow manure.

The ability of serotype O157:H7 to tolerate acidity was substantiated in 1993, when another acidic food was implicated in a series of restaurant outbreaks that infected at least 48 persons. Although the source of the outbreaks was not conclusively identified, epidemiologic investigations and other data implicated mayonnaise or mayonnaise-based dressing and sauces. Samples of mayonnaise had a pH of 3.6 to 3.9, and the sauces prepared from it were also acidic, with pH levels of 3.6 to 4.4 (10). After this outbreak, several studies confirmed that although isolates of serotype O157:H7 do not multiply under these conditions, they can persist in commercial mayonnaise up to 55 days at 5°C (10,11). How the mayonnaise became contaminated with serotype O157:H7 was not determined; however, improper handling of bulk mayonnaise or cross-contamination with meat juices or meat products was suspected.

## Water

Several recent incidents show that both drinking water and recreational water can serve as vehicles for transmitting serotype O157:H7 infections.

The first and largest waterborne outbreak associated with this pathogen occurred in Missouri in 1989 (12). Of the more than 240 people infected, 32 were hospitalized, and four died. The source of the outbreak was not identified, but backflow during a water main break might have contaminated the drinking water supply (12). Like most *E. coli*, serotype O157:H7 isolates are susceptible to the effects of chlorine. Hence, adjustments in the chlorination of the drinking water supply during repairs to the water main might have prevented the outbreak (12).

An outbreak caused by serotype O157:H7 and *S. sonnei* in 1991 may have involved recreational lake water in the vicinity of Portland, Oregon. Of the 59 people affected, 21 (all children) were infected by serotype O157:H7 (13). An epidemiologic survey showed that those who became ill had swum in the lake during the previous 3-week period. Transmission probably occurred when the swimmers swallowed lake water that was fecally contaminated by other bathers. The lengthy period during which people became infected suggests that these pathogens can remain viable in water for a long time, or that the water was repeatedly recontaminated. Fecal contamination of recreational water by bathers, especially small children, is not uncommon; however, the contaminants are usually diluted quickly by the large volume of water in recreational lakes, bays, or rivers. That swallowing a small amount of lake water can cause illness suggests that the pathogen has a low infectious dose (13). This fact is already well established for *Shigella* and seems to be consistent with recent epidemiologic data from foodborne outbreaks associated with serotype O157:H7.

A similar incident, implicating water from a children's paddling pool, was reported in Scotland in 1992 (14). Although epidemiologic evidence was not conclusive, the available data suggested that a child with diarrhea had played in the pool and fecally contaminated the water with serotype O157. Because the pool water was not changed or disinfected, it became the vehicle of infection for two other neighborhood children, who in turn infected others by person-to-person contact.

## Other vehicles of transmission

Recently, several other unique vehicles have been implicated in foodborne outbreaks associated with serotype O157:H7. A 1993 outbreak in an Oregon restaurant was apparently caused by the consumption of cantaloupe or other items from the salad bar, which were most likely cross-contaminated by meat products during preparation. One study showed that

serotype O157:H7 can survive and grow on salad vegetables stored at 12°C and 21°C for up to 14 days (15). An outbreak in the United Kingdom in 1991 was traced to the consumption of yogurt, which infected 16 persons, 11 of them children (16). Although consumption of raw milk has caused past outbreaks, serotype O157:H7 is susceptible to heat treatment and thus does not usually survive the pasteurization process. Even though the implicated yogurt was prepared from pasteurized milk, the milk might have become contaminated with serotype O157:H7 after pasteurization.

A puzzling incident was reported from northern Italy, where 15 cases of HUS, caused by serotype O157 and other EHEC serotypes, was recorded over a 5-month period in 1993 (17). These cases occurred in small towns scattered over a large area with little apparent connection to each other; therefore, common food vehicles and exposure to cattle were eliminated as possible sources of infection. However, data from the epidemiologic investigations suggested that contact with live poultry or with chicken coops may have been the source of infection, even though no toxin-producing EHEC strains were isolated from poultry feces. A recent study showed that inoculating 1-day-old chicks with strains of serotype O157:H7 resulted in rapid colonization of the cecal tissue of the chicks. The chicks then became long-term (up to 11 months) shedders of serotype O157:H7, and this microorganism was subsequently recovered from the shells of their eggs (18). It is conceivable, therefore, that live poultry were the source of infection in the outbreaks reported from northern Italy.

In December 1994, dry cured salami was implicated as the source of serotype O157:H7 in a disease outbreak in the state of Washington (19). A prior study showed that although isolates of serotype O157:H7 do not grow in seeded sausage batter, they can tolerate the acidity produced during sausage fermentation and survive the drying and the cold storage associated with the preparation of dry sausages (20). Fermented sausages can attain a pH as low as 4.8 (20). The ability of serotype O157:H7 isolates to persist under these conditions is consistent with the acid-tolerant properties this organism exhibited in the previously discussed studies with apple cider (7) and mayonnaise (10).

Although the consumption of bovine products still accounts for most of the serotype O157:H7 infections, the incidents described above show that other food types can also serve as vehicles in transmitting infections with this serotype.

## Emergence of Phenotypic Variants

Multilocus enzyme electrophoresis of *E. coli* strains associated with enteric disease show that serotype O157:H7 is in a well-defined group only distantly related to other SLT-producing serotypes (4,5). Recently, however, several phenotypic variants of this serotype were isolated in Europe. Thus, in addition to causing infections through food vehicles, the problems associated with serotype O157:H7 are compounded by the emergence of phenotypic variants, which may have an impact on diagnostic assays used to detect this pathogen.

The clonal nature of serotype O157:H7 has facilitated its phenotypic identification. Unlike other *E. coli*, isolates of serotype O157:H7 do not ferment sorbitol in 24 hours (21) and are negative in the methyl-umbelliferyl glucuronide assay (22), which measures glucuronidase activity (23). These phenotypes, especially the absence of sorbitol fermentation, are used extensively to distinguish isolates of serotype O157:H7 from related bacteria. Isolation of serotype O157:H7 from foods, on selective media, such as hemorrhagic colitis agar (24) and cefixime-tellurite sorbitol-MacConkey agar (25) is based on the sorbitol phenotype. Similarly, sorbitol-MacConkey agar (26) is used in the clinical laboratory as the primary screening medium to analyze patient specimens for the presence of serotype O157:H7. Prompt culturing of bloody stools with this agar has been very effective in isolating serotype O157:H7 from stool specimens (1).

Although extremely useful, isolating and identifying the pathogen exclusively on the absence of sorbitol fermentation has some limitations. Other enteric bacteria, such as *E. hermanii* and *Hafnia* spp., share similar phenotypes and resemble serotype O157:H7 on sorbitol-containing medium. Likewise, strains of O157, of non-H7 serotype that are not pathogenic and do not ferment sorbitol have occasionally been isolated from foods (27). Because of the presence of phenotypically similar species sorbitol negative isolates must be serologically confirmed with O157 and H7 antisera (28).

Though intended solely to select for serotype O157:H7, sorbitol-containing media may also exclude the isolation of other pathogenic *E. coli* serotypes, many of which ferment sorbitol. It appears that serotype O157:H7 is the predominant pathogenic serotype worldwide; however, a large number of other serotypes also produce SLT (1,2). Although many of these have not been implicated in disease or are known to cause only nonbloody diarrhea, some reports indicate that selected SLT-producing, non-O157:H7 serotypes may have caused cases of hemorrhagic colitis and HUS in Europe (29,30). In the United States, disease caused by non-O157:H7 serotype is rare; however, a recent outbreak of bloody diarrhea in Montana was suspected to have been caused by a SLT-II-producing *E. coli* of serotype O104:H21 (31).

A more relevant finding, and one that has stronger implications regarding the reliance on sor-

bitol phenotype for identifying pathogens, comes from a recent study which showed that isolates of serotype O157:H7 in sorbitol-containing foods can mutate from a sorbitol-nonfermenting to a sorbitol-fermenting phenotype (32). Moreover, the frequency of isolation of sorbitol-fermenting O157 strains in Europe appears to be increasing. In Germany, for instance, strains of serotype O157:H$^-$ that produce SLT-II have been isolated from HUS patients (33). Unlike serotype O157:H7, these strains fermented sorbitol and were positive in the methyl-umbelliferyl glucuronide assay. Initially, these strains were considered atypical. However, other studies confirmed that pathogenic, sorbitol-fermenting, serotype O157:H$^-$ strains were fairly prevalent in HUS patients in central Europe (34). In another report, serologic and biochemical characterization of 41 SLT-producing, O157 strains (including H7 and H$^-$ serotypes) determined that as many as 25% of the isolates were sorbitol positive. Furthermore, there was considerable variation among pathogenic serotype O157 isolates not only with respect to sorbitol fermentation, but also with respect to other phenotypic characteristics (6). These variants are not detected by sorbitol-containing media and may not be identified by the routine biochemical tests used to characterize serotype O157:H7.

The notoriety of recent outbreaks has stimulated the development of many new assays to detect serotype O157:H7; some of them may also be useful for detecting phenotypic variants. Many of these assays use molecular techniques, and some are commercially available. Several new molecular subtyping methods have also been introduced. Although typing methods will not be discussed here, such techniques as ribotyping, pulsed-field gel electrophoresis (35), lambda restriction fragment length polymorphism (36), and others have been extremely useful in studying the epidemiology of serotype O157:H7 in foodborne outbreaks.

Phenotypic variants of serotype O157:H7 retain the O157 antigen; hence, antibodies to O157 antigen can be used to identify both serotype O157:H7 and its variants. In the clinical laboratory, anti-O157 sera are used effectively in agglutination or latex agglutination tests to rapidly screen or serologically confirm isolates. Some anti-O157 antibodies have also been coupled to magnetic beads and used to selectively isolate this pathogen from foods (37) or have been incorporated into enzyme immunoassays to directly detect serotype O157:H7 in foods and clinical specimens. The latter application of anti-O157 sera, however, has had some drawbacks. Many preparations of anti-O157 sera cross-react with other bacteria, including *Citrobacter freundii* (38), *E. hermanii*, and *Yersinia enterocolitica* O:9 (39). Moreover, the O157 antigen is present on other non-H7 *E. coli* serotypes (6,40), many of which are not pathogenic. For example, when anti-O157 serum was used in an analysis of various food products, nonpathogenic O157 isolates were found that neither produced SLT nor were of the H7 serotype (27). Therefore, positive test results of food samples tested with assays that use anti-O157 sera should be confirmed by other methods. Pre-absorption of diagnostic antisera to remove cross-reacting antibodies or the use of antibodies specific for other non-O157 surface antigens of serotype O157:H7 may reduce the frequency of serologic cross-reactions (41).

Phenotypic variants also appear to retain the pathogenicity of serotype O157:H7 (6,33); therefore, assays specific for virulence factors are not affected by the phenotypic variations described above. For example, anti-SLT antibodies can be used to screen fecal specimens for toxins, and SLT gene-specific DNA probes and polymerase chain reaction (PCR) can be used to identify all SLT-producing pathogens regardless of phenotype. However, assays specific for SLT or SLT genes do not provide sufficient data for epidemiologic investigations and "trace-back" studies. More than 60 *E. coli* serotypes have been found to produce SLT (1,2), and even strains from more distantly related genera, such as *C. freundii*, reportedly produce SLT-II-like cytotoxins (42). Many of these SLT-producing *E. coli* serotypes have not been implicated in disease; therefore, the mere detection of potential SLT-producing strains in foods or in patients' specimens by these assays is not conclusive evidence that the bacteria caused the illness.

Some new assays do not have these limitations. One PCR assay, designed as a mismatch amplification mutation assay, preferentially amplifies an allele in the *uid*A gene that is unique only to serotype O157:H7, including its phenotypic variants of serotype O157:H$^-$ that are sorbitol and methyl-umbelliferyl glucuronide positive (43). Coupled with primers specific for SLT genes, this multiplex PCR assay can simultaneously identify isolates of serotype O157:H7 and the type of SLT they encode (44). Analysis of pure culture isolates showed that this assay detected all SLT-producing serotypes and was able to distinguish isolates of serotype O157:H7, including the phenotypic variants.

Advantages of these new molecular methods include specificity, sensitivity, and the ability to detect phenotypic variants of serotype O157:H7. However, these assays are far too complex and costly for use in the routine analysis of food or clinical specimens. Furthermore, although the emergence of phenotypic variants is of concern, they have only been observed sporadically and are not prevalent worldwide. Nonetheless, should the frequency of isolation or the incidence of infection caused by these variants increase or should other SLT-producing serotypes of *E. coli* become more firmly established as causative

agents of illness, other media or assays may need to be incorporated into existing diagnostic methods. In the interim, the continued use of a sorbitol-containing medium such as sorbitol-MacConkey agar to screen bloody stool specimens is a useful and economical laboratory procedure for the early diagnosis of serotype O157:H7 infections.

## Conclusions

Bovine products have most often been implicated in foodborne infections with *E. coli* serotype O157:H7. However, recent outbreaks indicate that other food types may also serve as vehicles of transmission for this pathogen. Most notably, acidic foods that were once thought to be of low risk can no longer be considered safe because of the acid-tolerant properties of this bacterium. Most microbiologic media and diagnostic assays have been designed specifically to detect serotype O157:H7. However, there is phenotypic diversity within the serogroup. Should these variants become more prevalent in infections, the use of sorbitol medium alone may become inadequate in detecting the diversity of strains in this pathogenic serotype.

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# Epidemic-Associated *Neisseria meningitidis* Detected by Multilocus Enzyme Electrophoresis

In Oregon and parts of Washington State, the incidence of serogroup B meningococcal disease increased substantially in 1994 (1). Multilocus enzyme electrophoresis (MEE) subtyping of *N. meningitidis* serogroup B strains collected in these areas during 1993 and 1994 suggested that these increases were due to a group of genetically related strains of the enzyme type-5 (ET-5) complex. ET-5 *N. meningitidis* serogroup B were first recognized in Norway in 1974 as the cause of a meningococcal disease epidemic that persisted through 1991. Since 1974, serogroup B meningococci of the ET-5 complex have caused epidemics in Europe, Cuba, and South America; these epidemics elevated disease rates for many years in the affected areas (2,3) and led to sustained efforts for vaccine development. This report describes the use of MEE to compare invasive *N. meningitidis* serogroup B meningococcal strains from Oregon and Washington with epidemic serogroup B strains from other countries and with serogroup B strains that have caused endemic disease in other parts of the United States.

MEE, first described in 1966 as a molecular approach to the study of genetic variation in eukaryotic systems, has only gradually been adopted by microbiologists and epidemiologists. The fundamental concept underlying MEE is that differences in the electrophoretic mobility of constitutive enzymes (resulting from amino acid substitutions) reflect the chromosomal genotype of strains and thereby allow the calculation of a genetic-relatedness index (Figure 1). As recently as 1984, only one bacterial species, *Escherichia coli*, had been studied by MEE. Since then, however, MEE has been used to characterize genetic variation among populations of *Legionella* spp., *Bordetella* spp., *Haemophilus influenzae*, *Streptococcus* spp., *Listeria monocytogenes*, *Neisseria meningitidis*, and other bacteria (4).

To carry out MEE, crude aqueous extracts of bacteria are electrophoresed in a block of 11% to 12% starch in the presence of a dilute buffer (pH 8.0). The block is then cut into thin slices, which are stained to detect specific enzymes. The distance traveled by each enzyme is used to create a series of numbers representing the set of enzyme mobilities characteristic of individual strains. The number of enzymes used is somewhat arbitrary and varies between organisms; 15 to 24 enzymes have usually been adequate to characterize genetic diversity among bacterial populations. For this investigation, electrophoretic variations in 24 enzymes were used to describe genetic variability among isolates of *N. meningitidis* serogroup B. *N. meningitidis* strains used for this analysis were collected from Oregon (1993-1994, n = 64) and part of Washington State (1993-1994, n = 17; 1992, n = 2; 1990, n = 1; unknown, n = 2); serogroup B meningococcal epidemics outside the United States (1976-1993; Norway n = 1; Cuba n = 1; Brazil n = 1; and Chile n = 2); and active population-based surveillance for meningococcal disease in selected areas of the United States (1991-1994, from the San Francisco Bay area, Georgia, Maryland, Oklahoma, and Tennessee, n = 57). The epidemic strains tested from Norway and Cuba are the type used for the outer membrane protein vaccines developed and tested in these countries.

The MEE data analyzed here (Figure 1) suggest that the increased rates of disease in Oregon and part of Washington are caused by highly genetically related *N. meningitidis* serogroup B strains of the ET-5 complex. These strains have been relatively rare in the United States. Oregon and Washington strains match a strain isolated in Santiago, Chile, during 1993. The prolonged duration of some ET-5 serogroup B meningococcal epidemics in large regions (e.g., Brazil, Argentina, and Chile) demands careful monitoring of this organism in the United States. Efforts to identify potentially modifiable risk factors for the disease and develop a vaccine have been intensified. MEE will continue to be the primary means for epidemiologic tracking and surveillance of ET-5 complex *N. meningitidis* serogroup B in the United States.

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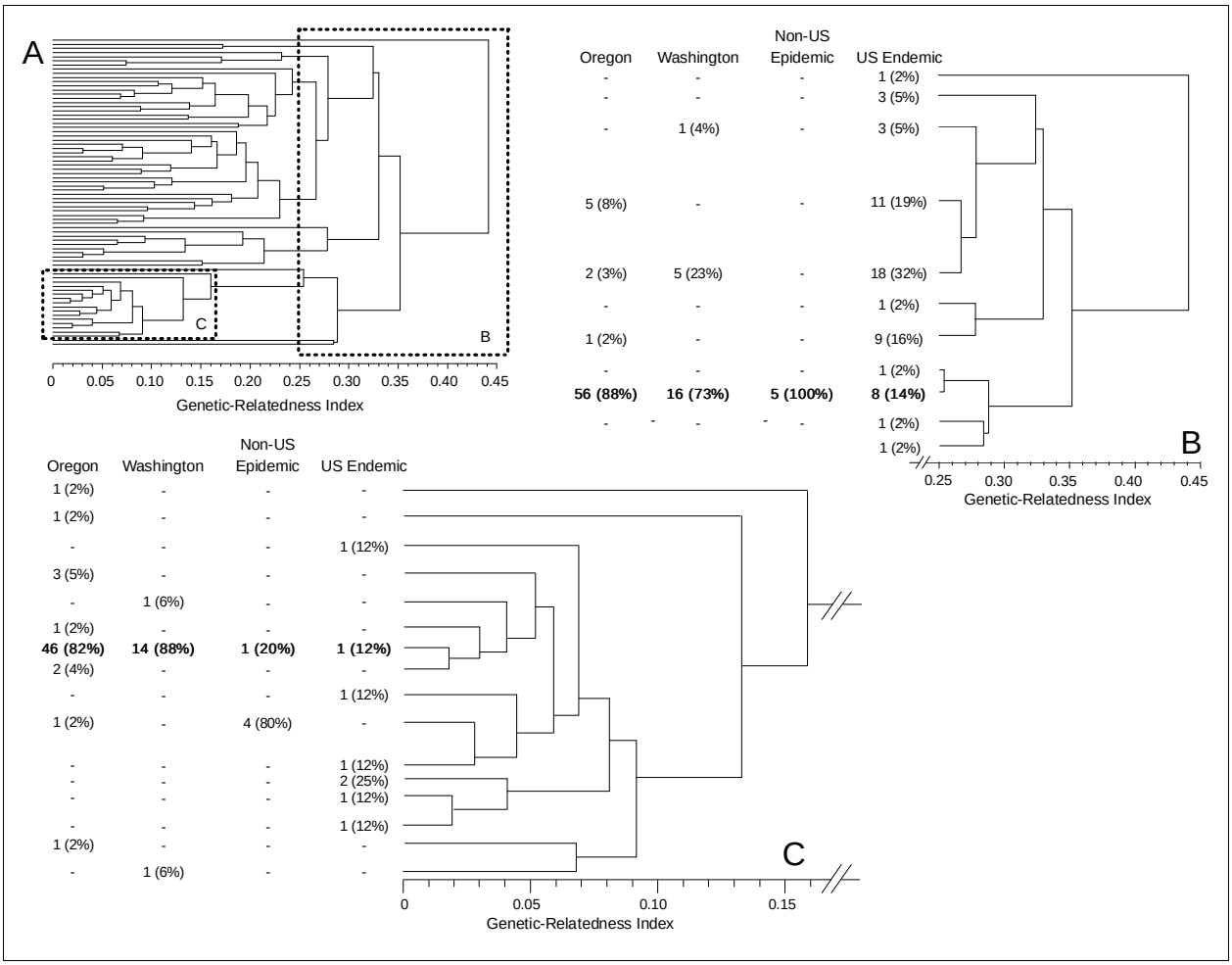


Figure 1. Genetic relatedness of serogroup B strains of *Neisseria meningitidis* from Oregon, Washington, other countries, and endemic-disease cases in the United States.

**A**. *Computer-generated dendrogram for all isolates*; 68 enzyme types (ETs) were identified with the 24 enzymes used in this study. To determine the relatedness of two ETs, start at the left side of the dendrogram at the line (or leg) representing the ET of interest and follow the leg horizontally to the right angle turn (up or down), allowing a path to the other ET. The point on the x-axis at which a right angle turn (up or down) is made to move horizontally back to the left indicates the genetic relatedness. For example, the ET at the top of the dendrogram is related to the other ETs at slightly more than 0.44; the next two ETs are related to each other at an index of approximately 0.17.

**B**. *Expanded view of dendrogram with a genetic-relatedness index of 0.25 to 0.45*. The ET-5 complex cluster is shown in bold type. This portion of the dendrogram represents the population structure of group B meningococci in this study; all strains with a genetic-relatedness level of 0.25 or less are shown as single legs or "complexes" of related strains. The distribution of serogroup B meningococcal strains by site and epidemiologic type (Oregon, Washington, non-U.S. epidemic, and U.S. endemic) and by ET group (or complex) is shown in columns to the left of the dendrogram. At a genetic-relatedness level of 0.25, the dendrogram is divided into 11 ET complexes. The ET-5 complex is the ninth leg down (or third from the bottom), shown in bold type. Of strains endemic in the United States, the highest proportion, 18 (32%) of 56, comprise an ET complex located at the fifth leg from the top and are related to the ET-5 complex at a genetic-relatedness index of just over 0.35. In contrast, 56 (88%) of 64 Oregon strains, 16 (73%) of 22 Washington strains, and 5 of 5 non-U.S. epidemic strains are in the ET-5 complex. Only 8 (14%) of 57 strains endemic in the United States are in the ET-5 complex.

**C.** *Expanded view of ET-5 portion of the dendrogram with genetic-relatedness index of 0 to 0.16*. The distribution of strains by site and epidemiologic type, within the ET-5 complex, is shown to the left of the dendrogram; 16 ETs are represented in the ET-5 complex. The eight strains endemic in the United States in the ET-5 complex are distributed among seven ETs. Forty-six (82%) of 56 Oregon strains and 14 (88%) of 16 Washington strains are clustered at the seventh leg down. One of the five non-U.S. epidemic strains, one of the two strains from Chile, and one (of 56) of strains endemic in the United States match these strains. The other four non-U.S. epidemic strains are located at the tenth leg down, along with one strain from Oregon; these are related to the cluster at the seventh leg at a genetic-relatedness index of approximately 0.06. This slight difference in relatedness results from a difference in the electrophoretic mobility of a single enzyme.

Bacteria strains were provided by K. Steingart, Southwest Washington Health Dist., and M. Goldoft, Washington Dept. of Health.

# Dengue/Dengue Hemorrhagic Fever: The Emergence of a Global Health Problem

Dengue and dengue hemorrhagic fever (DHF) are caused by one of four closely related, but antigenically distinct, virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4), of the genus *Flavivirus* (1). Infection with one of these serotypes does not provide cross-protective immunity, so persons living in a dengue-endemic area can have four dengue infections during their lifetimes. Dengue is primarily an urban disease of the tropics, and the viruses that cause it are maintained in a cycle that involves humans and *Aedes aegypti*, a domestic, day-biting mosquito that prefers to feed on humans. Infection with a dengue virus serotype can produce a spectrum of clinical illness, ranging from a nonspecific viral syndrome to severe and fatal hemorrhagic disease. Important risk factors for DHF include the strain and serotype of the virus involved, as well as the age, immune status, and genetic predisposition of the patient.

The first reported epidemics of dengue fever occurred in 1779-1780 in Asia, Africa, and North America; the near simultaneous occurrence of outbreaks on three continents indicates that these viruses and their mosquito vector have had a worldwide distribution in the tropics for more than 200 years. During most of this time, dengue fever was considered a benign, nonfatal disease of visitors to the tropics. Generally, there were long intervals (10-40 years) between major epidemics, mainly because the viruses and their mosquito vector could only be transported between population centers by sailing vessels.

A global pandemic of dengue begun in Southeast Asia after World War II and has intensified during the last 15 years. Epidemics caused by multiple serotypes (hyperendemicity) are more frequent, the geographic distribution of dengue viruses has expanded, and DHF has emerged in the Pacific region and the Americas (1,2). In Southeast Asia, epidemic DHF first appeared in the 1950s, but by 1975 it had become a leading cause of hospitalization and death among children in many countries. In the 1980s, DHF began a second expansion into Asia when Sri Lanka, India, and the Maldive Islands had their first major DHF epidemics; Pakistan first reported an epidemic of dengue fever in 1994. The recent epidemics in Sri Lanka and India were associated with multiple dengue virus serotypes, but DEN-3 was predominant and was genetically distinct from DEN-3 viruses previously isolated from infected persons in those countries (3).

After an absence of 35 years, epidemic dengue fever occurred in both Taiwan and the People's Republic of China in the 1980s. The People's Republic of China had a series of epidemics caused by all four serotypes, and its first major epidemic of DHF, caused by DEN-2, was reported on Hainan Island in 1985 (4). Singapore also had a resurgence of dengue/DHF from 1990 to 1994 after a successful control program had prevented significant transmission for over 20 years (5). In other countries of Asia where DHF is endemic, the epidemics have become progressively larger in the last 15 years.

In the Pacific, dengue viruses were reintroduced in the early 1970s after an absence of more than 25 years. Epidemic activity caused by all four serotypes has intensified in recent years with major epidemics of DHF on several islands (6).

Despite poor surveillance for dengue in Africa, we know that epidemic dengue fever caused by all four serotypes has increased dramatically since 1980. Most activity has occurred in East Africa, and major epidemics were reported for the first time in the Seychelles (1977), Kenya (1982, DEN-2), Mozambique (1985, DEN-3), Djibouti (1991-92, DEN-2), Somalia (1982, 1993, DEN-2), and Saudi Arabia (1994, DEN-2) (1,6, CDC, unpublished data). Epidemic DHF has been reported in neither Africa nor the Middle East, but sporadic cases clinically compatible with DHF have been reported from Mozambique, Djibouti, and Saudi Arabia (CDC, unpublished data).

The emergence of dengue/DHF as a major public health problem has been most dramatic in the American region. In an effort to prevent urban yellow fever, which is also transmitted by *Ae. aegypti*, the Pan American Health Organization organized a campaign that eradicated *Ae. aegypti* from most Central and South American countries in the 1950s and 1960s. As a result, epidemic dengue occurred only sporadically in some Caribbean islands during this period. The *Ae. aegypti* eradication program, which was officially discontinued in the United States in 1970, gradually eroded elsewhere, and this species began to reinfest countries from which it had been eradicated. In 1995, the geographic distribution of *Ae. aegypti* was similar to its distribution before the eradication program (Figure 1).

In 1970, only DEN-2 virus was present in the Americas, although DEN-3 may have had a focal distribution in Colombia and Puerto Rico (7). In 1977, DEN-1 was introduced and caused major epidemics throughout the region over a 16-year period (7). DEN-4 was introduced in 1981 and caused similar widespread epidemics (7). Also in 1981, a new strain of DEN-2 from Southeast Asia caused the first major DHF epidemic in the Americas (Cuba) (7).

This strain has spread rapidly throughout the region and has caused outbreaks of DHF in Venezuela, Colombia, Brazil, French Guiana, Suriname, and Puerto Rico. By 1995, 14 countries in the American region had reported confirmed DHF cases (Figure 2), and DHF is endemic in many of these countries.

DEN-3 virus recently reappeared in the Americas after an absence of 16 years. This serotype was first detected in association with a 1994 dengue/DHF epidemic in Nicaragua (8). Almost simultaneously, DEN-3 was confirmed in Panama and, in early 1995, in Costa Rica (8, CDC, unpublished data). In Nicaragua, considerable numbers of DHF were associated with the epidemic, which was apparently caused by DEN-3. In Panama and Costa Rica, the cases were classic dengue fever.

Viral envelope gene sequence data from the DEN-3 strains isolated from Panama and Nicaragua have shown that this new American DEN-3 virus strain was likely a recent introduction from Asia since it is genetically distinct from the DEN-3 strain found previously in the Americas, but is identical to the DEN-3 virus serotype that caused major DHF epidemics in Sri Lanka and India in the 1980s (R. Lanciotti; unpublished data). The new DEN-3 strain, and the susceptibility of the population in the American tropics to it, suggests that DEN-3 will spread rapidly throughout the region and likely will cause major epidemics of dengue/DHF in the near future.

In 1995, dengue is the most important mosquito-borne viral disease affecting humans; its global distribution is comparable to that of malaria, and an estimated 2.5 billion people are living in areas at risk for epidemic transmission (Figure 3). Each year, tens of millions of cases of dengue fever occur and, depending on the year, up to hundreds of thousands of cases of DHF. The case-fatality rate of DHF in most countries is about 5%: most fatal cases are among children.

There is a small, but significant, risk for dengue outbreaks in the continental United States. Two competent mosquito vectors, *Ae. aegypti* and *Aedes albopictus*, are present and, under certain circumstances, each could transmit dengue viruses. This type of transmission has been detected twice in the last 15 years in south Texas (1980 and 1986) and has been associated with dengue epidemics in northern Mexico (7). Moreover, numerous viruses are introduced annually by travelers returning from tropical areas where dengue viruses are endemic. From 1977 to 1994, a total of 2,248 suspected cases of imported dengue were reported in the United States (9, CDC, unpublished data). Although some specimens collected were not adequate for laboratory diagnosis, preliminary data indicate that 481 (21%) cases were confirmed as dengue (9, CDC, unpublished data). Many more cases probably go unreported each year because surveillance in the United States is passive and relies on physicians to recognize the disease, inquire about the patient's travel history, obtain proper diagnostic samples, and report the case. These data underscore the fact that southern Texas and the southeastern United States, where *Ae. aegypti* is found, are at risk for dengue transmission and sporadic outbreaks.

The reasons for this dramatic global emergence of dengue/DHF as a major public health problem are complex and not well understood (10). However, several important factors can be identified. First, effective mosquito control is virtually nonexistent in most dengue-endemic countries. Considerable emphasis for the past 20 years has been placed on ultra-low-volume insecticide space sprays for adult mosquito control, a relatively ineffective approach for controlling *Ae. aegypti*. Second, major global demographic changes have occurred, the most important of which have been uncontrolled urbanization and concurrent population growth. These demographic changes have resulted in substandard

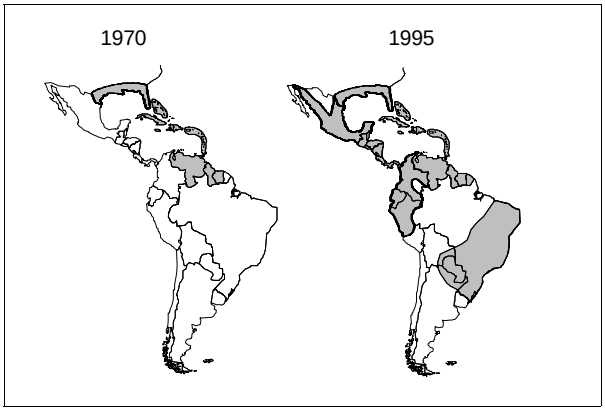


Figure 1. Distribution of *Aedes aegypti* (shaded areas) in the Americas in 1970, at the end of the mosquito eradication program, and in 1995.

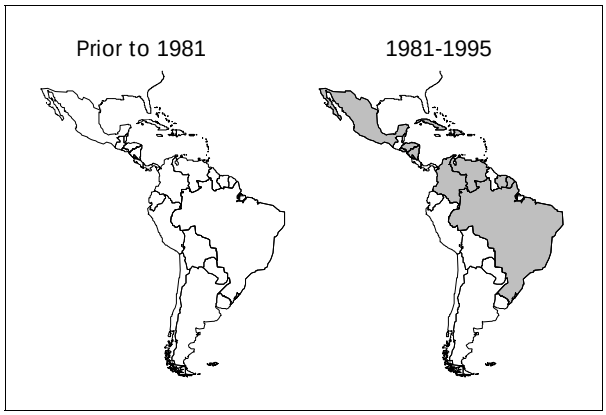


Figure 2. American countries with laboratory-confirmed hemorrhagic fever (shaded areas), prior to 1981 and from 1981 to 1995.

housing and inadequate water, sewer, and waste management systems, all of which increase *Ae. aegypti* population densities and facilitate transmission of *Ae. aegypti*-borne disease. Third, increased travel by airplane provides the ideal mechanism for transporting dengue viruses between population centers of the tropics, resulting in a constant exchange of dengue viruses and other pathogens. Lastly, in most countries the public health infrastructure has deteriorated. Limited financial and human resources and competing priorities have resulted in a "crisis mentality" with emphasis on implementing so-called emergency control methods in response to epidemics rather than on developing programs to prevent epidemic transmission. This approach has been particularly detrimental to dengue control because, in most countries, surveillance is very inadequate; the system to detect increased transmission normally relies on reports by local physicians who often do not consider dengue in their diagnoses. As a result, an epidemic has often reached or passed the peak of transmission before it is detected.

No dengue vaccine is available. Recently, however, attenuated candidate vaccine viruses have been developed in Thailand. These vaccines are safe and immunogenic when given in various formulations, including a quadrivalent vaccine for all four dengue virus serotypes. Unfortunately, efficacy trials in human volunteers have yet to be initiated. Research is also being conducted to develop second-generation recombinant vaccine viruses; the Thailand attenuated viruses are used as a template. However, an effective dengue vaccine for public use will not be available for 5 to 10 years.

Prospects for reversing the recent trend of increased epidemic activity and geographic expansion of dengue are not promising. New dengue virus strains and serotypes will likely continue to be introduced into many areas where the population densities of *Ae. aegypti* are at high levels. With no new mosquito control technology available, in recent years public health authorities have emphasized disease prevention and mosquito control through community efforts to reduce larval breeding sources (11). Although this approach will probably be effective in the long run, it is unlikely to impact disease transmission in the near future. We must, therefore, develop improved, proactive, laboratory-based surveillance systems that can provide early warning of an impending dengue epidemic. At the very least, surveillance results can alert the public to take action and physicians to diagnose and properly treat dengue/DHF cases.

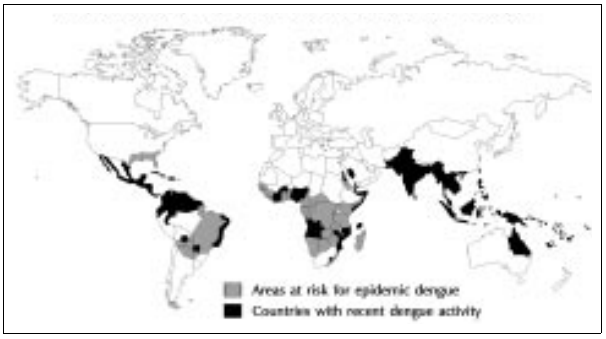


Figure 3. World distribution of dengue viruses and their mosquito vector, *Aedes aegypti*, in 1995.

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# Progress Toward the Eradication of Dracunculiasis (Guinea Worm Disease): 1994

Dracunculiasis, or guinea worm disease (GWD), is a disabling infection caused by the nematode parasite *Dracunculus medinensis*. The disease is endemic in India, Africa, and the Middle East. People become infected when they drink water containing tiny crustaceans, called copepods or "water fleas," that act as intermediate hosts of the organism and harbor infective larvae. When the ingested copepods are killed by the digestive juices in the stomach, the larvae are released and move to the small intestine. They penetrate the intestinal wall and migrate to the connective tissues of the thorax, where male and female larvae mature and mate 60 to 90 days after infection. Over the next year, female worms grow to maturity, reach a length of 70 cm or more (2–3 feet), and slowly migrate to the surface of the body. Worms emerge from the lower extremities in about 90% of cases, but they can also appear in the upper extremities, the trunk, buttocks, genitalia, or other parts of the body.

Infected persons remain asymptomatic for approximately a year after infection when the mature female worm approaches the skin and forms a painful papule in the dermis. This papule can become a blister within 24 hours or may enlarge for several days before becoming a blister. Eventually it ruptures, exposing the worm. Shortly before the skin lesion forms, pronounced systemic symptoms may occur, including erythema and urticarial rash with intense pruritus, nausea, vomiting, diarrhea, and dizziness. On contact with fresh water, a loop of the worm's uterus opens and discharges a swarm of motile larvae. This process may be repeated, if the lesion is resubmerged in water, until the entire brood of larvae is discharged. Larvae ingested by copepods mature in the body cavity of the intermediate host in about 2 weeks. Stagnant sources of drinking water such as ponds, cisterns, pools in dried-up river beds, temporary hand-dug wells, and step-wells commonly harbor populations of freshwater copepods and are the usual sites where the infection is transmitted.

As the worm emerges through the skin lesion, the affected person pulls it out slowly and carefully, usually by winding a few centimeters each day on a stick. This very painful process may last many weeks. Pain and other symptoms may lessen with the rupture of the blister, but at this time pyogenic organisms often invade the superficial lesion and worm tract and aggravate the condition. If the worm breaks during traction, an intense inflammatory reaction occurs, with pain, swelling, and cellulitis along the worm track. Infected persons are often incapacitated for several weeks, or for months, if complications caused by secondary bacterial infections ensue.

Infected persons do not develop immunity, and there is no cure for GWD. Pain from emerging worms may be relieved by applying wet compresses to the lesion or by immersing the lesion in a container of water, and then safely disposing of the released larvae. Placing an occlusive bandage on the wound to keep it clean prevents the patient from contaminating sources of drinking water. Once the worm appears, oral medications to relieve the associated pain and topical antiseptics or antibiotic ointment to minimize the risk for secondary infections allows the worm to be removed by gentle traction over a number of days.

The World Health Organization (WHO) has targeted GWD for eradication by the end of 1995. A coalition of agencies, institutions, and organizations are providing technical and financial assistance to national eradication programs.

An understanding of the factors that contribute to the emergence of dracunculiasis provides the basis for the current elimination program. GWD can be eradicated for several reasons: 1) there is no human carrier beyond the 1-year incubation period; 2) there is no known animal reservoir; 3) detection of patent infections (i.e., worms protruding from skin lesions) is an easy way to assess the presence of the disease in communities, and protrusion of the worm is required for transmission; 4) transmission of the disease is markedly seasonal, facilitating the timing and effectiveness of surveillance and control interventions, including containment of cases; 5) the methods for controlling transmission are simple, and 6) the disease is well recognized by the local population in areas where it is endemic.

The overall strategy of national GWD eradication programs includes three operational phases: l) conducting baseline surveys to identify villages where the disease is endemic; 2) training village-based health workers to use case registries for monthly surveillance and to implement control interventions in affected communities; and 3) using case-containment, i.e., prompt detection of all remaining cases (before or within 24 hours of worm emergence), treatment of the lesions to prevent transmission, and close supervision of village-based health workers to ensure that each case has been properly contained (1).

The thrust of control interventions is to educate affected persons about the origin of the disease and about the measures they and their communities can take to prevent it. Affected households are provided

with cloth filters and taught how to safely use them to remove the copepods from water. Household members are also taught that those with emerging worms should be kept from entering sources of drinking water. Other control interventions include providing safe drinking water to affected villages and selectively using the insecticide temefos to reduce copepod populations.

In 1986, an estimated 3.32 million cases of GWD occurred in Africa (2). That same year, only 22,610 cases of GWD were reported from India, the only affected country with a national eradication campaign then underway. Worldwide cases reported to WHO have declined from 781,219 in 1988 to 221,055 in 1993 (3). By 1990, 9 of the 19 affected countries had initiated eradication programs and had conducted baseline surveys to assess the extent of GWD (Figure 1, Table 1). At the end of 1994, all 19 countries were actively combating the diseases, and the provisional number of cases reported to WHO was 164,750 (Figure 2, Table 2). The number of affected villages was reduced from more than 23,000 in 1993 to fewer than 10,000; 52% of the affected villages were in the case-containment phase.

In 1994, Pakistan had no cases (4). (Pakistan is the first country where GWD was endemic during the 1980s to have eliminated indigenous transmission of the disease for 1 year.) Yemen reported small foci of disease transmission for the first time in several years, and Sudan reported 28,899 GWD cases during active village-based searches, and through its passive surveillance system (5). In early March 1995, Sudan revised the number of cases reported to WHO for 1994 to 53,092 cases. Although

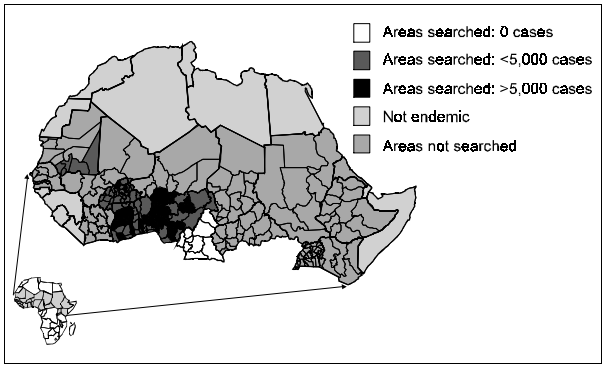


Figure 1. Status of dracunculiasis eradication in Africa: 1990.

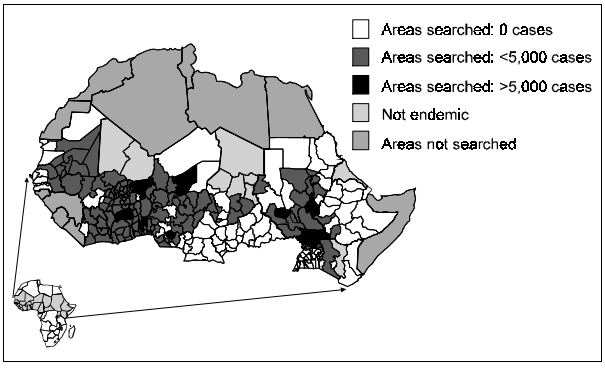


Figure 2. Status of dracunculiasis eradication in Africa: 1994.

**Table 1. Dracunculiasis cases reported, 1990***

| Country | Number of cases |
|---|---|
| Nigeria | 394,082 |
| Ghana | 117,034 |
| Burkina Faso | 42,187 |
| Benin | 37,414 |
| Mauritania | 8,036 |
| India | 4,798 |
| Togo | 3,042 |
| Cameroon | 742 |
| Pakistan | 160 |
| Chad† | – |
| Côte d'Ivoire† | – |
| Ethiopia† | – |
| Kenya† | – |
| Mali† | – |
| Niger† | – |
| Senegal† | – |
| Sudan† | – |
| Uganda† | – |
| Yemen† | – |

*Cases reported to the World Health organization from countries completing national case searches or from active village-based surveillance.
† No information available at this time.

**Table 2. Dracunculiasis cases reported, 1994***

| Country | Number of cases |
|---|---|
| Sudan | 53,092 |
| Nigeria | 39,774 |
| Niger | 23,568 |
| Uganda | 10,409 |
| Ghana | 8,432 |
| Burkina Faso | 6,859 |
| Mali | 5,396 |
| Togo | 5,045 |
| Mauritania | 5,029 |
| Côte d'Ivoire | 4,700 |
| Benin | 3,440 |
| Ethiopia | 1,252 |
| Chad | 640 |
| India | 371 |
| Senegal | 186 |
| Yemen | 74 |
| Kenya | 37 |
| Cameroon | 30 |
| Pakistan | 0 |

*Provisional data reported to the World Health Organization from countries completing national case searches or from active village-based surveillance. Sudan's report to WHO includes cases reported by the Passive Surveillance System.

the number of cases reported by Sudan may not be precise, their relative magnitude likely reflects that the incidence of disease there exceeds that reported by any of the other affected countries.

The global GWD eradication campaign faces two major challenges in 1995: to complete implementation of the case-containment strategy and other control interventions, such as insecticide use, in villages of all affected countries and to mobilize greater public support in these countries. The countries that pose the greatest eradication challenge are Sudan, Niger, and Nigeria. However, even in the countries with the fewest cases, markedly tighter control measures will be required to completely interrupt transmission of GWD by the end of 1995. Success of the eradication campaign depends on continued funding and on the ability of national programs and collaborating agencies and organizations to complete the needed surveillance and control interventions.

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# Heat-Stable Enterotoxin-Producing *Escherichia coli* O169:H41 in Japan

**To the Editor:** Enterotoxigenic *Escherichia coli* (ETEC) cause diarrhea by producing either a heat-labile enterotoxin, a heat-stable enterotoxin (ST), or both. Consequently, ETEC can be identified either by detecting the enterotoxin in the culture fluid by immunologic assays or by detecting enterotoxin-coding genes with DNA probes or polymerase chain reaction (PCR) amplification. For many clinical laboratories, however, serologic typing is the most common test used to determine if isolates are members of known pathogenic groups.

Although *E. coli* serotype O169:H8 has been recognized as one of the ETEC strains (1), serotype O169:H41 is not established worldwide as one of the diarrheagenic *E. coli* of the ETEC group. Ando et al. first reported that an outbreak at a school for physiologically handicapped children in Saitama Prefecture was due to ST-producing *E. coli* serotype O169:H41 (2). We report an outbreak of diarrhea caused by *E. coli* O169:H41 that predates the outbreak reported by Ando et al. and information about additional outbreaks in Japan since 1991.

In June 1991, we isolated toxigenic *E. coli* from the stool of two of three ill members of an eight-member family during an outbreak of diarrheal illness in Osaka, Japan. An epidemiologic investigation implicated pickles (kimchi) purchased during a visit to Korea; only the three members of the family who ate the pickles became ill. The major symptoms were diarrhea (3/3), abdominal pain (2/3), and fever (2/3) of 38° C. The incubation period was estimated at 33 hours. The serotype of these ST-producing *E. coli* isolates was not recognized immediately because the cultures were non-typable by the lot of *E. coli* antiserum available when the cultures were first isolated. The cultures were identified as *E. coli* O169:H41 when a new lot of antiserum became available.

In another outbreak investigated by the Osaka City Department of Environment and Health and the Osaka Prefecture Department of Environment and Health, food poisoning occurred among 776 of 1,242 guests of wedding receptions held at a wedding facility during September 1993. The main symptoms were diarrhea (98%) and abdominal cramps (74%), and the mean incubation period was 40.5 hours. *E. coli* O169:H41 was isolated from the stool specimens of 7 of 14 patients. A strain of *E. coli* O169:H41 was isolated from frozen, ready-to-eat seafood recovered from a distributor who provided foods to the wedding facility.

In addition to the outbreaks mentioned above, Japanese surveillance reports describe foodborne outbreaks in different prefectures between January 1991 and September 1994. In addition to being cultured for *E. coli*, stools were also routinely cultured for *Shigella*, *Salmonella* (including *typhi* and *paratyphi*), *Vibrio*, *Clostridium*, *Aeromonas*, *Plesiomonas*, *Bacillus cereus*, and *Staphylococcus aureus*. Stools were also examined for rotaviruses and small round viruses by electron microscopy. *E. coli* serotype O169:H41 was isolated from patients' stools in 11 of 40 outbreaks; recovery rates were 10%-100%. In 7 of the 11 outbreaks, recovery rates of serotype O169:H41 exceeded 75%. PCR was used to examine the diarrheagenicity of 31 *E. coli* isolates selected from reported outbreaks that occurred from 1991 to 1994 (3). ST production of the 31 isolates was also examined by COLI ST EIA (Denka Seiken Co., Ltd., Tokyo, Japan), a competitive enzyme-linked immunosorbent assay (ELISA) for toxigenic and invasive strains of *E. coli*. Strains were grown in Casamino Acids-Yeast Extract broth shaken at 37° C for 18 hours. The supernatant obtained after the centrifugation of cells was used for the test according to the manufacturer's instructions.

Thirty of the 31 *E. coli* O169:H41 isolates tested demonstrated toxigenicity by both PCR and ELISA. Collaborative studies are in progress to further characterize these isolates and to study the relationships between different isolates by molecular epidemiologic methods. Five cultures of *E. coli* O169:H41 have been ribotyped by a digoxigenin-labeled (Genius System, Boehringer Mannheim) probe prepared from pKK3535 according to the manufacturer's instructions. The resulting patterns were indistinguishable when the restriction enzymes *Eco*RI, *Sma*I, *Bgl*II, *Bam*HI, *Sal*I, *Pst*I, or *Hind*III were used to digest chromosomal DNA.

We suggest that this comparatively new serotype of ETEC may be spreading across Japan and urge that studies be conducted to determine its distribution and association with gastroenteritis worldwide.

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## The GAP Project in Southeastern Turkey: The Potential for Emergence of Diseases

**To the Editor**: The undersigned, representing interested scientists from both Turkey and the United States, recently visited the water development projects in southeastern Anatolia, Turkey. This letter describes our observations and projections on the possible health-related consequences of these projects with specific emphasis on infectious diseases.

When new irrigation schemes are introduced into previously dry areas, disease frequently follows the new water. The Southeastern Anatolia Irrigation Project or GAP (its Turkish acronym) is one of the largest projects ever undertaken in Turkey. This water resources development program includes the construction of 22 dams and 19 hydroelectric plants on the Euphrates and Tigris rivers in southeastern Turkey. Upon completion, the project will also include an irrigation network for 1.7 million hectares of land, covering eight provinces corresponding to approximately 10% of Turkey's total population and surface area (1). In its entirety, GAP comprises investments in development projects linked to agriculture, energy, transportation, telecommunications, health care, education, and urban and rural infrastructures. To ensure the success of the project, an agency has been established (the Republic of Turkey Prime Ministry Southeastern Anatolia Project Regional Development Administration) to oversee and implement all of these projects.

The largest of the completed dams on the Euphrates River is the Ataturk Dam. It is the sixth-largest rock-filled dam in the world; its hydroelectric systems have already produced more than seven billion kilowatt hours of power since 1992 (2). Water from the Ataturk Dam reservoir is diverted to the plains of upper Mesopotamia through the Sanliurfa Irrigation Tunnel System. This system consists of two parallel tunnels, each 26.5-km long and 7.62 m in diameter, and numerous other irrigation networks and canal systems. The first water started to flow to the plains of Harran in November 1994. Additional lands will be incorporated into the irrigation scheme as the canals are completed. (The year 2020 is the target date for completion.) When fully operational, GAP is expected to double Turkey's hydroelectric production, increase irrigated areas by 50%, more than double the per capita income in the region, more than quadruple the gross national product, and create two million new jobs in the coming decade (3). The total surface area affected by the irrigation scheme is about 75,000 $km^2$; of this, 46.2% is cultivated (36% semiarid rain-fed farmland), 33.3% is dry pastures, 20.5% is forest and bush.

One of GAP's major goals is to remove the socioeconomic disparity between the country's more developed regions and the project area. For GAP to reach its targeted and sustainable economic aims, projects in various other sectors also need to be considered and integrated. In this context, the public health consequences of emerging diseases in this setting must be anticipated so that appropriate health education and disease prevention measures can be implemented.

To anticipate changing patterns in disease associated with microclimatic and other environmental changes, knowledge of existing diseases in the region is vital. Since arthropods, reservoir animals, and other intermediate hosts are involved in the transmission of many waterborne parasitic diseases, a clear understanding of the existing species—especially of insect vectors—is equally important.

Historically, occasional cases of malaria have occurred in the region; however, limited records show that this disease is clearly on the rise. Cutaneous leishmaniasis is also endemic and on the rise, but few data are available on the prevalence of the visceral form of the disease. Other common diseases in the region include bacterial and helminthic gastrointestinal infections as well as trachoma.

According to data from the Malaria Division of the Turkish Health Ministry, the reported cases of *Plasmodium vivax* malaria rose from 8,680 in 1990 to 18,676 in 1992 (4). The province of Sanliurfa (population one million in 1990), which is at the heart of the irrigated plains in GAP, has reported that malaria cases increased from 785 in 1990 to 5,125 in 1993. The numbers of cases in the first 9 months of 1994 alone were already significantly higher than those reported in 1993 (S. Aksoy, unpublished data). Although presumably *P. vivax* malaria is most common, cases of *P. falciparum* malaria have also been reported in the country. Three cases of *P. falciparum* malaria were recently documented in Izmir, which is on the Aegean Sea coast of Turkey (4). No cases of drug-resistant malaria have been reported.

Another endemic disease on the rise in the southeastern region is leishmaniasis, transmitted by biting sand flies. In Sanliurfa the number of documented cases of the cutaneous form of this disease has risen from 552 in 1990 to 1,955 in 1993. In the first 9 months of 1994 alone, the number of reported cases was more than 3,000 (S. Aksoy, unpublished data). At Sanliurfa's Diyarbakir Hospital, in 1991, in addition to cases of the cutaneous forms of the disease, there were 80 potential cases of visceral leishmaniasis (kala-azar) in children ages 2 to 10 (5). *Leishmania donovani* is often the causative agent of kala-azar, but both *L. tropica* and *L. infantum* may also be involved (6). As the economic oppor-

tunities in the GAP provinces attract populations to the region, visceral leishmaniasis may become a greater threat. The prevalence of the sand-fly species in the region, their habitats, and the future implications of the microclimatic changes for these habitats must be studied to anticipate future disease patterns.

Other prevalent pathogens in the region include *Entamoeba histolytica, Giardia lamblia, and Ascaris lumbricoides.* Of 22,468 stool samples examined in one study, over 90% carried intestinal parasites; in children from infancy to 5 years of age, 60% contained *Giardia intestinalis* (7). In a second study in Diyarbakir involving 4,670 patients (ages <1–65 years), the incidence of protozoan and helminthic infection was approximately 16% (53%, *E. histolytica*; 31%, *G. lamblia*; and 10%, *A. lumbricoides*) (8). In both studies, the incidence of amebiasis was approximately 8% to 9%. In 1989, a survey conducted among 1,001 children in four elementary schools in Sanliurfa found parasites in 88% of the stool samples examined (50% *Ascaris*, 53% *Trichuris trichiura*, 22% *Giardia*, 11% *Entamoeba coli)* (9). Ancylostomiasis, which occurs in the eastern Mediterranean, is a potential danger for the region (10).

The emergence of schistosomiasis, which can quickly reach epidemic proportions in water-related projects unless measures are taken, should not be ignored. A recent study in Sanliurfa has identified *Bulinus truncatus*, the snail vector of *Schistosoma haematobium* in the region (11). Whether other regions in GAP also harbor this species is not known, although there have been reports of these snails in the Nusaybin and Mardin regions (12). A few decades ago, sporadic cases of disease were also reported from southeastern regions (13). As microclimatic changes occur in the GAP area, the presence of these snails and the potential emergence of schistosomiasis should be closely monitored.

The costs of combating epidemic diseases can be very large, whereas the costs of prevention are much lower. Large national projects that anticipate economic benefits may sometimes overlook the distant prospects of disease. Ideally, health planning should be built into a project from its inception for small funds invested for prevalence studies early on can bring high returns later. Earlier dam projects in Senegal, Lake Volta, and Egypt have shown that unless effective measures are taken early, infections can quickly reach epidemic levels (14). The establishment of good surveillance and recording systems is an important first step.

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# Action Plan for Drug-Resistant *Streptococcus pneumoniae*

*Streptococcus pneumoniae* is a leading cause of illness and death in the United States. It accounts for an estimated 3,000 cases of meningitis, 50,000 cases of bacteremia, 500,000 cases of pneumonia, and more than seven million cases of otitis media annually (1, 2). *S. pneumoniae* had been almost uniformly susceptible to penicillin; however, with the development and worldwide spread of drug-resistant *S. pneumoniae* (DRSP), a public health challenge has emerged. Studies from Australia, Southeast Asia, Africa, and Europe have reported pneumococcal strains resistant to penicillin and other drugs (3). Surveillance data collected at the Centers for Disease Control and Prevention (CDC) have shown that high-level resistance to penicillin increased more than 60-fold—from 0.02% for 1979-1987 to 1.3% in 1992—for pneumococcal isolates from invasive infections (4). In some communities, at least 30% of isolates are nonsusceptible to penicillin (5; CDC, unpublished data).

Pneumococcal resistance has been reported for beta-lactams, macrolides, chloramphenicol, and sulfonamides. As multidrug-resistant strains become increasingly prevalent, treatment options will become limited. The clinical impact of antimicrobial resistance on the outcome of invasive and noninvasive DRSP infections remains largely unknown. Vancomycin has been required to treat patients with pneumococcal meningitis caused by strains resistant to extended-spectrum cephalosporins (e.g., cefotaxime and ceftriaxone) (6). Optimal treatment regimens for DRSP infections remain to be defined; CDC is organizing a working group to develop consensus guidelines for the management of pneumococcal infections.

The prevalence of pneumococcal resistance to antimicrobial drugs is not known for most areas of the United States since DRSP infection has not been a reportable condition. Some studies have suggested great geographic and temporal variation in levels of resistance; prevalence rates are 2% to 30% (5,7). In addition, DRSP can spread rapidly through a population, and the prevalence of resistant isolates can differ in adults and children. To make appropriate empiric antimicrobial choices, clinicians need a reliable and current assessment of the level of antimicrobial resistance in the community.

To address the growing problem of DRSP, a working group of public health practitioners, health care providers, clinical laboratorians, and representatives of key professional societies was formed in June 1994. The group identified the development of an electronic, laboratory-based surveillance system for DRSP as the essential first step to address this concern. The group has issued a comprehensive plan, "A National Strategy for the Surveillance, Applied Research, Control, and Prevention of DRSP," to be published in June 1995. This plan focuses on three public health priorities: 1) to define and monitor the prevalence and geographic distribution of DRSP and recognize the emergence of patterns of resistance, 2) to study further the epidemiology of DRSP, and 3) to minimize the complications of DRSP infections through control and prevention.

The working group has begun piloting an electronic, laboratory-based surveillance network to detect serious illness due to DRSP. For isolates nonsusceptible to oxacillin (zone size <20 mm), minimal inhibitory concentrations should routinely be determined for penicillin, an extended-spectrum cephalosporin, chloramphenicol, vancomycin, and other clinically relevant drugs. These data will be analyzed to determine community-specific levels of resistance and will be made available to clinicians to improve antimicrobial use. Additionally, aggregate data will be sent to CDC so that national trends in pneumococcal resistance can be identified and reported.

Clinical laboratory directors, large commercial laboratory operators, and laboratory software manufacturers indicate that many laboratory software systems can accommodate a paperless, automated mechanism for reporting communicable disease information directly to public health authorities. DRSP surveillance may thus serve as a model for electronic, laboratory-based reporting for other laboratory-reportable conditions. Although improved data flow should increase the number and timeliness of reported cases, a strategy for ensuring quality control of data will be required.

In the era of emerging antimicrobial resistance, prevention of pneumococcal infections is paramount; vaccination strategies offer an important approach to controlling DRSP. An existing pneumococcal polysaccharide vaccine that can prevent a substantial number of pneumococcal infections, including those caused by DRSP, is underutilized. The vaccine is recommended by the Advisory Committee for Immunization Practices (ACIP) for use in persons older than 2 years of age who have certain underlying medical conditions and for all persons older than 65 years of age (8). It is not recommended for routine use among children under 2 years of age because it does not provide immunity consistently in this age group. An effective vaccine is needed to prevent pneumococcal infections in this population, which has the highest risk for otitis media and meningitis caused by DRSP. If the prevalence of pneumococcal infection (and therefore antimicrobial use) can be substantially reduced by vaccination, the impact of DRSP may diminish. Novel vaccine demonstration projects supported by federal and state health agencies are under way to explore means of increasing

coverage with the effective 23-valent pneumococcal polysaccharide vaccine.

Applied research is also needed to address the problem of DRSP. CDC has recently funded population-based investigations to define risk factors, patterns of transmission, costs, and health outcomes associated with DRSP. Public health programs for control and prevention of DRSP are being designed for use at local, state, and federal levels. Because unnecessary use of antimicrobial agents has contributed to the emergence of resistant bacteria (1, 3, 5), educational materials and campaigns are being developed for both health care providers and consumers to raise awareness of the link between excessive antimicrobial use and the emergence of drug-resistant organisms. Through a multifaceted approach, the growing problem of DRSP can be addressed to minimize the complications and costs of resistant pneumococcal infections. Surveillance for DRSP is an important starting point from which control and prevention solutions can proceed.

For more information regarding DRSP activities at CDC, write to Division of Bacterial and Mycotic Diseases, NCID, CDC Mailstop C-09, 1600 Clifton Rd., Atlanta, GA 30333 or send an e-mail to DRSP@ciddbd1.em.cdc.gov.

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## WHONET: An Information System for Monitoring Antimicrobial Resistance

WHONET is an information system developed to support The World Health Organization's (WHO) goal of global surveillance of bacterial resistance to antimicrobial agents. Microbiologists, clinicians and infection control workers may use its software to enhance monitoring of drug resistance in their hospitals and communities and to merge their files into national, regional, and global networks for surveillance of drug resistance. WHONET software can be installed on personal computers and be configured for the locations of the patients a laboratory serves and for the antimicrobial agents it tests. The program accepts susceptibility test results and allows printing of reports and logbooks and retrieval of data. If the laboratory already has a computerized reporting system, a translation program can be created to download the laboratory's files into WHONET. Either way, the microbiologists and other infectious disease specialists gain new analytical tools to monitor and manage susceptibility test quality and the spread of drug resistance locally and outside their area.

WHONET can also analyze stored data. From a single screen, a WHONET user selects the type of analysis to run, the species of bacteria to analyze, the subsets of isolates to include (e.g., all, isolates from urine only, and isolates resistant to gentamicin and from certain locations), and the antimicrobial agents and period to examine. Types of analyses include percentage of data categorized as resistant, intermediate, or susceptible by standard or other breakpoints; distributions of test measurements (zone diameter, minimal inhibitory concentration) in the form of histograms; scatterplots comparing measurements for different agents or methods for the same isolates; and line listings of isolates grouped by combinations of agents to which they are resistant (antibiotypes) to trace distinctive strains. Isolates with uncommon antibiotypes can also be flagged on entry so that they may be rechecked while still available, and local outbreaks can be detected early.

Although test results are entered and monitored locally on software configured for local use, they are filed in a universal file format so that any copy of the program can analyze the files of any laboratory. This feature has enabled groups of users in 10 countries to set up passive surveillance systems by pooling and analyzing their files collaboratively. WHONET assists such initiatives by providing file encryption options to ensure confidentiality before data are pooled and analyzed.

Ongoing local analysis by local workers is the foundation of the system. It detects local problems in testing, which no laboratory can avoid entirely, and thus improves the overall quality of the files. It delineates local spread of drug-resistant strains, which aids infection control and can explain and correct uncommon prevalence of certain types of drug resistance at certain sites. It allows local workers to distinguish their problems from those of other sites and focus on infection control or antimicrobial use that might be related to those problems.

Expansion of the system has been recommended by the WHO Scientific Working Group on Monitoring and Management of Bacterial Resistance to Antimicrobial Agents. For more information or for participation contact

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## Recommendations for Preventing the Spread of Vancomycin Resistance

CDC's Hospital Infection Control Practices Advisory Committee (HICPAC) has published "Recommendations for Preventing the Spread of Vancomycin Resistance." The recommendations focus on vancomycin-resistant enterococci (VRE).

The reported incidence of infection and colonization with VRE in U.S. hospitals has increased rapidly in the last 5 years. This increase has compounded the need for antimicrobial drugs to treat VRE infections. Most VRE are also resistant to multiple other drugs (e.g., aminoglycoside and ampicillin), which have been used for treating VRE infections. In addition, the possibility that the vancomycin-resistance genes present in VRE may be transferred to other gram-positive microorganisms, especially *Staphylococcus aureus*, is a serious public health concern.

Although the epidemiology of VRE has not been fully elucidated, and most enterococcal infections have been attributed to the patient's endogenous flora, recent studies have demonstrated that enterococci, including VRE, can be spread directly from patient to patient or indirectly by transient carriage on the hands of personnel or contaminated environmental surfaces and patient-care equipment.

In its recommendations, HICPAC stresses that the prevention and control of vancomycin resistance will require a coordinated, concerted effort from various departments of a hospital. Because the rec-

ommendations were developed with limited data and further research is needed to find cost-effective ways to control the spread of vancomycin resistance, HICPAC strongly encourages hospitals to develop their own institution-specific plans, which should stress the following elements: 1) prudent vancomycin use by clinicians, 2) education of hospital staff regarding vancomycin resistance, 3) early detection and prompt reporting of vancomycin resistance in enterococci and other gram-positive microorganisms by the hospital microbiology laboratory, and 4) immediate implementation of appropriate infection-control measures to prevent person-to-person transmission of VRE.

The recommendations were developed by HICPAC's Subcommittee on the Prevention and Control of Antimicrobial-Resistant Microorganisms in Hospitals and subject-matter experts and representatives of the American Hospital Association, American Society for Microbiology, Association for Professionals in Infection Control and Epidemiology, Infectious Diseases Society of America, Society for Healthcare Epidemiology of America, and Surgical Infection Society. The recommendations were published in February in *Infection Control and Hospital Epidemiology* 1995;16:105-13 and will also be published in the April 1995 issue of the *American Journal for Infection Control.*

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## Waterborne Cryptosporidiosis Threat Addressed

*Cryptosporidium parvum* was first recognized as a cause of human illness in 1976. From 1976 to 1982, the disease was reported rarely in the United States, primarily among the immunocompromised. In 1982, the number of reported cases began to increase dramatically along with the number of HIV-infected persons; outbreaks among immunocompetent populations also were reported. Recent municipal waterborne outbreaks of cryptosporidiosis in Texas (1984), Georgia (1987), and Oregon (1992), and a massive outbreak in Wisconsin in 1993 that affected more than 400,000 persons have raised awareness about the waterborne transmission of cryptosporidiosis. Since 1993, several smaller cryptosporidiosis outbreaks were reported in the United States: two were related to drinking water, six were linked to recreational water, and one was foodborne.

Cryptosporidiosis is caused by ingestion of the environmentally tough oocysts of the protozoan parasite *C. parvum*, an intracellular organism that can replicate in the gut epithelial cells of most mammals. Its oocyst is extremely resistant to chlorine, which is commonly used to treat municipal water.

In healthy persons, the disease lasts 1 to 2 weeks and can have considerable economic impact through absenteeism of those affected. In the immunocompromised, the disease is often severe, lifelong, and life-threatening. No effective therapy is available.

The magnitude of the 1993 Wisconsin outbreak and its association with a municipal water plant operating within existing state and federal regulations underlined the need for improved surveillance and coordination among public health agencies and spurred efforts for regulatory standards for *Cryptosporidium* in drinking water. During 1995-1996, the U.S. Environmental Protection Agency (EPA) intends to implement the Information Collection Rule, which requires utilities that serve populations of 100,000 or more and use surface water (lakes, rivers, streams) to test that water routinely for *Cryptosporidium* oocysts. If oocysts are found, the utility may also have to test finished water (tap water). Utilities that serve populations of 10,000 to 99,000 will also have to test source water, but for a shorter period. They will not be required to test tap water, even if oocysts are found. Authority to issue boil water advisories if oocysts are found varies from state to state. The health risks from ingesting low levels of *Cryptosporidium* are unknown. More than 300 representatives from 40 states and more than 25 regulatory, public health, water utility, and advocacy groups met at the Centers for Disease Control and Prevention (CDC) in Atlanta in September 1994 to discuss the prevention and control of waterborne cryptosporidiosis. Recommendations from the CDC workshop will be published in the next 2 to 3 months.

CDC held the first meeting of the Working Group on Waterborne Cryptosporidiosis in November 1994. The working group convenes biweekly by teleconference. For more information about the group, contact Margaret Hurd (phone: 404-488-7769, fax: 404-488-7761).

The working group has three main purposes: 1) promote a regular exchange of ideas, goals, activities, and proposals among individual scientists, agencies, and organizations interested in waterborne cryptosporidiosis; 2) make decisions on public health issues related to waterborne cryptosporidiosis; and 3) assemble smaller, more focused, task forces with expertise to develop, implement, and evaluate projects of the working group.

The working group has created task forces to assist local, state, and national public health departments, water utilities, and regulatory agencies in preparing for and managing outbreaks. The task forces have the following responsibilities:

- Develop and evaluate informational materials about cryptosporidiosis.
- Develop guidelines on when to initiate or end boil water advisories.
- Help formulate the language for EPA's Information Collection Rule.
- Identify officials with authority to issue boil water advisories. Examine legal issues associated with boil water advisories and the environmental testing, surveillance, and diagnostic requirements for waterborne *Cryptosporidium*.

In addition, technical task forces will collect data to develop guidelines for persons who may want to use bottled water and personal-use water filters and provide updates on the status of environmental sampling, water testing, and surrogate indicators of *Cryptosporidium* oocysts. These task forces will also report on the status of clinical diagnostic and serologic tools and provide local cryptosporidiosis infection rates to use in assessing the risk for waterborne transmission.

*C. parvum* oocysts are present in most surface water supplies; better technological tools and epidemiologic assessments are needed to determine the public health risks from these oocysts. Until the risks are fully known, efforts should be made to inform the public about cryptosporidiosis. Information on opportunistic infections, including cryptosporidiosis, for physicians who treat diseases in immunocompromised patients will be published this fall in a supplement to *Clinical Infectious Diseases* by authors from CDC and the Infectious Diseases Society of America.

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